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Yoghurt Science and technology

Second edition

A. Y. Tamime and R. K. Robinson

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Yoghurt: science and technology is a standard work in its field for industry professionals and those involved in applied research. Because manufacture is still, essentially, a natural biological process, it remains difficult to control the quality of the final product. Such control depends on a thorough understanding of the nature of yoghurt and both the biochemical changes and process technologies involved in production. *Yoghurt: science and technology* provides just such an understanding.

Since the last edition the industry has been transformed by the introduction of mild-tasting 'bio-yoghurts', changing both consumer markets and manufacturing practices. The new edition has been comprehensively revised to take on board this and other major changes in the industry, including new technological developments such as the production of strained yoghurt by ultrafiltration, and the latest advances in mechanisation and automation.

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YOGHURT

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Second edition

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Contents

This book is dedicated to our families

Preface to second edition

When the first edition of this book was published in 1985, the retail markets in Australasia, Europe and North America were dominated by just one product – stirred fruit yoghurt, with natural set yoghurt occupying a well-defined niche. Some traditional products like *labneh* and *drinking yoghurt* were manufactured on a small scale but, in general, the choice available to consumers was strictly limited.

Over the last ten years, this scenario has changed. Initially, competition for a share of the lucrative market for fermented milks gave rise to numerous variants of the basic products, but a more dramatic impact was achieved by the introduction of mild-tasting bio-yoghurts. In these latter products, selected bacteria with prophylactic/therapeutic properties are involved with the fermentation and, whilst many aspects of the yoghurt-making process remain the same, the introduction of these new cultures has led to some significant changes in both consumer attitudes and manufacturing practices.

In light of these recent developments, it became apparent that a revision of this book was long overdue, and it is to be hoped that readers will appreciate the introduction of bio-yoghurt and the additional information about this remarkable sector of the dairy industry.

Automation in yoghurt-making involves complex engineering and design and this technology has been covered by Mr J. Bird and Mr I. Chester who represent two of the foremost equipment manufacturers in the world. We would like to acknowledge their assistance and that of all the companies who provided us with technical information and illustrations. Last but not least, we are grateful to Mrs A. Peacock (SAC) for her patience in typing the manuscript, and Mrs Y. Gamble and E. McCall (SAC) for their skills in taking the necessary photographs and drawing the illustrations.

A. Y. Tamime
R. K. Robinson

Preface to first edition

Although there are numerous fermented milks produced on a local basis around the world, only yoghurt has achieved a truly international distribution. This popularity stems from a number of sources: the pleasant, aromatic flavour of natural yoghurt, its reputation as a foodstuff associated with good health, but perhaps above all from the fact that the thick, creamy consistency makes it an ideal vehicle for fruit. Thus, it was the natural compatibility with fruit that really brought yoghurt into the retail markets, and since the introduction of fruit yoghurts during the 1950s sales have climbed steadily upwards.

Today millions of gallons of yoghurt are produced each year, and yet because manufacture is still, in essence, a natural biological process, success can never be taken for granted. It is this capricious nature of the fermentation that makes it so fascinating, and indeed if the system were not so prone to variation, then there would have been little motivation to produce this book at all. Some aspects of production have, of course, become fairly standard, but so many areas of potential difficulty remain that only a thorough appreciation of the nature of yoghurt can provide those associated with its production and distribution with the confidence that eliminates product failure.

It goes without saying that the best teacher is experience, but if this book can offer some preliminary guidance on the intricacies of handling yoghurt, then its compilation will have been worthwhile.

1983

A. Y. Tamime
R. K. Robinson

1

Historical background

1.1   Introduction

Fermentation is one of the oldest methods practised by human beings for the trans-
formation of milk into products with an extended shelf life. The exact origin(s) of
the making of fermented milks is difficult to establish, but it could date from some
10–15 000 years ago as the way of life of human beings changed from being food
gatherer to food producer (Pederson, 1979). This change also included the domes-
tication of animals (i.e. cow, sheep, goat, buffalo and camel), and it is most likely
that the transition occurred at different times in different parts of the world. Archae-
ological evidence shows that some civilisations (e.g. the Sumarians and Baby-
lonians in Mesopotania, the Pharoes in north-east Africa and the Indians in Asia)
were well advanced in agricultural and husbandry methods, and in the production
of fermented milks such as yoghurt.

Although there are no records available regarding the origin of yoghurt, the
belief in its beneficial influence on human health and nutrition has existed in many
civilisations over a long period of time. According to Persian tradition, Abraham
owed his fecundity and longevity to yoghurt and, in more recent times, Emperor
Francis I of France was said to have been cured of a debilitating illness by con-
suming yoghurt made from goat's milk (Rosell, 1932).

It is likely, however, that the origin of yoghurt was the Middle East, and the evo-
lution of this fermented product through the ages can be attributed to the culinary
skills of the nomadic people living in that part of the world. Today, fermented milk
products are manufactured in many countries (Campbell-Platt, 1987; Kurmann *et
al.*, 1992), although few are of commercial significance.

1.2   Evolution of the process

The production of milk in the Middle East has always been seasonal, being restricted
usually to no more than a few months of the year. The main reason for this limited

availability of milk is that intensive animal production has never really existed, so that, as in early history, farming is in the hands of nomadic peoples who move from one area to another following the pastures. This type of existence forces nomads to be in the wilderness for months at a time, far away from populated cities and villages where they could sell their animal produce. Another major factor is that the Middle East has a subtropical climate and summer temperatures can reach as high as 40°C. In such a climate, milk turns sour and coagulates within a short time of milking, particularly as the milk is produced under primitive conditions. Thus, the animals are hand milked, no cooling of the milk is possible, and the risk of contamination by micro-organisms from the air, the animal, the feeding stuff or the hands of the milker is extremely high. Under these conditions the possibility of transporting or even keeping milk for any length of time is non-existent. As a result the bulk of the population consume milk only rarely, and even the nomadic people have to utilise the milk virtually as it is produced.

However, it may well have been evident even at an early stage that the souring of milk was by no means a uniform process. Thus, the fermentation brought about by non-lactic acid bacteria gives rise to a product which is insipid and stale and,

Table 1.1 Selection of yoghurt and yoghurt-like products that have been identified in the Middle East and elsewhere

Traditional name	Country
Jugurt/eyran/ayran	Turkey
Busa	Turkestan
Kissel mleka/naja/yaourt	Balkans
Urgotnic	Balkan mountains
Leban/laban or laban rayeb	Lebanon and some Arab countries
Zabady/zabade	Egypt and Sudan
Mast/dough/doogh	Iran and Afghanistan
Roba/rob	Iraq
Dahi/dadhi/dahee	India
Mazun/matzoon, matsun, matsoni, madzoon	Armenia
Katyk	Transcaucasia
Yiaourti	Greece
Cieddu	Italy
Mezzoradu	Sicily
Gioddu	Sardinia
Tarho/taho	Hungary
Viili	Finland
Filmjolk/fillbunke/filbunk/surmelk/taettemjolk/tettemelk	Scandinavia
Iogurte	Brazil and Portugal
Skyr	Iceland
Gruzovina	Yugoslavia
Donskaya/varenetes/kurugna/ryzhenka/guslyanka	Russia
Tarag	Mongolia
Shosim/sho/thara	Nepal
Yoghurt/yogurt/yaort/yourt/yaourti/ yahourth/yogur/yaghourt	Rest of the world ("Y" is replaced by "J" in some cases)

After: Tamime and Deeth (1980), Accolas *et al.* (1978), Tokita *et al.* (1982) and Kosikowski and Mistry (1997).

furthermore, the coagulum is irregular, filled with gas holes and shows extreme whey syneresis. Lactic acid bacteria, however, act on milk to produce a fermented product which is pleasant to eat or drink; this latter product was usually referred to as sour milk.

The animals that are raised by the nomadic peoples of the Middle East are cows, goats, sheep and camels, and gradually the nomadic tribes evolved a fermentation process which brought under control the souring of these various milks. In particular, the process might have included:

- use of the same vessels, or the addition of fresh milk to an on-going fermentation, relying mainly on the indigenous microflora to sour the milk;
- heating the milk over an open fire to concentrate the milk slightly, so that the final coagulum would acquire an attractive viscosity due to the modified properties of the casein, again a change which would have improved the quality of the end product;
- seeding the heat-treated and cooled milk (blood or ambient temperature) with sour milk from a previous batch, so enabling the thermophilic strains of lactic acid bacteria to become predominant;
- gradual selection of lactic acid bacteria capable of tolerating high levels of lactic acid and of giving the product its distinctive flavour;
- eradication of any pathogenic micro-organisms present in the milk.

Although the evolution of the process was strictly intuitive, the production of sour milk soon became the established pattern of preservation, and since the early 1900s, defined micro-organisms have been used to prepare these products on a large scale in factories. Gradually other communities learnt of this simple preservative treatment for milk and one such product became known as yoghurt from the Turkish word "jugurt"; numerous variants of this word have appeared over the years and a selection of alternatives is shown in Table 1.1.

1.3 Diversity of fermented milks

Around 400 generic names are applied to the traditional and industrialised fermented milk products manufactured throughout the world (Kurmann *et al.*, 1992). Although these products may have different names, they are practically the same, and a more accurate list might include only a few varieties. Taking into account the type of milk used, the microbial species which dominate(s) the flora and their principal metabolic products, Robinson and Tamime (1990) proposed a scheme of classification for fermented milks which divided them into three broad categories: (a) lactic fermentations, (b) yeast–lactic fermentations and (c) mould–lactic fermentations (Fig. 1.1). Recently, these products have been extensively reviewed by Tamime and Marshall (1997).

Although yoghurt has many desirable properties, it is still prone to deterioration, especially at ambient temperature, within a matter of days, and one discernible trend in the Middle East has been the search for simple techniques to extend the keeping quality.

The first step in this process turned out to be relatively simple because the containers traditionally used by the nomads for the production of yoghurt were made from animal skins. In normal use the yoghurt would have been consumed fairly
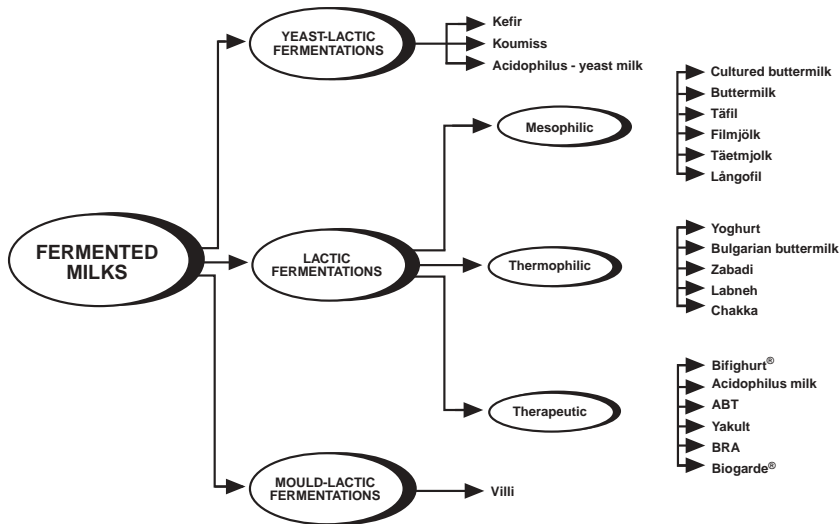
Fig. 1.1 Scheme of classification of fermented milk products (for details see Table 5.15) ABT, *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Streptococcus thermophilus*; BRA, *Bifidobacterium infantis*, *Lactobacillus reuteri* and *L. acidophilus.* Adapted from Robinson and Tamime (1990)

rapidly but, if left hanging in the skin for any length of time, the nature of the product altered dramatically. Thus, as the whey seeped through the skin and evaporated, the total solids content of the yoghurt rose and with it the acidity. The end result was a condensed or concentrated yoghurt with an acidity of >2.0% lactic acid and a total solids content in the region of $25 \text{ g} 100 \text{ g}^{-1}$; the original yoghurt might have had a solids content of $12–13 \text{ g} 100 \text{ g}^{-1}$ and an acidity of around 1.5% lactic acid. To the nomadic people, whose main sources of wealth and nourishment are the animals that can be raised and the milk that they produce, the relative resistance of the condensed yoghurt to spoilage must have appeared attractive.

Evidence of this trend can be found in Armenia where the mazun (Armenian yoghurt) is usually pressed to yield a product called tan or than. Similarly, surplus milk production in remote villages in Turkey is turned into concentrated yoghurt by the daily addition of milk to yoghurt hanging in goat or sheep skins. Another method of concentration of yoghurt is where the product is placed in an earthenware vessel; the Egyptians call this product leben zeer.

Nevertheless, even condensed yoghurt becomes unpalatable within a week or two, and it was for this reason that salted yoghurt rapidly became popular. Salting is an age-old method used by humans to preserve food, but the incorporation of salt into concentrated yoghurt also acts as a neutralising agent to reduce the acid taste of the product. Thus, different types of concentrated yoghurt are made in Turkey by the addition of various quantities of salt. Another traditional way of prolonging the keeping quality of concentrated yoghurt is employed in Lebanon, where the salted product is made into small balls about 2 cm in diameter and placed in the sun to dry. Afterwards the yoghurt balls (which are partially dried) are placed in either glazed earthenware pots or glass jars and covered with olive oil. The product is then referred to as winter yoghurt, that is, it is available when natural yoghurt is out of
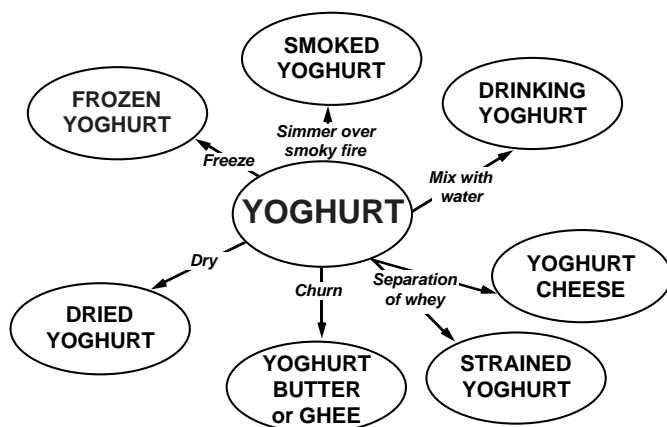
Fig. 1.2 Schematic illustration showing the different processes for the manufacture of yoghurt-related products

season and it has a storage life of up to 18 months; the product is spread easily on bread and consumed.

An alternative preservation process involves heating yoghurt for a few hours over low fires of a special type of wood; the end product is referred to as smoked yoghurt. This type of yoghurt is also preserved over the winter months by placing it in jars and covering it with either olive oil or tallow.

In some countries (Turkey, Lebanon, Syria, Iraq and Iran) the concentrated yoghurt is processed even further to produce a totally different product of almost indefinite keeping quality. This is a dried form of yoghurt; milk is processed into yoghurt in the traditional manner and wheat flour, semolina or parboiled wheat, known locally as burghol, is rubbed into it. The yoghurt–wheat mixture is shaped into small nuggets and placed in the sun to dry. This product is called kishk and it is sold either as nuggets or in a ground-up form as flour. Kishk (as a dish) is prepared by reconstituting the yoghurt–wheat mixture with water and then simmering the mix gently over a fire. The consistency of this product, which is normally consumed with bread, is similar to porridge.

The concentrated yoghurt can be also processed into a different product called chanklich. Here again the product is partially dried, but is then mixed with spices and herbs (presumably to assist in preservation). The mixture is then formed into balls, placed into glass jars and finally covered with olive oil. It is evident that many different products can be manufactured from yoghurt and Fig. 1.2 illustrates some examples; the relationship between these various products is discussed further in Chapter 5.

1.4 Patterns of consumption

As refrigeration became widespread, so interest in these traditional products declined, except among certain communities in the Middle East. In their place, a new generation of yoghurts emerged, with production typically centred on a large modern creamery, and success in the market place depending on the existence of a

Table 1.2 Per capita annual consumption (kg head^{-1}) of fermented milks in some selected countries

Country	1975[a] Bᵇ	Yᵇ	Oᵇ	1980 B	Y	O	1985 B	Y	O	1990 B	Y	O	1993 B	Y	O
Australia	–	1.0		–	1.8	–	–	2.8	–	–	3.5	–	–	4.8	–
Austria	0.6	3.4	3.9	2.0	5.8	2.0	2.2	6.6	2.2	2.9	7.5	–	1.9	8.6	2.5
Belgium	6.7	5.1	–	2.8	4.9	–	2.3	6.0	0.06	1.9	6.5	1.2	2.3	5.7	3.9
Canada	4.4	0.7	–	0.6	1.7	–	0.6	2.5	–	0.5	3.2	–	0.4	3.1	–
Chile					1.4			2.5			3.9				
Czechoslovakia	0.9	1.3	1.7	3.1	1.7	2.5	3.9	2.5	3.1						
Denmark	23.1	5.9	7.1	9.8	9.1	7.8	8.4	8.0	7.5	6.9	7.8	6.9	5.6	8.3	6.8
Federal Germany	7.7	4.6	4.2	2.2	6.7	1.2	2.1	7.9	1.1	2.8ᶜ	10.6ᶜ	0.8ᶜ	2.6ᶜ	11.3ᶜ	0.9ᶜ
Finland	7.2	6.3	29.1	4.1	8.4	28.5	1.9	9.4	28.0	1.2	11.7	25.4	1.1	13.3	23.7
France	1.8	← 7.8ᵈ →			← 9.3ᵈ →			← 12.7ᵈ →			← 16.4ᵈ →			← 17.3ᵈ →	
Iceland		1.7			5.7			6.2	14.1		9.9	14.7		9.8	16.1
India					3.7	–	18.8	4.0	–	23.1	4.7	–			
Ireland	12.6	1.0	–	5.7	2.0	–		← 3.4ᵈ →		3.2	← 3.1ᵈ →				
Israel		3.4	10.7		4.7	9.6		6.8	9.1						
Italy				–	1.3		–	1.6	1.3	–	2.6	1.4	–	← 5.0ᶠ →	
Japan	–	0.8	1.7	–	1.0	1.4	–	2.9	5.0	–	3.9	3.9	–	4.8	3.7
Luxemburg	1.9	3.3	0.2		← 5.1ᵈ →			← 6.8ᵈ →		1.8	6.1				
Netherlands	10.5	14.2	–	9.5	17.8	–	8.5	18.1	–	10.7	21.8	–	9.0	20.7	–
Norway	–	1.2	7.9	–	2.2	7.9	–	3.1	10.9	–	4.3	10.6	–	6.3	
Poland		← 3.2ᵈ →		1.3	0.1	0.6	0.9	0.4	0.6						
Spain		3.4			← 6.0ᵈ →			5.5			8.0			← 9.8ᵈ →	
Sweden	4.2	2.3	17.6	0.1	4.2	19.7	0.03	5.4	21.9	0.002	7.4	21.7		7.5	21.1
Switzerland	5.5	10.9	–	1.0	13.8	–	1.2	16.2	–	1.7	17.3	–	1.7	17.0	
UK	–	1.7	–	–	2.8	–	–	3.1	–	–	4.3	0.1		4.6	0.2
USA	9.0	0.9	–	1.9	1.2	–	2.0	1.8	–				1.4	2.1	
Former USSR	–	–	7.2	–	–	6.2	–	–	7.5						

ᵃ Data for buttermilk also includes skimmed milk. ᵇ B,Y,O: buttermilk, yoghurt and other fermented milks, respectively. ᶜ Data includes German Democratic Republic. ᵈ Data represent yoghurt and other fermented milk products.

Dash (–) indicates product is not manufactured; blank space indicates data are not available.
Data compiled from IDF (1977, 1982, 1987, 1992, 1995).

network of retail outlets with storage facilities at <7°C. Initially, production was confined to natural yoghurt and the market was limited, in large measure, to those who believed that yoghurt was beneficial to health. Gradually, however, attitudes towards yoghurt changed, and the advent of fruit yoghurts during the 1950s gave the product an entirely fresh image. Instead of being a speciality item for the health food market, it became a popular and inexpensive snack food or dessert. Production figures reflect the expanding market. In the U.K., for example, the value of yoghurt sold per annum in 1990 ran to around £400 million (sterling) (Barrantes *et al.*, 1994), and such figures are now commonplace around the world. Indeed total production is still rising, a trend confirmed by the data shown in Table 1.2.

It is evident from Table 1.2 that fermented milks, and in particular yoghurt, are widely consumed around the world and according to Kurmann (1984), the factors that can influence consumption are:

- availability of milk
- food habits
- level of income
- advertising
- range of fermented milks available in the market
- distribution system
- relation to consumption of other dairy products
- religion.

However, the consumption of buttermilk is not properly classified in most countries because: (a) traditional or natural buttermilk is the by-product of butter making from ripened or cultured cream, (b) cultured buttermilk is produced by the fermentation of skimmed milk with the addition of butter flakes, and (c) there is sweet buttermilk which is not fermented; the data for buttermilk shown in Table 1.2 have to be assessed in a cautious manner. Nevertheless, fermented milk products made with mesophilic lactic acid bacteria (see Fig. 1.1) are widely consumed in the Scandinavian countries, while the yeast–lactic fermented milks are popular in the former USSR, eastern European countries and Mongolia.

1.5 Methods of production and classification

The methods of production of yoghurt have, in essence, changed little over the years and although there have been some refinements, especially in relation to lactic acid bacteria, that bring about fermentation, the essential steps in the process are still the same, namely:

- Raising the level of total solids in the process milk to around 14–16 g $100 g^{-1}$.
- Heating the milk, ideally by some method that allows the milk to be held at high temperature for a period of 5–30 min; the precise time will depend on the temperature selected.
- Inoculating the milk with a bacterial culture in which *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* are the dominant organisms.

- Incubating the inoculated milk, in bulk or retail units, under conditions that promote the formation of a smooth viscous coagulum and the desired aromatic flavour/aroma.
- Cooling and, if desired, further processing, e.g. the admixture of fruit and other ingredients, pasteurisation or concentration (see Chapter 5).
- Packaging for distribution to the consumer under chilled conditions.

At present there are many different types of yoghurt produced worldwide, and Tamime and Deeth (1980) have proposed a scheme of classification that separates all types of yoghurt into four categories based on the physical characteristic of the product. This approach is illustrated in Table 1.3. However, these products and in particular yoghurt are subdivided into different groupings based on the following aspects:

- Legal standards (i.e. existing or proposed) to classify the product on the basis of chemical composition or fat content (full, semi-skimmed/medium or skimmed/low fat).
- Physical nature of the product, i.e. set, stirred or fluid/drinking; the latter is considered stirred yoghurt of low viscosity.
- Flavours (plain/natural, fruit or flavoured; the latter two types are normally sweetened).
- Post-fermentation processing (vitamin addition or heat treatment).

Figure 1.3 illustrates a scheme for the classification of yoghurt based on the above-mentioned criteria.

The fact that all commercial processes share this common "core" has led to the word yoghurt being applied to a whole range of products, for example, dried yoghurt, frozen yoghurt and even pasteurised yoghurt. The inclusion of these varieties under the banner of yoghurt offends some people, because yoghurt *per se* must, by virtue of the process, contain an abundance of viable bacteria originating from the starter culture. However, popular usage appears to have determined that, as long as a carton is clearly labelled with information about the nature of the finishing process, for example, pasteurised yoghurt, the integrity of the basic product has not been compromised. Common sense would suggest that this view will prevail.

This approach also implies that yoghurt manufacture must always include a fermentation stage, that is a coagulum produced by the direct addition of lactic acid should never be designated as a yoghurt or even yoghurt-like, yet it is this very stage

Table 1.3 Proposed scheme for the classification of all yoghurt products

Category	Physical state	Yoghurt products
I	Liquid/viscous	Yoghurt
II	Semi-solid	Concentrated/strained
III	Solid	Frozen
IV	Powder	Dried

Adapted from Tamime and Deeth (1980), Robinson and Tamime (1990) and Tamime and Marshall (1997).
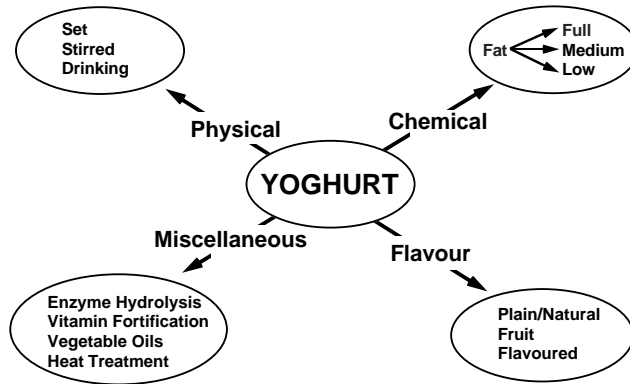
Fig. 1.3 Generalised scheme for the classification of yoghurt

that can, in commercial practice, prove extremely temperamental. Variations in milk composition, irregular behaviour of the starter organisms, faulty regulation of the incubation temperature, along with a number of other process variables, can all give rise to an end product that is deficient in respect of overall quality, and only a thorough understanding of the fermentation can provide an operative with the foresight to reduce the risk of product failure. It is with this background in mind that the relevant issues have been isolated for discussion, for although the different steps in production are interrelated, it is convenient to discuss them within the confines of an individual compartment. The following chapters are a reflection of this view.

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2

Background to manufacturing practice

2.1   Introduction

The process of yoghurt making is an ancient craft which dates back thousands of years and possibly even to the domestication of the cow, sheep or goat, but it is safe to assume that prior to the nineteenth century the various stages involved in the production of yoghurt were little understood. The survival of the process through the ages can be attributed, therefore, to the fact that the scale of manufacture was relatively small, and hence the craft was handed down from parents to children. However, over the last few decades the process has become more rational, mainly due to various discoveries and/or improvements in such disciplines as:

• microbiology and enzymology
• physics and engineering
• chemistry and biochemistry.

Yet by today's standards of industrial technology, the process of yoghurt making is still a complex process which combines both art and science together.

The micro-organisms of the yoghurt starter cultures play an important role during the production of yoghurt, for example, the development of acid and flavour. Their classification, behaviour and characteristics are discussed in detail in Chapter 7. However, in order to understand the principles of yoghurt making, it will be useful to describe separately the various stages of manufacture and their consequent effects on the quality of yoghurt. The technology of the process, that is, the equipment required for small and large scale production, will be discussed in Chapter 3.

The traditional and the improved methods for the manufacture of yoghurt are illustrated in Fig. 2.1. It can be observed that the former process has several drawbacks, such as:

• Successive inoculations of the starter culture tend to upset the ratio between *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, or may lead to mutation beyond the 15–20th subculturing.
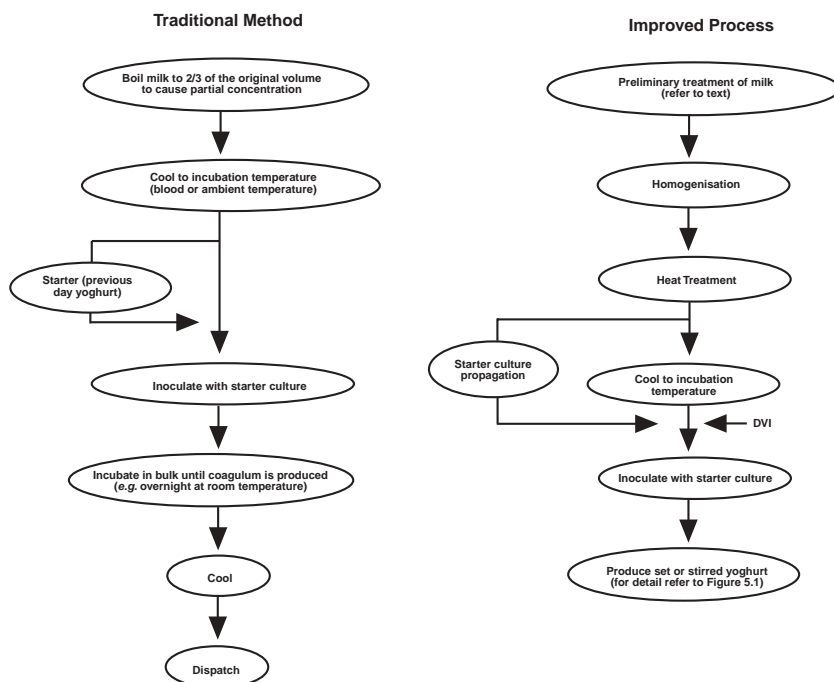
Fig. 2.1 Generalised scheme illustrating the different methods for the production of yoghurt

- The low incubation temperature, for example, ambient, results in slow acidification of the milk (18 hours or more), compared with the optimum conditions of 40–45°C for $2\frac{1}{2}$–3 hours.
- The slow rate of acid development may promote undesirable side effects, for example, whey syneresis, which can adversely affect the quality of yoghurt.
- The traditional process provides no control over the level of lactic acid produced during the fermentation stage.

Nevertheless, despite these drawbacks it is obvious that the traditional process has laid the basic foundation for the production of yoghurt as practised in the industry at the present time (see Fig. 2.1). In reality, the basic changes depend on the following:

- the purity of the yoghurt starter cultures which can be obtained from commercial starter manufacturers, starter banks or research establishments;
- the ability of dairies to propagate these cultures in sterile milk under aseptic conditions, so giving rise to active reliable starters; however, at present direct-to-vat inoculation (DVI) of the starter culture is widely used;
- the temperature of incubation can be accurately controlled, so that the rate of acid development and the processing time is known in advance;
- the cooling of the yoghurt can be carried out quickly at the desired level of acidity, and the quality of yoghurt is more uniform;

- the development of easy methods for measuring the rate of acid development in milk (using pH meters and/or acidimeters) enables even a semi-skilled operator to control the process adequately.

2.2 Preliminary treatment of the milk base

The bulk chemical composition of milk is mainly of water, but it also contains a mixture of complex components such as proteins, carbohydrate, fats, minerals and vitamins which are the main source of food for the young mammal. A detailed breakdown of these components is shown in Fig. 2.2. The characteristics of each chemical component have been discussed elsewhere in detail and the reader is
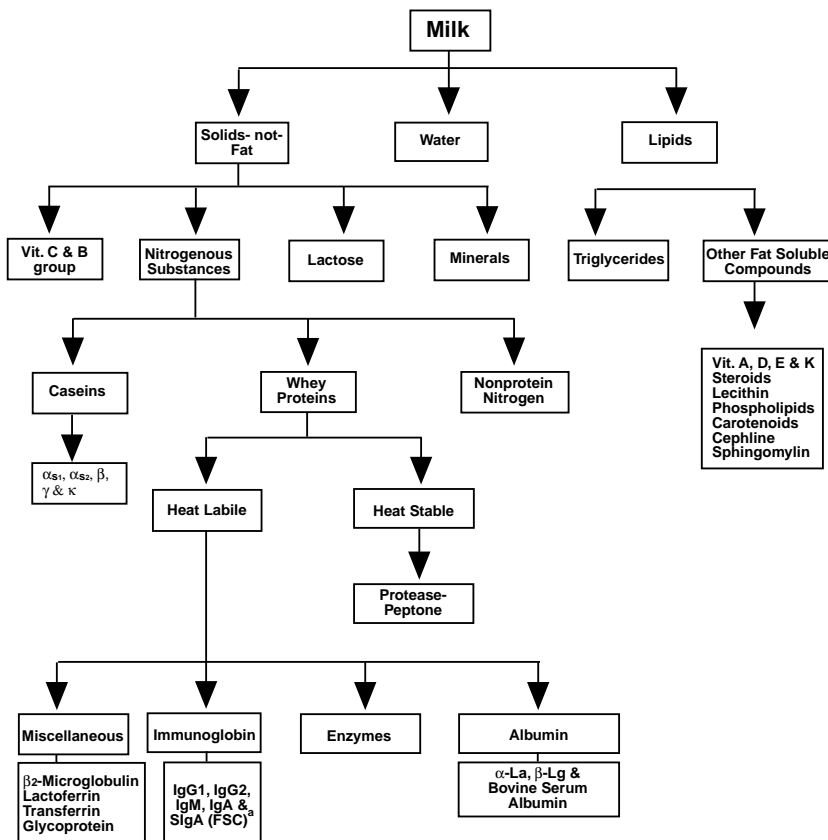
Fig. 2.2 Typical example of the main chemical components of cow's milk

[a] IgA could be also associated with another secretory component and the complex may occur in a free state.

Note: The milk also contains dissolved gases (O_2, CO_2 and N_2), enzymes (lipases, reductases, proteases, phosphatases, lactoperoxidases, catalases, oxidases, etc.), cellular matter (epithelial cells, leucocytes), micro-organisms (bacteria, yeasts and moulds) and contaminants due to carelessness during milking (straw, leaves, soil, disinfectant, etc.).

Adapted from Ling *et al*. (1961), Larson and Smith (1974c), Walstra and Jenness (1984), and Scott (1986).

referred to some reviews for a more complete discussion (Fox, 1992, 1994, 1997; Jakob, 1994; Pearce, 1995; Swaisgood, 1996).

2.2.1 Milk as a raw material

Milks of different species of mammals have been used for the production of yoghurt. Table 2.1 illustrates the major differences in the chemical composition of these milks. As a result, variations in the quality of yoghurt do occur, depending on the type of milk used. For example, milk containing a high percentage of fat (sheep, buffalo and reindeer) produces a rich and creamy yoghurt with an excellent "mouth-feel" compared with yoghurt manufactured from milk containing a low level of fat, or milk deprived of its fat content, for example skimmed milk. The lactose in milk provides the energy source for the yoghurt starter organisms, but the protein plays an important role in the formation of the coagulum and hence the consistency/viscosity of the product is directly proportional to the level of protein present; yoghurt produced from unfortified mare's and ass's milk would be less viscous than yoghurt made from sheep's or reindeer's milk.

Although the flavour of yoghurt is mainly the result of complex biochemical reactions initiated by microbial activity, the flavour of the milk base varies from species to species and this characteristic is reflected in the end product.

Since cow's milk is widely available in most countries around the world, the emphasis will be on the use of this type of milk for the manufacture of yoghurt, although even when considering cow's milk, there are quite large differences in composition (Table 2.2). The major constituents of milk are: water, fat, protein, lactose and minerals (ash). A detailed breakdown of these components is shown in Fig. 2.2.

Inevitably, the chemical composition of fresh milk varies from day to day within any particular breed depending on such factors as stage of lactation and age of the cow, milk intervals, season of the year and environmental temperature, breed of cows and breeding policy, efficiency and intervals between milking, nutrition, hormones and/or disease of the udder. The following are recommended for further reading regarding aspects of dairy cow husbandry (Larson and Smith, 1974a, b, c; Larson, 1978; Phillips, 1996). Figure 2.3 illustrates the monthly variations in the fat

Table 2.1 Chemical composition $(g\,100\,g^{-1})$ of milk of different species of mammals

Species	Water	Fat	Protein	Lactose	Ash
Ass	89.0	2.5	2.0	6.0	0.5
Buffalo	82.1	8.0	4.2	4.9	0.8
Camel	87.1	4.2	3.7	4.1	0.9
Cow	87.4	3.9	3.3	4.7	0.7
Goat	87.0	4.5	3.3	4.6	0.6
Horse	88.8	1.9	2.6	6.2	0.5
Reindeer	63.3	22.5	10.3	2.5	1.4
Sheep	81.6	7.5	5.6	4.4	0.9
Yak	82.7	6.5	5.3	4.6	0.9
Zebu	86.5	4.8	3.3	4.7	0.7

Adapted from Lentner (1981), Jenness (1988) and Holland *et al.* (1991).

Table 2.2 Commercial (average expected) composition of cow's milk (g 100 g^{-1})

Breed	Fat	Protein	Lactose	Ash
Ayrshire	3.85	3.35	4.95	0.69
Friesian	3.40	3.15	4.60	0.73
Guernsey	4.90	3.85	4.95	0.75
Jersey	5.14	3.80	5.00	0.75
Shorthorn	3.65	3.30	4.80	0.69

After Scott (1986).

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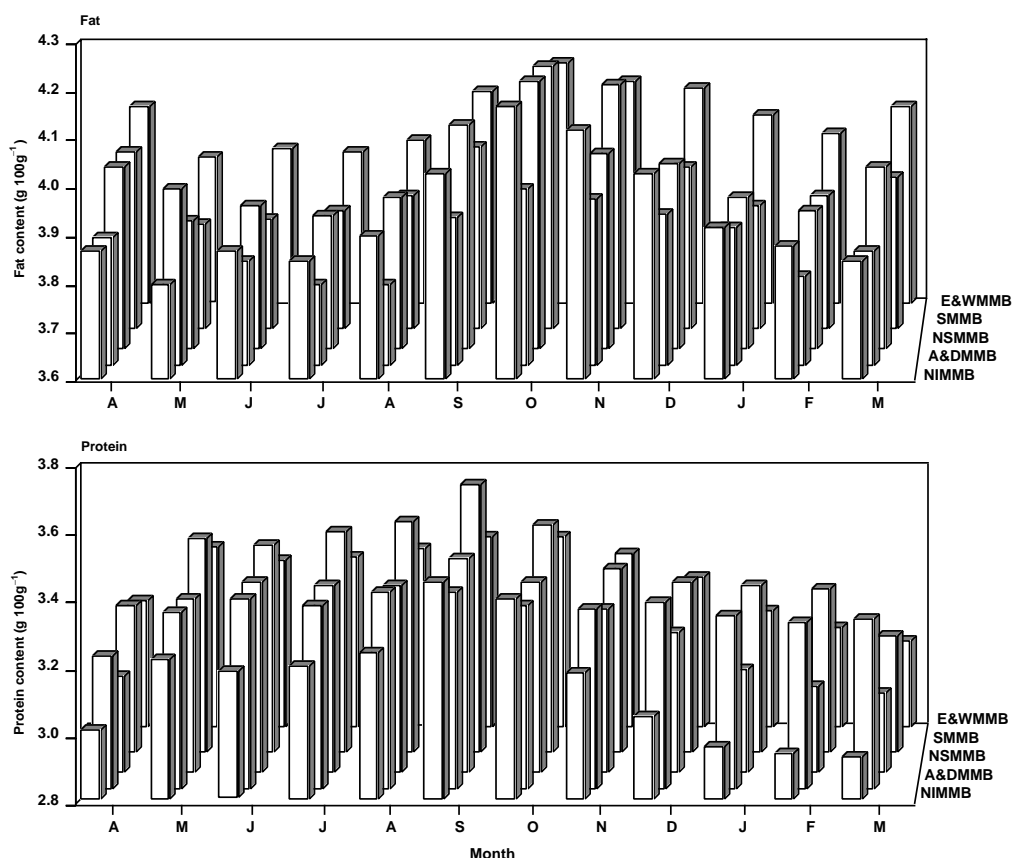
Fig. 2.3 Monthly variation (g 100 g^{-1}) of the fat and protein contents of milk obtained from the former Milk Marketing Boards in the U.K.

Since the ash and lactose contents in bulk milk vary little, figures of 0.75 and 4.5 g 100 g^{-1}, respectively, are taken as an annual averages. The data were obtained between April 1993 and March 1994, before these schemes were revoked on 31 October 1994.

England & Wales Milk Marketing Board (E&WMMB), Scottish Milk Marketing Board (SMMB), North of Scotland Milk Marketing Board (NSMMB), Aberdeen & District Milk Marketing Board (A&DMMB), and Northern Ireland Milk Marketing Board (NIMMB).

Data compiled from Pickett (1996).

and protein contents of milk from the former Milk Marketing Board regions in 1993–94 before these schemes were revoked on 31 October 1994 (Pickett, 1996). In order to overcome these inherent variations in composition, fresh liquid milk has to be standardised and/or fortified:

- to comply with existing or proposed legal standards for yoghurt, that is, the percentage of fat and/or solids-not-fat (see Chapter 10);
- to standardise the quality of yoghurt, that is, acidity, sweetness and consistency/viscosity of the coagulum to meet the demands of the consumer; the former two factors can be controlled during the production stages, but the consistency/viscosity of yoghurt is affected by the level of protein present in the milk and hence fortification of the milk solids-not-fat fraction is of primary importance.

2.2.2 Separation of cellular matter and other contaminants present in milk

Liquid milk may contain cellular material, for example, epithelial cells and leucocytes, which originates from the udder of the cow, and is, in some instances, due to carelessness during milk production. The milk is prone to further contamination with straw, leaves, hair, seeds, soil, etc. The primary objective of a milk processor is to remove such contaminants from the milk in order to ensure a better quality end product and while different methods are employed in dairies, the most universal system is the cloth filter. However, this method of filtration does have its limitations, one of which is that it can only remove the large debris present in the milk.

During the manufacture of some varieties of cheese, the presence of spore-forming organisms and/or cellular matter can affect the quality of the product and since the level of heat treatment of the cheese milk is limited to 72°C for 15 s, survival of the spores can lead to product loss.

Centrifugal clarification has been employed, with limited success, to remove spores, but unfortunately the treatment tends to break the bacterial clumps and the milk sours more quickly. However, the principle of centrifugation has been exploited in a high speed separator known as a bactofuge and this type of separator can remove many undesirable micro-organisms from milk together with a very small amount of the milk constituents. In practice, the separated fraction (bactofugate) amounts to around 2–3% of the total throughput of milk. The bactofuge is then subjected to a sterilisation treatment by live steam injection at 130–140°C for 3–4 s and, after cooling, is added back into the pasteurised cheese milk. Another method of removing spore formers from cheese milk known as microfiltration has become available. Details and illustrations of this method of processing have been reported by Tamime (1993). The application of this high heat treatment to only a small portion of the milk overcomes the problems associated with the presence of spore-forming organisms and at the same time does not affect the quality of the cheese.

However, the use of bactofuge separators or microfiltration on a yoghurt processing line is not really necessary since the heat treatment of the milk base (see Section 2.9.1) is high enough to eliminate, or at least reduce drastically, the undesirable organisms in the yoghurt milk and, in any case, organisms of this type do not cause any major problems in the yoghurt industry. Thus, the use of cloth filters is more than adequate for raw milk. In some instances, an in-line metal sieve has to

be installed when dried milk products are used to fortify the total solids in the milk; the metal sieves serve to separate any scorched or undissolved milk powder particles.

2.2.3 Milk reception and storage

Milk collection from farms in developing and industrialised countries is carried out in bulk, using a road tanker and, in some instances, rail tanker, or in churns; the facilities available for milk reception at a typical dairy are discussed in Chapter 3. However, the current practice of milk handling in dairies involves (a) ensuring that the temperature is about 5°C, (b) perhaps subjecting the milk to various treatments before storage such as thermising at about 65–67°C and cooling to <5°C, inoculating the milk with lactic acid bacteria or other microfloras to control the growth of psychrotrophic bacteria (Fetlinski *et al.*, 1982; Bianchi-Salvadori and Lavezzari, 1984), and/or (c) addition of formate or flushing with CO_2 (Singh and Shankar, 1984; Roberts and Torrey, 1988; Ruas-Madiedo *et al.*, 1996; Espie and Madden, 1997). Muir (1996) reviewed these methods of milk preservation and their effect on the quality of fresh dairy products. However, the use of CO_2 can cause the deposition of milk solids in a plate heat exchanger and degassing is recommended before heat treatment (Calvo and de Rafael, 1995). Milk containing somatic cells $>250\,000\,\mathrm{ml}^{-1}$ can affect the organoleptic properties of yoghurt (Rogers and Mitchell, 1994), and whilst preculturing the milk with proteolytic enzymes (from psychrotrophic bacteria or plasmin) or prolonged storage of milk for up to 6 days at about 7°C stimulates the growth of the starter culture, the yoghurt has substantially different physical properties (Reinheimer *et al.*, 1990; Gassen and Frank, 1991; Srinivas *et al.*, 1997; Prabba and Shankar, 1997).

In warm countries milk tends to deteriorate faster due to methods of production and handling. A handbook has been published by the International Dairy Federation (IDF, 1990) that addresses this topic in detail and the measures that are used to minimise the bacterial spoilage of milk. Furthermore, the lactoperoxidase (LP) system delays gel formation in cow's milk by 1.5 hours and affects the flavour of the yoghurt; the body and texture characteristics are not affected (Mehanna and Hefnawy, 1988; Kumar and Mathur, 1989; Abdou *et al.*, 1994; Nichol *et al.*, 1995; Nakada *et al.*, 1996).

2.3 Standardisation of fat content in milk

The fat content $(\mathrm{g}\,100\,\mathrm{g}^{-1})$ of yoghurt manufactured in different parts of the world can vary from as low as 0.1 to as high as 10 and in order to meet existing or proposed compositional standards for yoghurt, it is necessary to standardise the milk. For example, a typical average butterfat content in milk ranges from 3.7 to 4.2 g $100\,\mathrm{g}^{-1}$ (Fig. 2.3), but the fat content of commercial yoghurt averages around $1.5\,\mathrm{g}\,100\,\mathrm{g}^{-1}$ (medium fat yoghurt) or $0.5\,\mathrm{g}\,100\,\mathrm{g}^{-1}$ (low fat yoghurt). The methods employed for standardisation are as follows:

- removal of part of the fat content from milk
- mixing full cream milk with skimmed milk
- addition of cream to full fat milk or skimmed milk

- a process which may combine some of the methods mentioned above, that is, the use of standardising centrifuges.

The components required to achieve a standard milk, using one of the above methods, can be easily calculated using the Pearsons Square method.

Fat (g 100 g^{-1}) in A   Fat (g 100 g^{-1}) in standardised milk   C
1st raw material

(B – C) or (C – B)
= D parts of
   raw material A
+
(A – C) or (C – A)
= E parts of raw material B
D + E = F parts of a process milk
of correct composition

Fat (g 100 g^{-1}) in B
2nd raw material
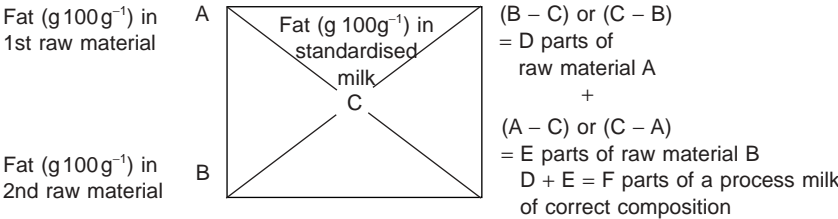
Alternatively, to calculate the amount of each type of raw material required, for example, per batch of a 1000 l of standardised milk:

$$A = \frac{(B - C) \text{ or } (C - B) \times 1000}{F}$$

$$B = \frac{(A - C) \text{ or } (C - A) \times 1000}{F}$$

1st example
How many litres of full cream milk (4 g fat 100 g^{-1}) and skimmed milk (0.1 g fat 100 g^{-1}) are required to produce 1000 l of yoghurt milk at 1.5 g fat 100 g^{-1}?

4   1.5   1.5 – 0.1 = 1.4

+

0.1   4.0 – 1.5 = $\underline{2.5}$
 3.9
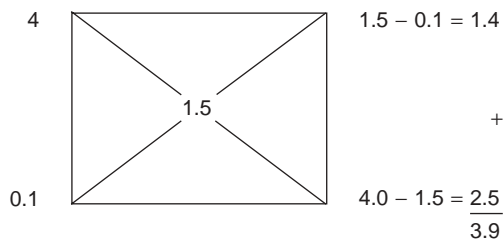
$$\text{The amount of full cream milk required} = \frac{1.4 \times 1000}{3.9} = 359 \text{ l}$$

$$\text{The amount of skimmed milk required} = \frac{2.5 \times 1000}{3.9} = 641 \text{ l}$$

$$\text{Total } \underline{\underline{1000 \text{ l}}}$$

2nd example
How many litres of cream (50 g 100 g^{-1}) and skimmed milk (0.1 g fat 100 g^{-1}) are required to produce 1000 l of yoghurt milk at 1.5 g fat 100 g^{-1}?

50   1.5   1.5 − 0.1 = 1.4

+

0.1   50.0 − 1.5 = 48.5 / 49.9
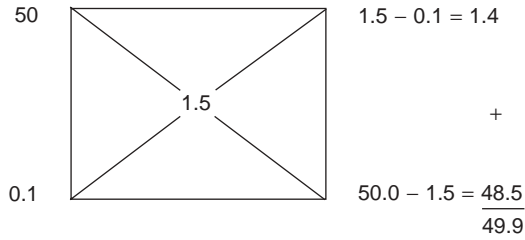
$$\text{The amount of cream required} = \frac{1.4 \times 1000}{49.9} = 28.1\,l$$

+

$$\text{The amount of skimmed milk required} = \frac{48.5 \times 1000}{49.9} = 971.9\,l$$

Total 1000.0 l

3rd example

How many litres of cream ($50\,g\,100\,g^{-1}$) and full cream milk ($4\,g$ fat $100\,g^{-1}$) are required to produce $1000\,l$ of yoghurt milk at $10\,g$ fat $100\,g^{-1}$?

4   10   50 − 10 = 40

+

50   10 − 4 = 6 / 46
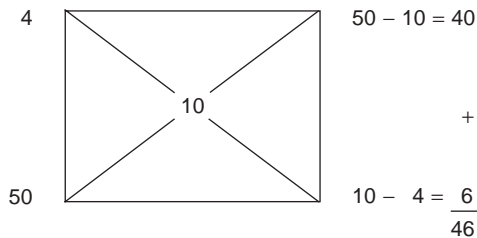
$$\text{The amount of full cream required} = \frac{40 \times 1000}{46} = 869.6\,l$$

$$\text{The amount of cream required} = \frac{6 \times 1000}{49.9} = 130.4\,l$$

Total 1000.0 l

2.4   Standardisation of the solids-not-fat content in milk

The percentage of solids-not-fat (SNF) (mainly the lactose, protein and mineral matter) in milk for the manufacture of yoghurt is governed either directly by legal standards of the country concerned, or indirectly by the manufacturer seeking to produce an end product with certain physical properties and flavour. In the case of existing legal standards, the required solids-not-fat content in yoghurt ranges from 8.2 to $8.6\,g\,100\,g^{-1}$ (see Chapter 10), and this minimum percentage seeks merely to protect the consumer; that is, the SNF level is roughly comparable to the level present in liquid milk. From the manufacturer's point of view, the physical

properties of yoghurt, for example, viscosity/consistency of the coagulum, are of great importance and, in general, the higher the level of solids in the yoghurt mix the greater the viscosity/consistency of the end product. The relationship between the level of solids in the milk and the consistency of yoghurt was studied by Tamime (1977), and he observed that this property was greatly improved as the milk solids increased from 12 to $20\,g\,100\,g^{-1}$. Figure 2.4 shows this improvement in consistency as measured by the penetrometer. It must be emphasised that the greater the depth of penetration, the softer the coagulum and vice versa. However, the change in consistency between 16% and 20% tends to be less pronounced and hence there may be little value, in terms of product quality, in using a solids level above $16\,g\,100\,g^{-1}$.

Since the 1970s, there have been many publications on the technology of yoghurt and other fermented milk products (Humphreys and Plunket, 1969; Robinson and Tamime, 1975, 1986, 1990, 1993; Rasic and Kurmann, 1978; Tamime and Deeth, 1980; Olano and Ramos, 1982; Bottazzi, 1983; Kilara and Treki; 1984; Merilainen, 1987; Shukla *et al*., 1987; Roginski, 1988; Tamime and Robinson, 1988; Morgensen, 1988; Chandan, 1989; Ferguson, 1989; Kroger *et al*., 1989, 1992; Schmidt, 1992; Chandan and Shahani, 1993, 1995; Rossi, 1994; Varnam and Sutherland, 1994; Sarkar, 1995; Tamime and Marshall, 1997; Oberman and Libudzisz, 1998). However, in a series of articles, Vedamuthu (1991a–h, 1992a, b) has reviewed the topic extensively, while Mann (1984, 1985, 1987, 1990a, b, 1992a, b, 1994a, b) regularly publishes a "Digest" of international dairy publications on yoghurt. Furthermore, the International Dairy Federation periodically publishes monographs updating the technological and scientific aspects of fermented milks (IDF, 1984, 1988a, 1992a).

The level of solids in milk (including the fat content) for the manufacture of yoghurt ranges from as low as $9\,g\,100\,g^{-1}$ in low fat yoghurt to as high as $30\,g\,100\,g^{-1}$ in other types of yoghurt. The best yoghurt is probably made from milk containing $15{-}16\,g\,100\,g^{-1}$ total solids (Tamime *et al*., 1987) and the composition of
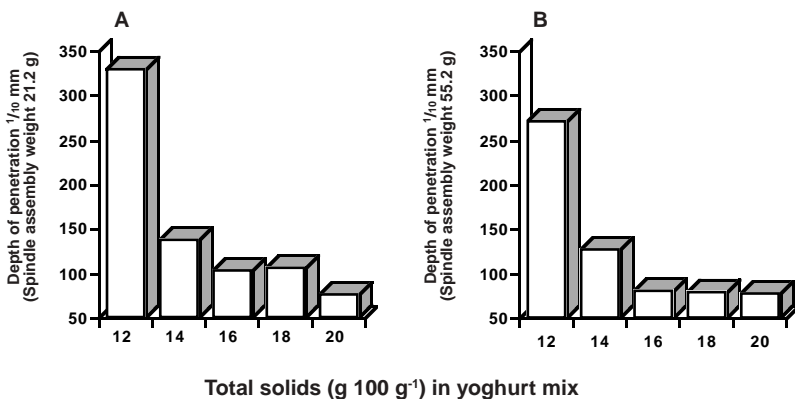
Fig. 2.4 Consistency measurement of yoghurt ($12{-}20\,g$ total solids $100\,g^{-1}$) directly at the end of the incubation period at 42°C (A) and after overnight storage in a refrigerator at 5–7°C (B)

Note: A standard penetrometer was employed.

After Tamime (1997).

Table 2.3 Effect of total solids in the mix in relation to natural acidity (NA), titratable acidity (TA) and developed acidity (DA) after incubation at 42°C

Total solids (g 100 g^{-1}) in yoghurt milk	Time of incubation (hours)	% Lactic acid		
		NA	TA	DA
12	3.5	0.15	0.80	0.65
14	3.5	0.19	0.84	0.65
16	3.0	0.21	0.83	0.62
18	2.5	0.24	0.88	0.64
20	3.0	0.29	0.93	0.64

Full cream spray dried milk powder was reconstituted to different levels of total solids in the mix.
Starter culture was CH-1 obtained from Chr. Hansen's Laboratorium A/S, Copenhagen, Denmark.
Adapted from Tamime (1977).

most commercial yoghurts falls within the range of 14–15 g 100 g^{-1}. Although 30 g 100 g^{-1} total solids has been suggested for the production of "super" yoghurt, the end product could well resemble "concentrated" yoghurt in its consistency rather than normal yoghurt (see Chapter 5). Furthermore, if the total solids level in the yoghurt mix is in excess of 25 g 100 g^{-1}, it can adversely affect the availability of moisture to certain strains of starter culture and this in turn can hinder their activity (Pulay and Krasz, 1974; Patel and Chakraborty, 1985).

As a result of increasing the level of SNF in the mix, the titratable acidity of the milk is raised due to the buffering action of the additional proteins, phosphates, citrates, lactates and other miscellaneous milk constituents (Walstra and Jenness, 1984) and this function can lead to a reduced gel formation time (Table 2.3). A similar view is held by Davis (1973), who reported that doubling the SNF content in milk resulted in a doubling of its titratable acidity. However, different levels of SNF in milk influenced the generation times and cell counts of the yoghurt starter culture; optimum conditions were 12 g and 14 g SNF 100 g^{-1} for *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, respectively (Al-Dabbagh and Allan, 1989).

The fortification of the total solids in the yoghurt mix can be achieved by a number of different methods, such as those described in the following sections.

2.4.1 Traditional process

The application of heat to milk has long been practised traditionally, that is, boiling to reduce the volume of the milk to two-thirds of its original value. Although the objective was to increase the concentration of total solids in the milk, the application of heat caused many physicochemical changes (refer to Section 2.9 on heat treatment). The degree of concentration achieved by the boiling process is rarely calculated with any accuracy, but if, for example, the total solids level in the milk is 13 g 100 g^{-1}, the result of boiling the milk to reduce its volume to two-thirds will be to raise the total solids content to around 19–20 g 100 g^{-1}. This method of fortification is still used in rural communities where the scale of yoghurt manufacture is very small.

2.4.2 Addition of milk powder

Milk powder (full cream or skimmed) is widely used in the industry to fortify liquid milk for the manufacture of a thick smooth yoghurt (Bøjgaard, 1987). Since the majority of the commercial yoghurt produced in the United Kingdom is of the low fat type, it is probable that skimmed milk powder (SMP) is the more popular ingredient. The rate of addition to the yoghurt mix may range from as little as 1% to as high as 6%, but the recommended level is 3–4%, since the addition of higher levels of milk powder may lead to a powdery taste in the yoghurt.

Good quality yoghurt has been produced by fortification of the yoghurt mix with (a) 2% SMP (Wolfschoon-Pomba *et al.*, 1984; Resubal *et al.*, 1987; Mehanna, 1988; Mehanna and Hefnawy, 1990), (b) mixing raw milk with recombined milk at a ratio of 1:1 (Kurwijila *et al.*, 1983; Caric *et al.*, 1986; Balasubramanyam *et al.*, 1988), (c) replacing half the water required for recombination of SMP with sweet whey (El-Safty and El-Zayat, 1984) or using only Cheddar cheese whey (Krishna *et al.*, 1984), and (d) addition of high protein SMP to increase the level of protein to $5.2 \, \mathrm{g} \, 100 \, \mathrm{g}^{-1}$ (Mistry and Hassan, 1992).

In some developing countries, yoghurt is manufactured totally using SMP and anhydrous milk fat (AMF), about $99.9 \, \mathrm{g}$ fat $100 \, \mathrm{g}^{-1}$, and the normal practice is to rehydrate the powder to about $12 \, \mathrm{g} \, 100 \, \mathrm{g}^{-1}$ SNF. The use of SMP during the manufacture of fermented milks is preferable to whole milk powder because of the problem(s) associated with oxidised flavour in the latter product (Harper, 1985). The latest approach in SMP production is the use of protein adjustment in order to overcome the seasonal variation in the protein content in milk, and to improve functional characteristics and storage stability (Kieseker and Healey, 1996). However, in some countries, for example Denmark and Italy, the fortification of the yoghurt milk with powder(s) is not permitted, and hence other methods are employed to increase the solids level.

High protein milk powders (whole or skimmed) are available in some markets, and these are produced by ultrafiltration followed by diafiltration in order to reduce the lactose content before drying (see Table 2.4) (Bjerre, 1990; Mistry and Hassan,

Table 2.4 Comparison of gross compositional quality $(\mathrm{g} \, 100 \, \mathrm{g}^{-1})$ of different powders used for the manufacture of yoghurt

Powder	Protein	Fat	Lactose
Whole milk			
Commercial	26.3	26.3	39.4
Retentate	41.7	41.7	9.3
Skimmed milk			
Commercial	36.1	0.6	52.9
Retentate[a]	62.8–80.5	0.9–1.5	5.5–23.9
Whey			
Commercial	12.2	1.3	78.0
Demineralised	14.5	1.0	80.5
Concentrate[a]	35.0–73.2	0.2	12.0–55.0
Buttermilk	34.1	5.0	51.0
Caseinate	87.3	0.2	–

[a] Range of different powders.

Adapted from Tamime and Marshall (1997).

1991a, b; Mistry *et al.*, 1992; Aguilar and Ziegler, 1994a, b). They have been used to produce firm yoghurts (El-Samragy *et al.*, 1993a, b; Thomopoulos *et al.*, 1993; Panfil-Kuncewicz *et al.*, 1994; Getler *et al.*, 1997), but are more expensive than SMP.

Since SMP is widely used for recombination during the manufacture of yoghurt, the specifications of the powder are important and can influence the quality of the product. The current specifications for powders published by the American Dairy Products Institute (ADPI, 1990) are universally recognised; previously the organisation was known as the American Dry Milk Institute (ADMI). In general, powders should be free from any inhibitory agents and be of good microbiological quality and physical standards. Critical reviews of dairy powder specifications, including an update of standards, have been reported by Sjollema (1988) and Kjaergaard-Jensen (1990). Some specific requirements of SMP used for recombination have been reported by Wilcek (1990) and include the following:

- whey protein nitrogen index, 4.5–5.9;
- cystein number, 38–48;
- thiol number, 7.5–9.4;
- heat number, 80–83.

These specifications classify the powder(s) as medium heat which is ideal for the production of fermented milks. Furthermore, the effect of thermal processing during whole milk powder production and storage temperature can affect its quality (Caric and Kalab, 1987; McKenna, 1997) and that of any yoghurt into which it is made (McKenna and Anema, 1993). As a consequence, New Zealand Milk Products have launched ALACO, a range of special powders that are texture improvers for yoghurt (Russel, 1994; Anon., 1994a; Harnett and Muller, 1995). A similar powder has been developed by DMV International in Holland called Excellion containing about 51–85 g protein $100 g^{-1}$ which is suitable as a SMP/stabiliser replacement (Maas, 1997); however, its functional characteristics are to improve the viscosity, texture and mouthfeel of yoghurt, and reduce syneresis.

The quality of yoghurt was studied using different commerical types of SMP (Klupsch, 1987, 1989; Blondeau and Goursaud, 1992) and the characteristics of the product (i.e. flavour, texture and acidity) differed considerably; some powders were suitable for set rather than stirred-type yoghurts. Recently, Chung *et al.* (1997a, b) reported that the use of old SMP affected the quality of yoghurt, so confirming that powder specifications can affect the quality of the manufactured yoghurt.

2.4.3   Addition of buttermilk powder

Buttermilk powder (BMP) is a by-product of sweet cream butter manufacture, but an acid type can also be obtained from the churning of cultured cream. This low fat powder is of value to the food and dairy industry because, due to the presence of high levels of phospholipids, it has considerable emulsifying properties and its chemical composition is similar to SMP. A method of manufacturing yoghurt from recombined dairy ingredients has been reported by Gilles and Lawrence (1979, 1982); the suggested formula is: 25 kg AMF, 125 kg SMP, 10 kg buttermilk powder and 840 kg water.

Buttermilk powder, used up to 50% as a replacement for SMP in the manufacture of low fat yoghurt, was acceptable and similar to the control product (Vijayalakshmi *et al.*, 1994). Fresh buttermilk fortified with SMP has been used

successfully to produce good quality yoghurt (El-Batawy *et al*., 1987; Vodickova *et al*., 1987; Mansour *et al*., 1994/95), but the use of fresh buttermilk concentrated by ultrafiltration (UF) or nanofiltration (NO) in low fat yoghurt production affected the consistency, flavour and aroma but not product stability (Reierstad, 1993).

2.4.4 Addition of whey powder and/or whey protein concentrates

This product originates in the cheese industry, and its utilisation in the food and the dairy industry was reviewed by Zadow (1983, 1994a, b), Alais and Blanc (1975), Smith (1976), Robinson and Tamime (1978), IDF (1988b) and Sienkiewicz and Riedel (1990). There are many different types of whey powder (WP) (e.g. whey protein concentrates (WPC), isolate (WPI) or hydrolysate (WPH), denatured whey protein, whey protein fraction and non-protein nitrogen product) available on the market and the characteristics of each are related to the processing technique applied before the drying stages, for example, demineralisation, lactose removal, whey protein concentrate or straightforward drying. The production and utilisation of concentrated whey proteins have been reported by Howel *et al*. (1990), Morr and Foegeding (1990), Dybing and Smith (1991), Wilmsen (1991, 1992), Harper (1992), IDF (1992b), Caric (1994), Barbut (1995), Blenford (1996) and Urbiene and Leskauskaite (1996). According to Jelen and Horbal (1974), Hartman (1975), Nielsen (1976) and Spurgeon (1976), the recommended level of addition of whey powder to the yoghurt mix is around 1–2%, since higher levels can impart an undesirable whey flavour. However, a process for the preparation of a yoghurt flavour is based on fermenting cheese whey followed by drying (van der Schaft, 1991) and the addition of such product to yoghurt improves its flavour and sweetens it.

Since the 1970s, there have been great developments in whey technology to produce various products of specific functional characteristics for yoghurt making. The heat stability of whey protein during the manufacture of yoghurt was reported by Buchheim *et al*. (1986), Jelen *et al*. (1987), Patocka *et al*. (1993) and Hollar *et al*. (1995). However, whey protein powder was used to fortify the yoghurt mix at levels ranging between 0.6% and 4% (Guirguis *et al*., 1984, 1987; Mehanna and Gonc, 1988; Rockell, 1989; Timmermans, 1993; Venkateshaiah and Jayaprakasha, 1995; Morris *et al*., 1995; Venkateshaiah *et al*., 1996; Kailasapathy and Supriadi, 1996; Kailasapathy *et al*., 1996a, b) and the results showed (a) that more acetaldehyde was produced, (b) increased viscosity and reduced syneresis, (c) improved sensory attributes and (d) enhanced buffering capacity at low pH. Good yoghurt could be produced from recombining SMP and sweet WP in a ratio of 75:25 (solids content about $12\,g\,100\,g^{-1}$), but a higher ratio of 50:50 was recommended for yoghurt made with 75% lactose hydrolysis; the latter product contained higher levels of soluble nitrogen due to:

- the addition of WP;
- the carry-over of yeast proteolytic activity in the β-D-galactosidase preparation, and
- the activity of the starter culture (Shah *et al*., 1993).

Replacement of SMP by whey–caseinate blends at 50% reduced the cost of manufacture and the yoghurt was acceptable, but the application of lactose hydrolysis during the manufacture of yoghurt has raised the cost slightly (Whalen *et al*., 1988). Furthermore, different processes for the manufacture of yoghurt and related products using whey protein powder(s) in the mix have been patented by

Koenraads and de Boer (1986), Tomita *et al.* (1992) and Hirano *et al.* (1994) (see also Hoffmann *et al.*, 1998).

WPC (i.e. about 14 g total solids (TS) 100 g^{-1}) have been used to fortify the yoghurt mix at a level up to 30% without affecting the quality of the product (Broome *et al.*, 1982; Greig and van Kan, 1984; Gruev and Flejtas, 1985; Tratnik and Krsev, 1985, 1988; Hofi *et al.*, 1994/95; Maric *et al.*, 1997). Greig and Harris (1983) observed a "cheesy" odour and a reduction in the viscosity of the yoghurt ($P < 0.01$) when the substitution of liquid milk was 40% with WPC, and the best results were obtained with 10% substitution, while Abou-Dawood *et al.* (1984) recommended the use of WPC to increase the SNF by 1 g 100 g^{-1}.

In Egypt, salted whey from Domiati cheese was demineralised twice (about 12.51 g TS 100 g by UF) to reduce the salt content, and the WPC was used successfully to replace 40% of the milk in the yoghurt mix (Abd-Rabo *et al.*, 1988). Alternatively, the salted whey could be UF and diafiltered using sweet whey rather than water, and finally the WPC was diluted with sweet whey to adjust the protein level to 3.5 g 100 g^{-1} (Abd El-Salam *et al.*, 1991). Such WPC was added to buffalo's milk up to 20% and the manufactured yoghurt had a better texture, mouthfeel and reduced syneresis. Cottage cheese whey was concentrated by vacuum evaporator (VE) to 40 g TS 100 g^{-1} by Baig and Prasad (1996) and part of this was acidified to pH 4.6 to produce a more acid WPC. Both whey concentrates were used separately to replace SMP in yoghurt making and the results were satisfactory, but it was observed that the incorporation of whey solids stimulated the growth of *S. thermophilus* and *Bifidobacterium bifidum*, whilst the counts of *L. delbrueckii* subsp. *bulgaricus* were reduced. Nevertheless, the firmness and syneresis of "cream" yoghurt (about 10 g fat 100 g^{-1}) made from milk and concentrated Camembert cheese whey were significantly influenced by the ratio of casein to whey and an optimum range recommended was 1.2–2.2 (Kulkarni *et al.*, 1990a, b; Plock and Kessler, 1992).

Recently, de Boer and Koenraads (1992) have reviewed the application of liquid WPC for partial replacement of skimmed milk during the manufacture of yoghurt in terms of: (i) legislation in most European countries is far from being uniform; however, based on the legal specifications in the Netherlands, the permitted and maximum replacement of SNF in milk with WPC was 10%, 20% and 30% for drinking yoghurt, plain stirred yoghurt and fruit stirred yoghurt, respectively, (ii) the microbial activity of the yoghurt starter during the fermentation stage was slightly enhanced, possibly due to shorter lag phase of *L. delbrueckii* subsp. *bulgaricus*; however, a stimulating effect was observed with *Lactobacillus acidophilus* (Marshall *et al.*, 1982), but contradictory results with yoghurt starter cultures have been reported in the literature which could be due to strain variation, and (iii) the rheological and sensory properties of yoghurt were, in some instances, improved and in other cases the flavour was affected. This could be attributed to: (a) the level of fortification of WPC used, and (b) the processing conditions applied during the preparation of the yoghurt mix. However, variations in the properties of the WPC during its preparation should not be overlooked.

2.4.5 Addition of casein powder

Different types of casein powder (e.g. acid or rennet casein, Na-, K-, Ca- or NH$_3$-caseinate and casein hydrolysate) are manufactured from skimmed milk and their

properties vary according to the technique used to precipitate the original casein, for example, acid casein (hydrochloric, lactic or sulphuric acid precipitation), coprecipitated casein and rennet casein. Casein powder, as the name indicates, consists mainly of casein and its addition to the yoghurt mix increases both the level of protein in the product and its viscosity (Sen, 1985; Hendrickx, 1996); the level of addition, compared with SMP, is comparatively low (see Fig. 2.5).

It is evident that different powders could be used to fortify the protein content in the yoghurt mix (see Table 2.4) and depending on the type of powder used, the physical and sensory properties could be influenced and/or modified. Caric (1994) has reviewed the different techniques used for the production of powders including whey protein concentrates. The functional properties of WPC have been reported by Kinsella (1986) and Kjaergaard-Jensen *et al.* (1987), whilst Robinson and Tamime (1986) have reviewed the role of protein(s) in yoghurt making.

The quality of yoghurt made with different dried ingredients has been investigated by many researchers in different laboratories around the world. Some examples of ingredients used to produce good quality yoghurt include: (a) mixing Ca-caseinate and whey powder in a ratio of 1:1 (Conc and Uysal, 1994), (b) the addition of Na-caseinate gave a firm yoghurt with little syneresis, whilst the differences between the addition of SMP and dried milk proteins were marginal; however, correlations between the sensory and rheological properties of the yoghurts made with different ingredients were influenced by the type of starter cultures used (Rohm and Kneifel, 1993), and (c) the susceptibility to syneresis of yoghurts made with different dried ingredients decreased in the following order: WPC 35 > Na-caseinate > WPC 45, 60 or 75 > SMP > BMP, whilst the viscosities, after a 25 min shear at a rate of $116.2\,s^{-1}$, decreased in the order: Na-caseinate > BMP, SMP, WPC 75, 60 or 45 > gelatin > WPC 35 (Guinee *et al.*, 1994, 1995; see also Rhom, 1993a; Rhom and Schmid, 1993) (note: the numbers refer to percentages of protein).

In some instances protein hydrolates have been recommended for use during the manufacture of yoghurt. Casein hydrolysates containing peptide lengths of about 1.5 stimulated the growth of *S. thermophilus* due to the increase in free amino
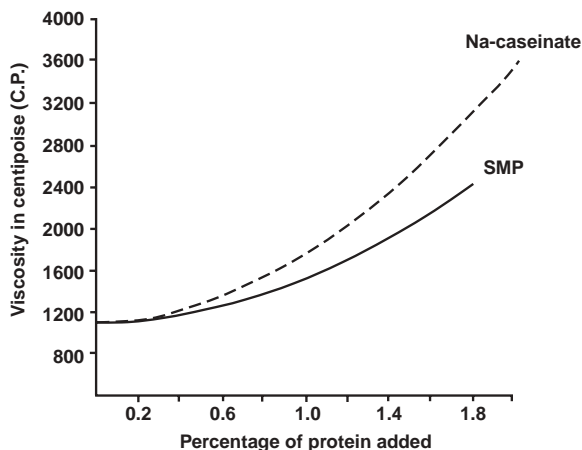
Fig. 2.5 Effect of an increase in protein content on vicosity of yoghurt
After van Gennip (1973, 1980, 1981a, b).

acids (Nakamura *et al*., 1991). However, the addition of up to 1% casein digest increased the viscosity by 16–87% and reduced syneresis by 26.5–30% in yoghurts made using strains of *S. thermophilus* (i.e. high and low viscosity) isolated from commercial yoghurts (Kim and Hwang, 1996). Alternatively, yeast autolysate and hydrolysed protein can be added at a rate of 0.5–0.3 g 100 g^{-1} to cultured milk products to control lipolysis and enhance the flavour (Akatsuka, 1984), whilst hydrolysate obtained from the muscle of mackerels (*Scomber japonicus*) has stimulated the growth of *S. thermophilus* in milk fortified by 0.2–1.0 g 100 g^{-1}, but not *L. delbrueckii* subsp. *bulgaricus* (Lee and Kim, 1986). Soy bean protein isolates and/or yeast extracts when added to milk have been found to stimulate the growth of *S. thermophilus* and different species of bifidobacteria (Yajima *et al*., 1992). However, the addition of such hydrolysates to fermented milks may be governed by statutory regulation.

2.4.6 Concentration by vacuum evaporation (VE)

This method of concentrating the total solids in the yoghurt mix is widely used in the industry. For an illustration of a typical plant refer to Chapter 3. The basic requirement is a single effect plate evaporator which can be easily incorporated into a yoghurt processing line. The evaporation and/or concentration process is carried out on the milk before the final heat treatment. In practice, the yoghurt milk must first be standardised, for example, the fat content, since the evaporation concentrates all the milk constituents with the exception of minor losses of volatile compounds in the condensate. The amount of water removed from the milk ranges from 10 to 25%, equivalent to an increase in the TS of 2–4 g 100 g^{-1}. However, Baltadzhieva *et al*. (1987) recommended VE of whole milk to 16–18 g TS 100 g^{-1} for the production of good quality yoghurt. Some other advantages claimed for the evaporation process are first, the removal of water from the milk takes place under vacuum which, in turn, aids the removal of entrapped air and hence improves the stability of the coagulum and reduces syneresis during storage (Gradhage and Thurell, 1978). Second, during the manufacture of goat's milk yoghurt, the evaporation process improves the consistency of and reduces the "goaty" flavour of the end product (Hadland and Hoffmann, 1974).

Incidentally, under commercial practice the yoghurt milk could be fortified and/or standardised using concentrated milk (whole or skimmed) or WPC from factories producing such concentrates before the drying stage. Typical gross compositions of concentrated milk products is shown in Table 2.5.

2.4.7 Concentration by membrane filtration

Membrane filtration is a process which was developed to concentrate and/or separate solids from an aqueous mixture. The usual membrane processes are reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF) and microfiltration (MF). The applications of RO and UF in the dairy industry have been reviewed by Glover *et al.* (1978), Hedrick (1983/84), Glover (1985), IDF (1979, 1992b, 1996), Kosikowski (1986), McGregor (1986), Cheryan (1986), Maubois (1989), Mohr *et al*. (1989), Rao *et al*. (1989), Degremont (1991a, b), Renner and Abd El-Salam (1991), Kessler *et al*. (1991), Grandison and Glover (1994), Caric (1994) and Bird (1996). The major functional differences between RO, NO, UF and MF are as follows:

Table 2.5 Comparison of gross compositional quality $(g\,100\,g^{-1})$ of concentrated milk products

Concentrate	Protein	Fat	Lactose
Evaporated milk			
Whole	6.8	7.6	10.0
Skimmed	7.5	0.2	11.3
Condensed			
Buttermilk	9.9	1.5	12.0
Whey	7.0	2.4	38.5
Retentate			
Whey[a]	12.2	NR	3.2

[a] Ultrafiltered; the volume of concentration factor is ×20.
NR: not reported.

Data compiled from Bassette and Acosta (1988) and Caric (1994).

- The RO process separates very low molecular weight solutes, i.e. about 100, and only water molecules are allowed to pass through the membrane. Thus, the membranes are basically impermeable (or slightly permeable) to organic compounds or inorganic ions and consequently the osmotic pressure becomes an important feature in the process. The RO system is operated at high pressures, i.e. 1–6 megapascals (MPa).
- The NO process is sometimes known as ultraosmosis. This system of filtration separates selectively low molecular weight solutes from aqueous solutions. The membranes are more permeable than RO, but less permeable than UF membranes. The NO system normally operates at pressures of 2–3 MPa.
- The UF process merely sieves or filters the milk and the membranes can only retain high molecular weight fractions, i.e. >2000. The operating pressures are, therefore, much lower than with the RO process, e.g. 0.1–1 MPa.
- The MF process operates at a very low pressure (about 0.01–0.05 MPa) and it is used to separate suspended particles up to 10 μm from an aqueous solution.

The material that passes through the membrane is referred to as the permeate, and the part of the feedstock which is retained by the membrane and contains the solute(s) or constitutent(s) to be concentrated is referred to as the retentate. Thus in principle, the permeate will be deficient in the solute(s) that are concentrated. Table 2.6 illustrates the permeability of solutes in milk (whole or skimmed) or whey using different membranes. The major difference between the permeates is that, while the RO permeate consists only of water, the UF permeate contains lactose, non-protein nitrogen, organic acids, ash and water-soluble vitamins besides water. A comparison of the chemical compositions of whole milk, skimmed milk and whey concentrated by RO and UF (and their permeates) is illustrated in Table 2.7.

Membrane filtration techniques are utilised in the dairy industry for specific processes (Bylund, 1995; Bird, 1996), and some examples are:

- RO is used for concentrating whey, UF permeate and, to a lesser degree, yoghurt milk.
- NF is applied for partial demineralisation/desalination of whey, UF permeate or retentate.

Table 2.6 Permeability of mik and/or whey components through different membrane systems

Milk/whey components	Type of membrane system			
	RO	NO	UF	MF
Water	✓	✓	✓	✓
Minerals	R	✓	✓	✓
Lactose, AA and NPN	R	R	✓	✓
Proteins	R	R	R	✓
Fat and bacteria	R	R	R	R

✓: passes through the membrane into the permeate; R: rejected by the membrane and retained in the retentate. AA: amino acids. NPN: non-protein nitrogen.

Adapted from Bird (1996).

Table 2.7 Chemical composition ($g\,100\,g^{-1}$) of the permeate and retentate of milk (whole and skimmed) and whey after concentration by UF or RO

Process	Product	Concentration factor	Total solids	Fat	Protein	NPN	Lactose
UF	Whole milk	×3	12.9	3.9	3.1	0.18	4.7
	Permeate		6.1	–	0.06	0.19	5.1
	Retentate		28.6	12.6	9.8	0.18	4.1
	Skimmed milk	×3	8.5	–	3.1	0.17	5.1
	Permeate		5.7	–	0.06	0.17	5.3
	Retentate		15.5	–	9.3	0.20	4.7
	Whey	×20	6.0	–	0.68	0.29	4.0
	Permeate		6.2	–	0.13	0.55	4.5
	Retentate		20.1	–	12.12	2.11	3.2
RO	Whole milk	×2	11.7	3.2	3.1	–	4.3
	Permeate		0.08	–	–	–	–
	Retentate[a]		22.6	6.4	6.1	–	8.6
	Skimmed milk	×2.2	8.8	–	3.1	–	4.7
	Permeate		0.33	–	–	–	–
	Retentate[a]		19.0	–	6.9	–	10.3
	Whey	×2.7	6.8	–	0.8	–	4.4
	Permeate		0.11	–	–	–	–
	Retentate[a]		18.2	–	2.2	–	11.9

NPN: non-protein nitrogen. Dashes (–) represent data not reported or in the case of RO filtration valve is nil.

[a] Data calculated from the membrane retention percentage reported.

Dated compiled from Glover (1971, 1985).

- UF process concentrates the fat and proteins in milk (see Fig. 2.6) for the standardisation of cheese milk or fortification of the yoghurt milk. It is also used to concentrate whey for the production of WPC.
- MF is basically used to reduce the number of micro-organisms in skimmed milk, whey and brine, and also to de-fat the whey used for the production of WPC or WPI.
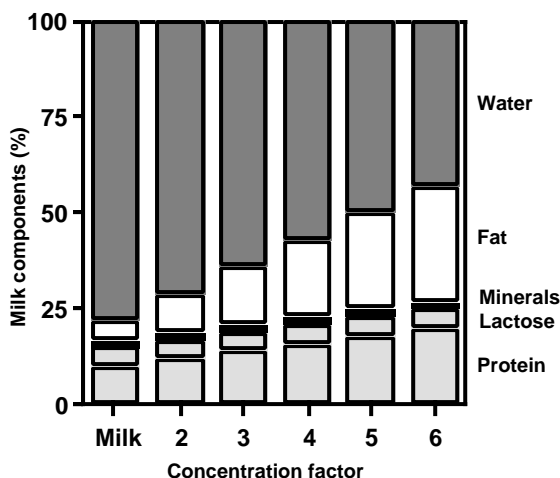
Fig. 2.6 An illustration showing the effect of concentration of components in whole milk UF retentate

Note: fat and protein contents increase in relation to the concentration factor whilst the lactose and minerals remain constant; the latter constitutent increases marginally at ≥ × 5 fold.

After Puhan (1990a, b).

The industrial-scale production of yoghurt from milk concentrated by RO or UF has been reported by Jepsen (1977, 1979) and according to the data compiled by Tamime and Deeth (1980) and Ferguson (1989), the qualities of yoghurt produced from RO and UF concentrated milks are as follows. First, whole milk concentrated by UF to $18-20\,g\,TS\,100\,g^{-1}$ produced a smooth, creamy yoghurt with a typical acid flavour; homogenisation was not required during subsequent treatment of the milk. Second, a process similar to the one mentioned above, but with the lactose content adjusted to $2\,g\,100\,g^{-1}$, resulted in a yoghurt rated as superior to ordinary brands. Third, skimmed milk concentrated by UF to $13\,g\,TS\,100\,g^{-1}$ was also suitable for yoghurt making. Fourth, the manufacture of yoghurt from skimmed milk concentrated by RO to $15\,g\,100\,g^{-1}$ total solids resulted in a yoghurt of similar quality (viscosity, acid and flavour) to yoghurt produced from skimmed milk fortified to $15\,g\,100\,g^{-1}$ total solids with SMP.

It is safe to assume that the application of RO in yoghurt making is very limited in the industry when compared with UF. Nevertheless, Dixon (1985) made yoghurt from RO retentate (i.e. the volume of milk was halved after concentration) which had a higher apparent viscosity and was less susceptible to syneresis than yoghurt where the milk was fortified with SMP. Some studies on yoghurt made from UF retentate suggest the following aspects and/or recommendations. First, Atamer *et al.* (1990) suggested that the total solids content should be $13.23\,g\,100\,g^{-1}$, but no data were given regarding the fat level; however, recombined SMP concentrated by UF to $12\,g\,100\,g^{-1}$ solids plus AMF and later made into yoghurt was highly rated by a taste panel (Mehanna *et al.*, 1988). Second, yoghurt made from UF retentate had a total free amino acids to protein ratio of 0.0375 and no significant difference was observed in particle size in low molecular weight peptides of yoghurt (Nakazawa *et al.*, 1991). However, concentrating milk more than two-fold resulted in a product which was too firm; curd tension was correlated with the degree of concentration

(Tomita *et al*., 1987). Third, low lactose (about $0.75 \, \text{g} \, 100 \, \text{g}^{-1}$) and low sodium yoghurt was produced by combining UF (about $20 \, \text{kDa}$) with addition of β-galactosidase to the retentate, and pectin, calcium phosphate and apple concentrate were added to the milk to compensate for calcium losses during filtration (Rasic *et al*., 1992). Fourth, the relative viscosity of skimmed milk UF retentate at pH 6.0 was influenced by protein content and $1 \, \text{g} \, \text{NaCl} \, 100 \, \text{g}^{-1}$ (Abd El-Salam *et al*., 1987). Fifth, skimmed milks concentrated by UF (about $10 \, \text{kDa}$) and RO were made into yoghurt; the former had a protein to lactose ratio of 1.2 and produced good yoghurt when the protein content was increased by 35% (Brazuelo *et al*., 1995).

It is evident that the activity of starter cultures in UF retentate is greater when an increase in conductance is observed, and the change in pH is decreased despite an increase in lactic acid content (Lanzanova *et al*., 1993). Such microbial behaviour is attributed to the buffering capacity of the UF retentate (Mistry and Kosikowski, 1985a, b, 1986a–c; see also Alvarez *et al*., 1998).

2.4.8 Addition of non-milk proteins

In countries where there is a shortage of milk production for human consumption, proteins that originate from plant, animals and other sources have been used in research laboratories to fortify the milk during the manufacture of yoghurt. Examples of using non-milk protein may including the following:

- Soy-milk and its protein derivates have been used extensively in food formulations, and soy-based yoghurt products are reviewed in detail in Chapter 5.
- Sweet potato (SP), milk, sucrose and gelatin mixtures were used to make yoghurt containing high amounts of protein (about $19 \, \text{g} \, 100 \, \text{g}^{-1}$). A good quality yoghurt was produced, but the product became darker in colour as the SP content was increased; overall, such yoghurts contained appreciable nutrients, for example, vitamin C $0.3–0.4 \, \text{mg} \, 100 \, \text{g}^{-1}$, vitamin A $971–1252$ retinol equivalent $100 \, \text{g}^{-1}$ and dietary fibre $2.5 \, \text{g} \, 100 \, \text{g}^{-1}$. No reduction in starter activity was reported (Collins *et al*., 1991a–c).
- Pulses or legumes, such as faba, cowpeas and mung beans, have been used in the preparation of yoghurt-like products. Faba bean yoghurt was highly rated in Egypt (Abou-Donia and Salam, 1981, 1982), but fermented milks made with cowpeas and mung beans were inferior to yoghurt, even though the sensory attributes were still within an acceptable range (Rao *et al*., 1988; see also Ibrahim *et al*., 1993).
- Egg white, soy-milk, gums, sugar, skimmed milk and vanilla extract were blended and processed into an acceptable and stable product (MaKenzie, 1983; Lin and Cunningham, 1984; see also Muller *et al*., 1987).
- Sunflower protein was used for partial replacement of the milk proteins in yoghurt making; such proteins appeared not to have any gel-forming ability, but interacted with the caseins to form a soft gel yoghurt (Bilani *et al*., 1989).
- Groundnut protein (flour or isolate) was blended with milk (whole with added SMP) to increase the total solids up to $23 \, \text{g} \, 100 \, \text{g}^{-1}$; heating the milk at 80°C for 30 min gave a curd after fermentation with increased yield stress and the strength was influenced by the concentration of groundnut protein (Ramana and Ramanathan, 1992; see also Venkateshaiah *et al*., 1994).

- Cottonseed proteins (i.e. different types) were used in yoghurt preparation and the most acceptable product, when compared with the control made from 100% milk powder, was obtained by mixing glandless cotton seed protein with whole milk powder in the ratios 1:1 and 1:3 (Abu-Foul *et al.*, 1992). Jiang *et al.* (1995) used a solution of fresh milk and low gossypol cottonseed protein at a ratio of 6:4, $1\,g\,100\,g^{-1}$ glucose and $0.1\,g\,100\,g^{-1}$ β-cyclodextrin to produce an acceptable yoghurt.
- Coconut milk fortified with SMP and the addition of $12\,g\,100\,g^{-1}$ sugar gave an acceptable product when compared with the control yoghurt (Sanchez and Rasco, 1984; Anon., 1985a, b; Davide, 1986).
- Dried egg white fortification of milk up to 3% enhanced acid development by *L. acidophilus*, *Lactobacillus paracasei* subsp. *paracasei* and *L. delbrueckii* subsp. *bulgaricus*, and the viscosity of the yoghurt was influenced by the amount of dried egg white used (Tae, 1997; Tae and Min, 1997).
- Miscellaneous protein additives such as soy-milk, oat flour and WP (Shirai *et al.*, 1992), wheat and milk proteins (Lorenzen, 1993), dried *Aloe vera* (Yongseo *et al.*, 1996; Lee and Yoon, 1997) and soy-milk and/or brown rice (Kisuk *et al.*, 1997) have been used to fortify milk to produce an acceptable yoghurt. The addition of mushroom extract ($1\,g\,100\,g^{-1}$) (*Lentinula edodes*) to reconstitued SMP enhanced the rate of acid development by *L. delbrueckii* subsp. *bulgaricus*, but the coagulum had a coarse structure (Vargas and Ohashi, 1996, 1997).

There are many methods of fortification/standardisation of the fat and/or SNF content of the milk base. A comparison of the chemical composition of these potential ingredients is given in Tables 2.4, 2.5 and 2.7. The choice of any one particular method of fortification in a given situation is governed primarily by the following factors:

- cost and availability of the raw materials
- scale of production
- capital investment in the processing equipment

but it is important to note that the degree of supplementation of each of the different milk constitutents does vary with the method used; the possible increases or decreases in the level of protein, lactose and fat contents in the yoghurt mix are dependent on the method of fortification/standardisation employed. However, other considerations may be equally relevant, and, for example, the addition of milk powder (whole or skimmed) beyond a certain level may result in a powdery flavour in the yoghurt and, due to the high level of lactose present in the mix, can also lead to excessive acid production during cold storage. Nevertheless, the viscosity/consistency of the coagulum is of primary importance during the manufacture of yoghurt and this feature is wholly dependent on the level of protein in the milk base; a relationship that is evident with respect to the variations in protein content of milk throughout the year (van Gennip, 1973, 1981a, b). Commercially, a high protein content in the yoghurt milk can be achieved by the addition of caseinate powder, concentrating the milk by the UF method or, to a lesser degree, by the addition of a high protein powder (whey or milk) and/or buttermilk powder (see Tables 2.4, 2.5 and 2.6).

Although, in broad terms, the overall level of protein in the mix affects the characteristics of the coagulum, the formation of the gel is entirely dependent on the

functional properties of the casein fraction (Rohm and Foissy, 1991). Thus, the lactic acid produced by the starter culture destabilises the casein micelles and at pH 4.6–4.7, in the presence of divalent ions (calcium and magnesium), the casein forms a three-dimensional network entrapping all the milk constituents including the aqueous phase. It is not surprising, therefore, that prior to the availability of high protein powders, the fortification of the milk base with casein or caseinate offered the following advantages:

- concentration of the milk, in order to increase the protein content, is not required;
- the natural flavour and texture of the yoghurt are maintained;
- it enhances the hydrophilic properties of the existing protein and so acts as a stabiliser;
- it improves the viscosity of yoghurt and decreases the problem of syneresis during cold storage;
- the recommended level of fortification, compared with skimmed milk powder, is in the proportion of 1 to 3, respectively. The efficacy of caseinate *vis-à-vis* skimmed milk powder in enchancing the consistency of yoghurt is shown in Fig. 2.5.

It is clearly feasible, therefore, to manufacture yoghurt from either concentrated or fortified milk. In an effort to isolate one particular method, Abrahamsen and Holmen (1980), Tamime *et al*. (1984), Becker and Puhan (1988, 1989) and Savello and Dargan (1995, 1997) compared the quality of yoghurt manufactured from a number of processed milks, that is, RO, UF, VE, and a product made from milk with added SMP. The chemical composition of the milk bases is illustrated in Table 2.8 and their conclusions can be summarised as follows:

Table 2.8 Chemical composition $(g\,100\,g^{-1})$ of yoghurt milks concentrated/fortified by different methods

Treatment	Total Solids	Fat	Protein	Lactose[a]	Ash
Abrahamsen and Holmen (1980)					
Control	11.84	3.43	3.12	4.45	0.84
VE	14.57	3.49	4.12	6.03	0.93
UF	14.13	3.60	4.97	4.63	0.93
RO	14.54	3.53	4.03	6.07	0.92
SMP	14.32	3.32	4.14	5.93	0.93
Tamime *et al*. (1984)[b]					
SMP	15.96	1.56	5.55	7.64	1.21
VE	15.11	1.62	5.24	7.11	1.15
UF	11.82	1.55	5.14	4.27	0.84
RO	15.79	1.60	5.51	7.53	1.15
Na-cn	12.87	1.53	5.36	5.13	0.85
Becker and Puhan (1989)					
SMP	13.79	3.50	3.72	5.68	0.89
VE	13.80	3.50	3.71	5.70	0.89
UF	13.71	3.50	4.09	5.13	0.85

[a] Figures for lactose (Abrahamsen and Holmen, 1980; Tamime *et al*., 1984) were calculated by difference. [b] Data is average using three different starter cultures.

VE: vacuum evaporated; UF: ultrafiltration; RO: reverse osmosis; SMP: skimmed milk powder and Na-cn: sodium caseinate.
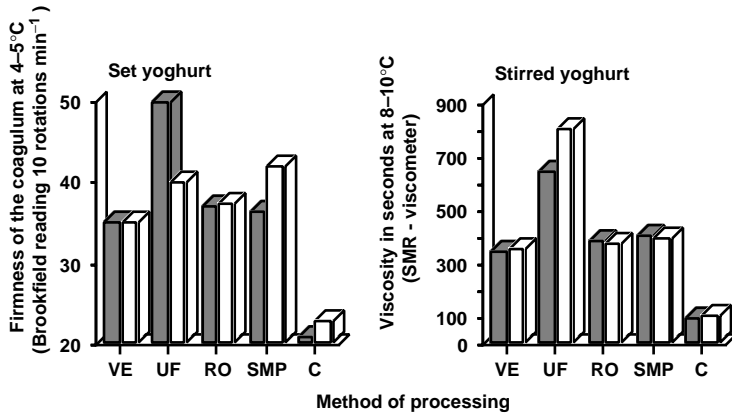
Fig. 2.7 Rheological properties of yoghurts manufactured from milk concentrated/fortified by different methods VE: vacuum evaporation; UF: ultrafiltration; RO: reverse osmosis; SMP: skimmed milk powder addition and C: control (liquid milk)

▨ One day old at 4°C. ☐ After 14 days storage at 4°C.

Adapted from Abrahamsen and Holmen (1980).

- UF and Na-caseinate yoghurts gave the highest reading for viscosity and firmness of the coagulum (see Fig. 2.7), but the mouthfeel of the latter product was not acceptable (Tamime *et al*., 1984).
- The favourable instrumental assessment of UF yoghurt was not supported by the organoleptic appraisal and the yoghurt prepared from VE milk proved the most popular (Abrahamsen and Holmen, 1980); however, such effects may not be observed if fruit and sugar are added to the yoghurt as reported by Romero Estevez (1988), Goh *et al*. (1990) and Biliaderis *et al*. (1992).
- The method of fortification can influence dramatically the chemical constituents of the yoghurt mix, but a target figure of about 5 g protein $100 g^{-1}$ is highly recommended.

However, the quality of yoghurt made from milk fortified by the different methods has been reported by many researchers. For example, Na-caseinate, whey proteins and UF milk were used to make yoghurt where the lactic acid content, level of D-lactate, firmness and sensory properties were influenced with the method of fortification used (Renner and Eiselt-Lomb, 1985a–c). In Canada, comparative studies on yoghurt made from milk fortified with different milk proteins (Modler and Kalab, 1983; Modler *et al*., 1983) suggest the following:

- The gel strength and syneresis were influenced by the method of fortification used and level of casein.
- The casein to non-casein ratio varied between 1.08:1 and 4.56:1.
- The micellar structure of the yoghurt, i.e. fusion of casein micelles, size of micelle chain and flocculated milk proteins, was influenced by the type of milk protein used (e.g. UF milk, caseinate, SMP and WPC using ion exchange, UF or electrodialysis).
- Sensory properties were influenced by method of fortification used.

During the preparation of the milk base, it is probable that a number of different dairy ingredients will be used and it is essential that the levels of SNF and fat are

calculated properly in order to achieve a balanced yoghurt milk. Two approaches can be considered: (i), an approximate formulation can be worked out by the Pearsons Square formula or, (ii), an algebraic method can be used to calculate exactly the quantities of fat and SNF that will be obtained from the various raw materials (Hyde and Rothwell, 1973). The former method of calculation is most satisfactory for small-scale yoghurt producers, but the algebraic method is usually recommended for large-scale manufacture, especially when considering the economics of the operation. Hypothetical examples of the above two methods of calculation are shown in Appendix IX.

2.5 Addition of stabilisers/emulsifiers

2.5.1 General background
Stabilisers and/or emulsifiers are used during the manufacture of some dairy products, but in yoghurt making only stabilisers are added to the milk base. Their application in most countries is governed by legislative regulation. At the international level, the FAO/WHO (1990) have drafted a list of compounds (with permitted concentrations) which can be used in the production of yoghurt and a similar approach has been adopted in the United Kingdom (Statutory Instruments (SI), 1995).

The classification of these food-grade stabilisers/emulsifiers has always proved something of a problem and a number of different schemes have been suggested, such as:

- All compounds to be referred to as polysaccharide materials
- The name to include the botanical origin
- Their general origin, i.e. plant, animal or synthetic
- Chemical grouping.

However, the latter approach has been modified by Glicksman (1969, 1979, 1982, 1983, 1985, 1986) and his proposed classification includes a reference to the processing technique, for example:

- Natural gums (those found in nature)
- Modified natural or semi-synthetic gums (i.e. chemical modifications of natural gums or gum-like materials)
- Synthetic gums (those prepared by chemical synthesis).

Some stabilisers permitted by FAO/WHO (1990) and SI (1995) are illustrated in Table 2.9 and, for convenience, Glicksman's method of classification has been used for the arrangement of the various product groups.

The primary aim of adding stabilisers to the milk base is to enhance and maintain the desirable characteristics in yoghurt, for example, body and texture, viscosity/consistency, appearance and mouthfeel. Thus, the yoghurt coagulum is often subjected to mechanical treatment during manufacture: (a) stirring of the coagulum in the fermentation tank at the end of the incubation period or for in-tank cooling, (b) pumping of the coagulum to a plate/tubular cooler, (c) mixing to incorporate the fruit/flavours into the coagulum, followed by pumping to the filling/packaging machine, and (d) subsequent postfermentation heat treatment of the coagulum for the manufacture of pasteurised, UHT or long-life yoghurt and as a result the

Table 2.9 Classification and functions of gums which could be used during the manufacture of yoghurt

Natural	Modified	Synthetic[a]
Plant	Cellulose derivatives (1)[b]	Polymers
Exudates	Carboxymethylcellulose	Polyvinyl derivatives
Arabic (1, 3)[b]	Methylcellulose	Polyethylene derivatives
Tragacanth (1)[b]	Hydroxyethylcellulose	
Karaya[b]	Hydroxypropylcellulose	
Extracts	Hydroxypropylmethylcellulose	
Pectins (2, 3)[b]	Microcrystallinecellulose	
Seed flour	Microbial fermentation	
Carob (1)[b]	Dextran	
Guar (1)[b]	Xanthan (1, 3)[b]	
Seaweeds	Miscellaneous derivatives[b]	
Extracts	Low-methoxy pectin	
Agar (2, 3)[b]	Propylene glycole alginate	
Alginates (1, 2, 3)[b]	Pregelatinised starches	
Carrageenan (2, 3)[b]	Modified starches	
Furcelleran (1, 2, 3)[b]	Carboxymethyl starch	
Cereal starches (1, 2, 3)	Hydroxyethyl starch	
Wheat	Hyroxypropyl starch	
Corn		
Animal		
Gelatin[b]		
Casein		
Vegetable		
Soy protein		

[a] Limited in their application in yoghurt. [b] Stabilisers permitted by FAO/WHO (1990), and the permitted level (singly or combination with others) is 5 g kg^{-1}, except for pectin, gelatin and/or starch derivatives where it is 10 g kg^{-1}.

Figures in parentheses indicate the function of the hydrocolloid: (1) thickener, (2) gelling agent and (3) stabiliser. The permitted level of these stabilising compounds is specified by the legislative regulations and they are not permitted in natural or unflavoured fermented milks.

Data compiled from Powell (1969), Glicksman (1969, 1979, 1982, 1983, 1985, 1986), Pedersen (1979), FAO/WHO (1990), Baird and Pettit (1991) and Gordon (1992).

yoghurt may become less viscous or, in extreme cases, may show whey separation: The addition of stabilisers can overcome these defects.

Stabilisers are sometimes referred to as hydrocolloids and their mode of action in yoghurt includes two basic functions: first, the binding of water and second, promotion of an increase in viscosity (Boyle, 1972; Thomas, 1982; Morley, 1984; Frost *et al.*, 1984; Phillips *et al.*, 1986, 1992, 1994; Rother, 1994; Doreau, 1994). Thus, the molecules of a stabiliser are capable of forming a network of linkages between the milk constituents and themselves, due to the presence of a negatively charged group, for example, hydrogen or carboxyl radical, or to the presence of a salt possessing the power to sequester calcium ions. These negative groups are concentrated at the interfacial areas and according to Boyle (1972), Dexter (1976), Ingenpass (1980), Baird and Pettit (1991), Kasapis *et al.* (1992), Gordon (1992) and Pedersen (1995), the binding of water into the milk base is achieved by the stabiliser as follows:
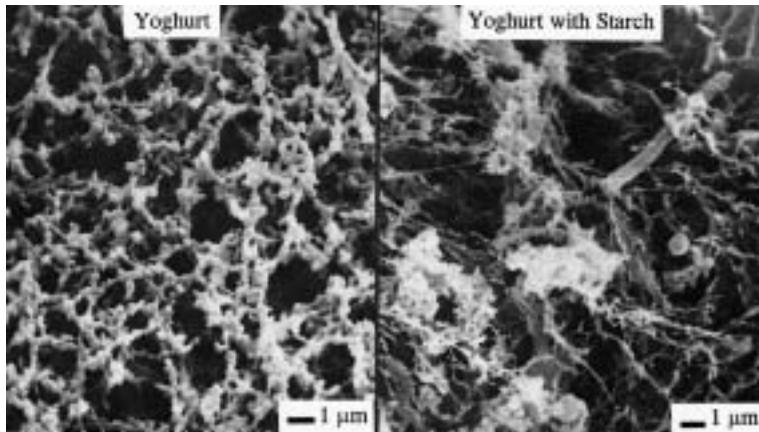
Fig. 2.8 Microstructure of yoghurt with or without added starch as shown by scanning electron microscopy (SEM)

After M. Kalab (personal communication).

- It binds the water as water of hydration;
- It reacts with the milk constituents (mainly the proteins) to increase their level of water hydration;
- It stabilises the protein molecules in the form of a network that retards the free movement of water (see Fig. 2.8 and 2.9).

Therefore, the functions of hydrocolloids in yoghurt are as: (a) gelling or thickening agents, and (b) stabilising agents (Rizzotti *et al.*, 1984; Schaffer, 1989; Thygesen, 1990). Table 2.9 shows the wide range of compounds which can be added to milk for the production of a viscous yoghurt and these stabilisers can be added as single compounds or as a blend. The latter approach is more widely used, since most commercial preparations are a mixture of stabilising compounds (unless it is declared otherwise). The object of blending these compounds together is to achieve a specific function or, in the majority of cases, to overcome one of the limiting properties associated with a specific compound. For example, a single stabilising compound (X) may be suitable for the manufacture of a fruit/flavoured yoghurt, but it may not be suitable on its own for the production of frozen, dried or pasteurised yoghurt. Hence the choice of a particular type of stabiliser is dependent on a multitude of factors, including those in the following sections.

2.5.2   Miscellaneous properties and conditions

2.5.2.1   Functional properties
These include the effect and/or mode of action of the selected stabiliser compound(s), and have to be considered in relation to the type of yoghurt produced. However, in most applications the rule of thumb is trial and error.

2.5.2.2   Optimum concentration
The optimum concentration of stabiliser(s) to be used in yoghurt is sometimes governed by legislation and/or side effects, that is, appearance or undesirable
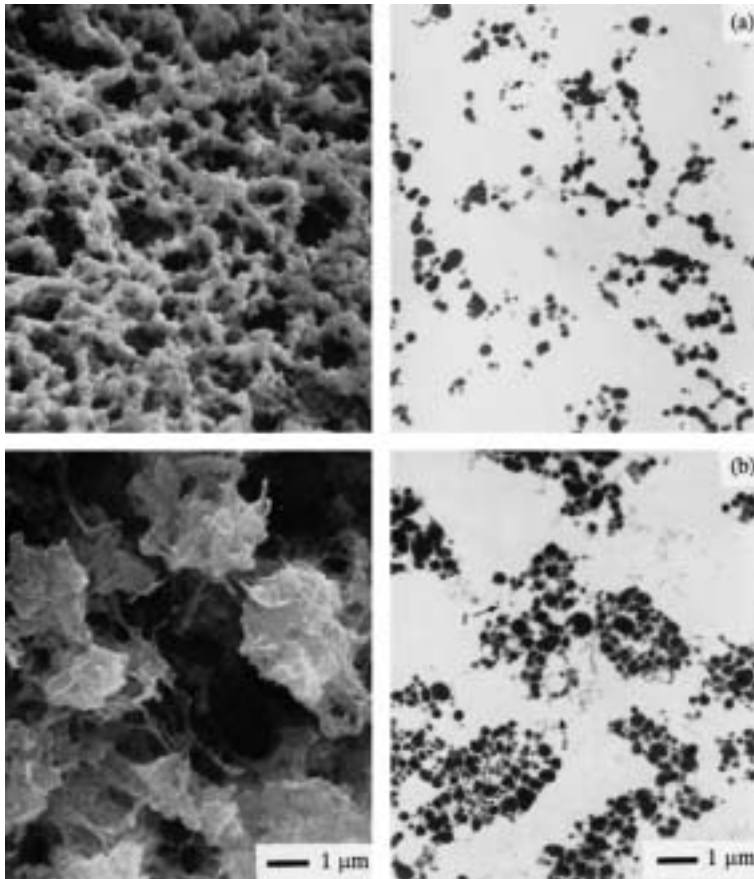
Fig. 2.9 Differences in the microstructure of yoghurt in the presence of different stabilising agents

Micrographs: (left-hand side) scanning electron microscopy (SEM), (right-hand side) transmission electron microscopy (TEM).

(a) Yoghurt supplemented by 0.4% carrageenan and (b) supplemented by 2% pregelatinised waxy maize starch.

The addition of carrageenan resulted in the formation of a fibrillar microstructure which connected large clusters of casein micelles. It can be observed that the fibres had no free terminations, but were thin and long. The presence of starch gave rise to short fibres and sheets and the fibres frequently had free terminations where some of them were connected to small clusters of casein micelles.

TEM showed no differences between the microstructure of yoghurt with starch or carrageenan stabilisers; however, SEM could be used to detect different additives in yoghurt.

Note: (g) fat globules, (f) flat fibres, (h) sheets, (m) casein micelles, magnification 6000 ×.

After Kalab *et al.* (1975). Reproduced by permission of *Journal of Dairy Research*.

mouthfeel, which could be caused by the addition of too large a quantity. Some recommended levels of stabiliser for the manufacture of yoghurt are:

• 0.02–0.7 g 100 g^{-1} of pectins or some modified starches (Winterton and Meiklejohn, 1978; Zmarlicki *et al.*, 1977; Gudnason *et al.*, 1983; Kratz *et al.*, 1989; Pedersen, 1993; Basak and Ramaswamy, 1994) or 1.2 g 100 g^{-1} carrageenan and 0.25 g 100 g^{-1} pectin (Petersen, 1989).

- 0.05–0.6 g 100 g^{-1} of agar-agar, locust (carob) gum, guar gum, alginate, gelatin, carrageenan or carboxymethyl cellulose (Volker, 1972; Schrieber, 1973; Ledder and Thomasow, 1975; Steinitz, 1975; Hannigan, 1982; van Coillie, 1989; Gonc *et al.*, 1994, Anon., 1995a; Sta, 1996); however, according to Fischer (1996), only high bloom gelatin should be used in yoghurt making due to improved gelatin/casein interactions, its higher melting point and stabilising ability.
- 1–2 g 100 g^{-1} of some starch preparations (Thomasow and Hoffmann, 1978; Chawal and Balachandran, 1986; Katz, 1991).
- 0.1–0.5 g 100 g^{-1} guar gum in an acidified milk sample (i.e. 0 to 20 g fat 100 g^{-1} and 6 to 12 g SNF 100 g^{-1}) did not affect the partition coefficients of acetaldehyde, ethanol or diacetyl (Lo *et al.*, 1996).
- 0.5–2 g 100 g^{-1} sugarbeet fibre improved the consistency of yoghurt and also gave an acceptable flavour (Saldamli and Babacan, 1997).
- 0.6 g 100 g^{-1} tapioca-based starch was able to replace 2 g 100 g^{-1} SNF (i.e. significant cost saving) without affecting the properties of yoghurt; 0.3 gelatin was less effective when compared with starch, especially in yoghurt containing 1.5 g fat 100 g^{-1} (McGlinchey, 1995, 1997); alternatively, a mixture of cooked wheat grains and sucrose has been used to improve the nutritional and organoleptic properties of yoghurt (Hamzawi and Kamaly, 1992).

Another factor which determines the level of stabiliser added to the yoghurt milk is the percentage of milk solids present. According to Hall (1975), the optimum concentrations (g 100 g^{-1}) for a gelatin/plant gum mixture were 0.5, 0.45, 0.4, 0.3 and 0.25 to yoghurt milks containing 12.5, 14.5, 16.5, 19.0 and 22.0 milk solids, respectively. Other recommended concentrations (g 100 g^{-1}) of stabiliser blends for the manufacture of yoghurt are: (a) 0.35 Gelodan (Mehanna and Mehanna, 1989), (b) 0.3 Na-alginate or gelatin + <1.5 starch (Jogdand *et al.*, 1991a, b), (c) 1 gelatin + 0.2 agar (Ajam *et al.*, 1993), (d) 0.2 Na-alginate + 0.1 β-cyclodextrin (Jiang *et al.*, 1995), (e) 0.06 carboxymethyl guar gum or leucaena gum + carrageenan at a ratio of 9:1 (El-Etriby *et al.*, 1994; Abd El-Salam *et al.*, 1996), (f) polymer solution of locust bean gum and carrageenan (Arnaud *et al.*, 1989), and (g) 2.0 Gelodan YF 358 (fibre + milk proteins) and 1.5 Gelodan YF 326 (fibre + gelatin) or 1.5 Gelodan YF 314 (fibre + milk proteins + low methoxyl pectin) and 1.5 Gelodan 361 (fibre + gelatin + low methoxyl pectin) (Carnell, 1989). However, studies evaluating different stabilisers during the manufacture of yoghurt have also been reported by Jamrichova (1985, 1990), Shukla *et al.* (1988), Shukla and Jain (1991) and Jawalekar *et al.* (1993).

2.5.2.3 Toxic or inhibitory effects
Some stabilisers, for example carrageenans, tragacanth and locust (carob) gum, are still awaiting toxicological clearance for use in foodstuffs, but in general, stabilising compounds do not inhibit the yoghurt organisms at the rates normally employed.

2.5.2.4 Legal aspects
These may differ with the country concerned and not all stabilising compounds are permitted for the production of yoghurt; hence, statutory regulations should be checked on a regular basis.

2.5.2.5 Solubility and dissolution

The solubility and dissolution of some starch preparations and Na-carrageenan are at an optimum at low temperature and hence they can be added to cold milk during the preparation of the milk base. The majority of the stabilising compounds are, however, only soluble at higher temperatures, for example, 50–85°C (with the exception of agar-agar at 90–95°C), so that in practice these stabilisers are added to warm milk before pasteurisation, or alternatively to hot milk after the heat treatment. In some instances, complete dissolution of a particular stabiliser blend, for example, one which contains a starch preparation, may necessitate a holding time at high temperature in order for the mixture to become active as a stabiliser.

In view of the different properties of these compounds, it is difficult to recommend one method for incorporation into the milk base, but the following points may help to overcome any problems:

- Follow the instructions provided by the manufacturer or in the absence of any information: (a) mix the stabiliser with the milk powder and add to the water or milk with high speed stirrer at the temperature recommended for the milk powder, or (b) mix the stabiliser with the sugar and add to the milk base under high speed agitation at the temperature recommended for the sugar.
- Hydrate the stabiliser (e.g. gelatin powder) in water or milk and then add to the basic mix with high speed stirring.

2.5.2.6 Casein

The effect on the casein of some hydrocolloids (Na-carboxymethyl cellulose, guar gum and locust bean gum) at levels as low as $0.05\,g\,100\,g^{-1}$ in sweet milk can destabilise the casein micelle (Powell, 1969) and, although the destabilised casein micelles will eventually coagulate, the matrix has a rather limited ability to retain water and syneresis becomes evident. Furthermore, such destabilised casein can give rise to a course coagulum with an open texture. The problem can be minimised, however, by blending the above compounds with carrageenan or aliginates (see also Dexter, 1976).

2.5.2.7 Processing conditions

The processing conditions for various yoghurt-based products have been developed (see Chapter 5) and the success of these is dependent on the addition of stabilisers. For example:

- Pasteurised, UHT or long-life yoghurt – it is recommended that a gelling agent is added consisting of a blend of locust gum and agar-agar and/or xanthan (Anon., 1980a); the presence of starch derivatives (diamylopectin glycerol ether or diamylopectin phosphate) can improve the appearance of heat-treated yoghurt (Vanderpoorten and Martens, 1976).
- Frozen yoghurt – an unspecified mixture of stabilisers/emulsifiers is recommended by Gautneb *et al.* (1979), but the addition of modified starch proved unsatisfactory (Winterton and Meiklejohn, 1978).
- Stirred yoghurt – a blend of $(g\,100\,g^{-1})$ 1 Na-proteinate (possibly Na-caseinate), 0.1 Frimulsion J5, 0.1 Genu gum CH 200, 0.3 Genu carrageenan with maltodextrin or 0.16 Frimulsion JQ improved the viscosity of the product (Luczynska *et al.*, 1978).

- Drinking yoghurt – an agar-agar based stabiliser is added at a rate of $0.25\,g$ $100\,g^{-1}$ and this helps to maintain the suspension of fruit in the product (Morley, 1978).
- Freeze-dried dahi – the quality of the product was improved by the addition of corn starch and lecithin or glycerol monostearate to the fermented milk prior to drying (Baisya and Bose, 1975).

Since casein precipitation may occur in sweet milk or during the development of acid, some of the stabilisers may be added to the yoghurt after the formation of the coagulum. In this case it is recommended that the stabilising compound (e.g. liquefied agar-agar and/or preswollen gelatin) is mixed with the sugar and then incorporated into the coagulum. Refer to Chapter 5 for further details and up-to-date information regarding the use of stabilisers in yoghurt-related products.

2.5.2.8   *Solidification characteristics*
The majority of stabilisers used in the production of yoghurt will exhibit solidification characteristics at ordinary refrigeration temperature, with the exception of gelatin and agar-agar which solidify at 25°C and 42–45°C, respectively. These latter stabilising compounds can, therefore, cause problems during the cooling stage, i.e. difficulty in pumping and/or packaging and, in addition, the use of gelatin may give the coagulum a rough texture. This latter fault can be reduced or eliminated by passing the coagulum through a fine mesh screen or sieve.

2.5.2.9   *Hygienic standards*
It is recommended that suitable hygienic standards be applied to the stabilisers. However, the temperature used during the processing of the yoghurt milk (85°C for 30 min or 90–95°C for 5–10 min) is high enough to destroy the majority of micro-organisms which could be present in the stabiliser. Stabilisers added to the coagulum after the incubation period must be of excellent microbiological quality, otherwise the shelf life of the product could be reduced.

2.6   Addition of sweetening agents

2.6.1   General introduction
Sweetening compounds are normally added during the manufacture of fruit/flavoured yoghurt and, in some instances, for the production of "sweet" natural yoghurt; the latter product is of limited demand.

The main object of the adding sweetening agents to yoghurt is to tone down the acidity of the product and the level of incorporation is dependent on:

- type of sweetening compound used,
- consumer preference,
- type of fruit used,
- possible inhibitory effects on the yoghurt starter organisms,
- legal aspects, and/or
- economic considerations.

On average, fruit/flavoured yoghurts may contain as high as $20\,g\,100\,g^{-1}$ carbohydrates and these are derived from: (a) residual milk sugars (lactose, galactose and

glucose) – the level varies in relation to the level of solids in the milk base and the method of fortification, (b) natural sugars present in the fruit (sucrose, fructose, glucose and maltose) and (c) sugars added by the yoghurt manufacturer and/or the fruit processor.

Fruit may contain different levels and types of natural carbohydrate and the total content ranges from as low at $1.6\,g\,100\,g^{-1}$ in lemon to as high as $65\,g\,100\,g^{-1}$ in raisins (Shallenberger and Birch, 1975; Holland *et al.*, 1991). The fruits, which are in regular demand, have the following natural carbohydrate content ($g\,100\,g^{-1}$):

Apricot	7.5
Black cherry	12.0
Blackcurrant	6.6
Mandarin	14.2
Peaches	9.0
Pineapple	11.6
Raspberry	5.6
Strawberry	6.2

The main carbohydrates present in fruits are glucose, fructose, sucrose and maltose, and hence the perceived sweetness of each type of fruit is dependent on the level and type of carbohydrate present. The comparative sweetness of various carbohydrates, including milk sugars and synthetic sweeteners, is illustrated in Table 2.10; sucrose is given a nominal rating of one.

The fruit preparations which are utilised by the yoghurt industry may be divided into two main categories, fruit preserves which do not contain any added sweeten-

Table 2.10 List of various sweetening compounds

Sweetening compound	Relative sweetness: sucrose = 1
Lactose	0.4
Dulcitol	0.4
Maltose	0.4
Sorbitol	0.5
Mannose	0.6
Galactose	0.6
Glucose	0.7
Xylose	0.7
Mannitol	0.7
Glycine	0.7
Invert sugar	0.7–0.9
Glycerol	0.8
Sucrose	1.0
Fructose	1.1–1.5
Cyclamate	30–80
Acesulfame K	150–200
Aspartame	200
Saccharin	240–350
Neohesperidin DC	1500–2000
Thaumatin	3000

After: Beck (1974), Crosby and Wingard (1979), Shallenberger and Birch (1975), Meade and Chen (1977), von Lipinski and Klein (1989), Canales (1991), von Lipinski (1990) and Stasell (1993).

ing agent and fruits with added sweeteners. The latter type is more popular and the level of added sweeteners in processed fruits for yoghurt manufacture ranges from 25 to 65 g 100 g^{-1}, with the most popular level being 30–35 g 100 g^{-1} (J.G. Spinks, personal communication).

It is now almost universal practice to add preserves and similar materials to the finished yoghurt, since the presence of carbohydrates in the milk base can inhibit the growth of the yoghurt organisms. Thus, Tramer (1973) reported a reduction in the rate of acid development by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in concentrated milk (16.5 g TS g 100 g^{-1}) as the sugar level was increased from 6 to 12 g 100 g^{-1}, and a microscopic examination of these different types of yoghurt showed that first, *S. thermophilus* was more tolerant of high sugar concentrations than *L. delbrueckii* subsp. *bulgaricus* (a view which was confirmed by Steinsholt and Abrahamsen, 1978; Marshall and Mabbitt, 1980) and second, that morphological changes occurred, that is, the cells were distorted, elongated and "unhealthy looking". Kim *et al.* (1995) reported that the use of >9 g 100 g^{-1} sugar in the milk base reduced the rate of acid development and decreased the viscosity of the yoghurt (see also Coghill, 1983; Grandi and Lopes-Andrade, 1989; Latrille *et al.*, 1992; Cislaghi *et al.*, 1995). However, in Finland, strawberry yoghurt containing 3.5 g fat 100 g^{-1} and sucrose 10 g 100 g^{-1} was highly rated by the male panellists, but not the female, and such observation may be used to segment consumers in order to predict product success (Tuorila *et al.*, 1993). It was evident, however, that the sugar tolerance of the starter cultures was strain dependent and it was recommended that the strains of starter culture to be employed in presweetened milks should be carefully screened.

Commercially available starter cultures are tolerant of sugar levels up to 12 g 100 g^{-1} in the milk base, but recently one such culture showed a slight delay in the fermentation period (i.e. about 30 min) when grown in milk containing 9 g sugar 100 g^{-1} (Tamime, unpublished data). However, in a recent study in Korea (Song *et al.*, 1996), the growth of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was inhibited by the following concentrations (g 100 g^{-1}) of sweeteners: sucrose ≥4, fructose ≥2.7, aspartame ≥0.02, fructo-oligosaccharide ≥7.3 and isomalto-oligosaccharide ≥7.7.

The inhibition of yoghurt starter cultures in milk (14–16 g TS 100 g^{-1}) plus added sugar (10–12 g 100 g^{-1}) is due mainly to the adverse osmotic effect of the solutes in the milk, but low water activity (Shallenberger and Birch, 1975; Labuza, 1980) may also be involved. The water activity (A_w) of a food is described as:

$$A_w = \frac{P_f}{P_o} = \frac{ERH}{100}$$

where A_w = water activity, P_f = vapour pressure of water in food, P_o = vapour pressure of pure water at the same temperature and ERH = equilibrium relative humitity.

This latter concept is important from a quality control point of view, since both microbial growth and enzyme activity in foods are related to the A_w (Acker, 1969), and hence it is possible to suggest that both osmotic pressure and A_w may be associated with the inhibitory effect on yoghurt starter organisms. However, starter cultures propagated in milks with high total solids, for example, 30 g TS 100 g^{-1}, can also show reduced activity (Zmarlicki *et al.*, 1974), a condition which could be entirely related to the A_w of the growth medium. This observation was also reported by

Tramer (1973), who observed the inhibition of yoghurt starter cultures propagated in milk ($21\,g\,TS\,100\,g^{-1}$) plus $3\,g\,100\,g^{-1}$ added sugar; the inhibitory effect was attributed to A_w, since it was considered unlikely that $3\,g$ sugar $100\,g^{-1}$ in solution could create enough osmotic pressure to retard the growth of the organisms.

In view of the above data, the normal methods used for the addition of sweetening agents are as follows: (a) the yoghurt manufacturer adds up to $5\,g\,100\,g^{-1}$ sweetener (sugar) to the milk base and (b) the sweetness desired in the final product is attained by the addition of a sweetened fruit preparation.

It is worthwhile pointing out at this stage that the sugar content of frozen yoghurt is much higher than in ordinary fruit/flavoured yoghurt. It is recommended that the quantity of sugar (sucrose) added to the milk base should not exceed $10\,g\,100\,g^{-1}$, with the balance being added to the cold yoghurt prior to freezing. Different types of carbohydrate may be used during the manufacture of sweetened fruit/flavoured yoghurt and some examples of these are given in the following section.

2.6.2   Types of carbohydrate sweetener

2.6.2.1   Sucrose (saccharose)
Sucrose is abundant in the plant kingdom and it is normally referred to as sugar. Sucrose has the empirical formula $C_{12}H_{22}O_{11}$ and the refined carbohydrate is obtained commercially from sugar cane or sugar beet. It is widely used in the food industry as a sweetening agent and can be obtained in a granulated or syrup form. The former type requires strong agitation/stirring for complete dissolution when added to liquid milk and, in practice, it is added with the rest of the dry ingredients at around 40°C. The syrup type, which contains $65-67\,g\,100\,g^{-1}$ sugar (saturated at 20°C), is easily mixed with the aqueous phase of the milk base but, since it contains $33-35\,g\,100\,g^{-1}$ moisture, it dilutes the level of solids in the yoghurt milk, and this added water must be allowed for when calculating a balanced mix.

The addition of sugar before the heat treatment of the milk is highly desirable, since it ensures the destruction of any vegetative contaminants, for example, osmophilic yeasts and moulds. However, if the sugar has to be added after the formation of the coagulum, steps must be taken to avoid uneven distribution of the sugar and excessive reduction in the consistency of the product.

2.6.2.2   Invert sugar
This type of carbohydrate results from the "inversion" of a sugar with dextrorotatory optical activity to one that is laevorotatory or vice versa. The different types of invert sugar depend on the raw material. For example, invert sucrose syrup is formed when sucrose undergoes acid hydrolysis in the presence of heat and the degree of inversion can range from 10 to 90%.

$$C_{12}H_{22}O_{11} + H_2O \quad \rightarrow \quad C_6H_{12}O_6 + C_6H_{12}O_6$$

sucrose	water	acid and heat	D-glucose (dextrose)	D-fructose (levulose)
			{invert sugar}	
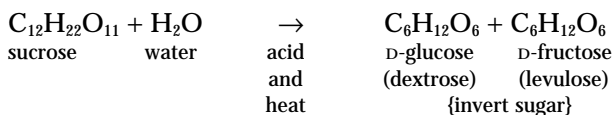
One advantage of this conversion is that a product (50% inversion) contains $23\,g$ $100\,g^{-1}$ moisture (Junk and Pancoast, 1973) and yet can be handled at this high sugar concentration without crystallisation. However, invert corn syrup is formed by the

hydrolysis of corn starch with the production of D-glucose (dextrose) and the degree of conversion is measured in terms of dextrose equivalent (DE), that is, types I (20–37 DE), II (38–57 DE), III (58–72 DE) and IV (>73 DE) (Junk and Pancoast, 1973). The process of hydrolysis is normally achieved by one of these methods:

- total acid hydrolysis
- acid liquefaction/enzyme saccharification
- total enzyme hydrolysis

In recent years starch syrups have also been processed into other types of sugars, for example, syrups high in maltose or fructose. The latter syrup has many potential applications in the food industry and according to Martin (1979), high fructose (corn) syrups are commercially produced in the United States containing 42 g, 55 g or 95 g fructose $100\,g^{-1}$; the corresponding sucrose equivalents, in terms of sweetness (sucrose = 1), are 1, 1.1–1.2 and 1.5, respectively (see also Dordovic *et al.*, 1981).

2.6.2.3 *Fructose (laevulose)*
Fructose or fruit sugar has the same empirical formula as glucose, $C_6H_{12}O_6$, and as can be seen from Table 2.10 is sweeter than both sucrose and glucose. Commercially, fructose is derived mainly from the conversion of starch, but recently, grape must containing fructose has been used at a rate of $20\,g\,100\,g^{-1}$ to sweeten yoghurt (Calvo *et al.*, 1995).

2.6.2.4 *Glucose (dextrose)*
Glucose has the same empirical formula as fructose, $C_6H_{12}O_6$, and is commercially produced from the hydrolysis of corn starch.

2.6.2.5 *Glucose/galactose syrup*
This is produced from whey, a by-product of the cheese and casein industries, and of the permeate of UF concentrated milk. The amount of lactose in whey is usually in the region of $5\,g\,100\,g^{-1}$ but, as illustrated in Table 2.10, the relative sweetness of lactose is only 0.4 compared with sucrose; hence the lactose has to be converted to its monomer constituents – glucose and galactose – before it can impart any real sweetness (see Table 2.10). The process of hydrolysis of lactose can be achieved using either acid or enzymes. The review by Sienkiewicz and Riedel (1990) provides details of these processes, the chemical composition of the different syrups and characterisation of the enzymes used, including commercial preparations and their countries of origin.

2.6.2.6 *Miscellaneous sweeteners*
Sorbitol is an alcohol, produced commercially from glucose by a hydrogenation process, that is, the aldehyde group (CHO) in the glucose molecule is converted to an alcohol group (CH_2OH). Although sorbitol has only half the sweetness of sucrose (see Table 2.10), it has a possible application in fruit/flavoured yoghurts for patients suffering from diabetes. Thus, the rate of absorption of sorbitol in the gut is slower than that of glucose and hence has little effect on the level of sugar in the blood. No recommended daily intake is given, since large intakes cause diarrhoea (Davidson *et al.*, 1979).

Saccharin and cyclamate are artificial sweeteners and their sweetness compared with sucrose is 240–350 and 30–80, respectively (Table 2.10). However, due to

Table 2.11 Reported characteristics of yoghurt made with artificial sweetners

Sweetener $(g\,100\,g^{-1})$	Comment	Reference
Xylitol 8, fructose 7, cyclamate 0.07 and xylitol 4 + saccharin 0.007	Xylitol retarded the starter culture growth and was only suitable when used with sucrose; the rates used were satisfactory alternatives to 8 g sucrose $100\,g^{-1}$.	Hyvonen and Slotte (1983)
Thaumatin 0.0002–0.0003 or up to 0.1	This is a protein sweetener derived from the fruit *Thaumatococcus danielli* and has been used in Japan.	Ohashi and Ochi (1983), Yasuda *et al.* (1985)
Aspartame 0.1–0.75	Sweetener was mixed with a stabilising solution $(g\,100\,g^{-1})$ consisting of: 3–8 low methoxyl pectin, 2–7 high methoxyl pectin and 0.2–2.5 Na-hexametaphosphate; the mixture was pasteurised and added to yoghurt at a ratio of 1:3–7.5 (v/v).	Malone and Miles (1984)
Aspartame 0.14	The added rate was equivalent to 2 g sucrose $100\,g^{-1}$, but 64% of panellists preferred sucrose because of the lingering aftertaste and slow development of sweetness when aspartame was used.	Greig *et al.* (1985)
High fructose corn syrup 4	Swiss-type yoghurt sweetened with fructose syrup (*c.* $90\,g\,100\,g^{-1}$) was highly rated ($P \le 0.001$) especially in the strawberry product; acetaldehyde and diacetyl contents were not influenced by the sweetener, but acetone content was highest in the experimental yoghurt.	Wilson-Walker (1982), McGregor and White (1986, 1987)
Aspartame 0.02 or sorbitol 7.4 plus polydextrose	Nine different sweeteners were evaluated, but sorbitol and aspartame were highly favoured; slight decrease in flavour was detected after 42 day storage period, but not after 28 days.	Keating and White (1990), White (1991)
NutraSweet®	Typical recommended quantities used were 400–500 mg l^{-1} for fruit yoghurt or 700 mg l^{-1} for mocha yoghurt; in France and India, low fat yoghurt sweetened with aspartame had high scores for overall preference.	Wiese (1988), Dupont (1989), Kumar and Atmaram (1991)

Actilight®	This sweetener consisted of fructo-oligosaccharide 1-kestose, nystose and fructosyl nystose; this product stimulated the growth of lactabacilli and bifidobacteria.	Thiriet (1989)
Aspartame	Stability of this sweetener in fruit preparation was $1\frac{1}{2}$, 4–6 or >6 months at 32.2°C, 21.1°C and 4.4°C, respectively; the stability in sundae-style yoghurt (i.e. with fruit in the bottom) was good.	Fellows *et al.* (1991a, b), Saldamli *et al.* (1991)
Different sweeteners	Yoghurt made with acesulfame remained stable during the storage period whilst the product sweetened with aspartame degraded slightly during the fermentation period, but was stable during storage.	Lotz *et al.* (1992)
Aspartame and acesulfame-K	Sensory tests on yoghurt revealed synergistic effects between these sweeteners, but no effect on the textural properties; recommended level of each type of sweetener in strawberry yoghurt was $0.016\,g\,100\,g^{-1}$.	Lotz *et al.* (1993), Anon. (1996)
Natren 0.3–0.4, aspartame 0.04 or lactose hydrolysed milk	Yoghurt made with natren and raspberry syrup was highly rated by the taste panellists.	Tosovic *et al.* (1994)
Different mixtures	Mixture of sweeteners (e.g. fructose + aspartame or fructose + aspartame + acesulfame) gave the highest sweetness intensity in yoghurt and the cost was lowest.	Rollet (1995)
Neohesperidine	This sweetener alone or in combination with acesulfame was stable in yoghurt after storage for 6 weeks at 3°C.	Montijano *et al.* (1995), Montijano (1996), Montijano and Borrego (1996)

possible toxic effects, cyclamate has been banned in many countries as an additive, and although saccharin is still permitted, its use is closely observed by food and drug administrators worldwide. The use of these sweeteners in the food industry is, therefore, restricted. In the present context, it should be noted that some information is available regarding the effect of the above sweetening agents on the activity of yoghurt starter cultures. Thus, Gautneb *et al.* (1979) reported an inhibition of acid production by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* when the yoghurt milk was fortified with a sweetening agent composed of 99.9 g 100 g^{-1} sorbitol and 0.1 g 100 g^{-1} saccharin. As a safeguard these types of sweetener should be added after the fermentation of the milk (see also Hyvonen and Slotte, 1983). Table 2.11 illustrates the latest reported information regarding the effect of synthetic sweeteners on the quality of yoghurt, growth characteristics of the starter cultures and stability of the sweetener(s) during the storage period. However, for further technical information on artificial sweetners (see Hough *et al.*, 1979; Grenby *et al.*, 1983; Grenby, 1987) and relevant data on yoghurt, the reader is referred to reviews by Hugill (1980), Harrison and Bernhard (1984), Homler (1984), van Tornout *et al.* (1985), Billaux (1989), Sasso (1989), Akahoshi *et al.* (1990), Pedersen (1991), Sardesai and Waldshan (1991), Farooq and Haque (1992) and Borrego and Montijano (1997).

Any of these different types of sweetening agents could be employed for the manufacture of fruit/flavoured yoghurts and the choice of any one particular sugar is determined by one or more of the following factors:

- Availability and cost of the sweetening compound: for these reasons it is probable that sucrose is the most widely used.
- Legal aspects: whether a certain sugar is permitted as a food additive, although since most sweetening agents are derived from natural products, with the exception of the artificial sweeteners, prohibition is unlikely.
- Storage facilities: granulated products are stored in multilayer bags or large silos and humidity control in the storage area is essential to prevent "caking"; details of bulk storage requirements are discussed by Junk and Pancoast (1973), Meade and Chen (1977), Kaplinsky (1989) and Chen and Chou (1993); syrups are mainly stored in large metal containers or silos.
- Nutritional aspects: fructose is a very sweet sugar and a sucrose/fructose syrup mixture used at a low level can provide both sweetness and a reduced calorie intake; in addition, fructose, like sorbitol, is absorbed only slowly into the bloodstream and its use in "diabetic" yoghurt production is a clear possibility.

2.7 Addition of miscellaneous compounds

During the preparation of the milk base, some yoghurt manufacturers add compounds to the milk in order to achieve specific objectives. Some examples of such additives are as follows.

2.7.1 Penicillinase

Intramammary injection of antibiotics is widely used for the treatment of mastitis in the dairy cow and residues of these compounds in milk can inhibit the growth of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (see Chapter 6). Relevant data on the structure, mode of action and other related biochemical activity(s) of anti-

microbial drugs can be found in Pratt and Fekety (1986) and Williams *et al*. (1996). Although statutory regulations have been introduced in different countries to limit the level of these inhibitory compounds in milk, even the permitted values can reduce the activity of the yoghurt starter culture. As a result, methods have been sought to inactivate the different antibiotics and notable success has been achieved in the case on penicillin. The inactivation of penicillin is carried out enzymatically using penicillinase (β-lactamase, EC 3.5.2.6), which is contained in the filtrate from different cultures of *Bacillus* species. One such preparation is commercially available under the name Bacto-Penase. β-Lactamase is specific in hydrolysing cyclic amides, that is, β-lactam in penicillin, thus producing an antibiotically ineffective compound; the structure of penicillin and the neutralising action of β-lactamase are illustrated in Fig. 2.10.

The activity of penicillinase preparations can be assayed by chemical or microbiological methods. Results from the former technique are expressed in Levy units (LU) or Kersey kinetic units (KKU), while the microbiological method measures the "units" of penicillin being inactivated. For example, 1 ml of Bacto-Penase has a potency of 2000 LU, 200 000 KKU, or can inactivate 1 000 000 units of penicillin G; the Bacto-Penase concentrate is ten times more active than the standard penicillinase preparation.

In commercial practice, penicillinase is added to the milk with the rest of the dry ingredients and it is recommended that it should be added at ambient temperature; high temperatures, for example, those employed in the heat treatment of yoghurt milk, can inactivate it. However, it is important to note that penicillinase is only effective against penicillin and that it should only be added to milk known to be contaminated with penicillin, a situation which is difficult to determine. Thus, routine
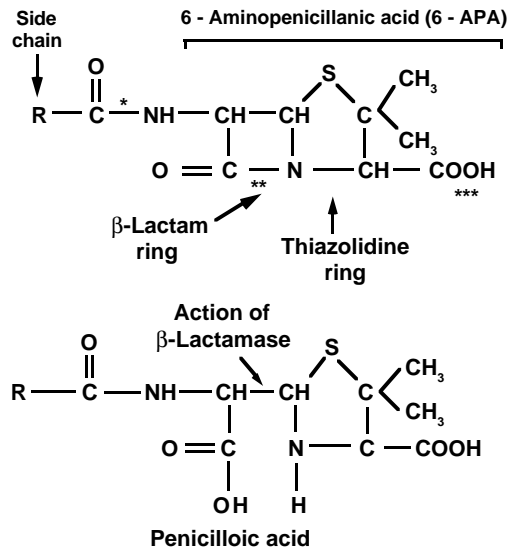
Fig. 2.10 Basic structure of penicillin and the mode of action of β-lactamase

*, Site of action of amidase; **, site of action of β-lactamase; ***, site of salt formation.

The β-lactam ring of the 6-APA is split by the action of β-lactamase to produce a bacteriologically inert penicilloic acid; however, the specific action if reduced or increased by the nature of the side chain.

Adapted from Edwards (1980) and Ball *et al*. (1983).

addition to the yoghurt milk may prove uneconomical in the long run, especially as 60% of the antibiotics used in the United Kingdom for mastitis therapy are not penicillin(s).

Another approach to inactivation of the penicillin content in milk was investigated in the U.K. and Poland, where the yoghurt milk was treated with selected strains of *Micrococcus* spp. (Reiter *et al.*, 1961; Czarnocka-Roczniakowa and Maciejska, 1985). In another study, *Micrococcus* spp. and lactic acid bacteria were inoculated simultaneously, and the latter micro-organisms were able to grow in the presence of low concentrations of penicillin about $0.3\,IU\,ml^{-1}$ (Maciejska and Czarnocka-Roczniakowa, 1985, 1989).

2.7.2 Preservatives

Different types of preservative are used in the food industry, including the processing of fruits, where they are effective growth inhibitors against yeast and moulds (Restaino *et al.*, 1982; Eklund, 1983; Andres, 1985). The addition of such fruits to yoghurt results in the carry-over of some of these compounds, and hence, in the United Kingdom, for example, the SI (1995) provides general information regarding preservatives which are permitted in fruit yoghurt, but not in natural yoghurt. A similar approach has also been adopted by FAO/WHO (1990) and the permitted preservatives in yoghurt, which come exclusively from the fruit preparations, are sorbic acid (including its Na-, K- and Ca-salts), sulphur dioxide and benzoic acid. The maximum permitted level in the final product is $50\,mg\,kg^{-1}$ (singly or in combination) (FAO/WHO, 1990).

In view of the fact that preservatives are allowed in fruit yoghurt, some manufacturers are inclined to fortify the milk base with one of the preservatives (e.g. sulphur dioxide, sorbic acid, benzoic acid, benzoates and/or ethyl, methyl or propyl *p*-hydroxybenzoate) in the hope of prolonging the keeping quality of the product. This approach is not, however, one to recommend, partly because the end products may not comply with the statutory regulations of an intended market and partly because the presence of such compounds in the milk may affect the growth of the starter culture. One preservative which might be an exception to this rule and which is widely used in the dairy industry (cheese and cheese products) is sorbic acid.

This compound is commercially available as a powder in the acid form ($CH_3.CH$-$CH.CH$-$CH.\,COOH$) or as the potassium or sodium salt ($CH_3.CH$-$CH.CH$-$CH.$ $COOK$ or Na), that is, potassium or sodium sorbate. These salts are used more commonly than the acid and their antimycotic activity is released at low pH, <6.5, where the salt is ionised to produce the free acid (Anon., 1974, 1981b). It should also be noted that K- or Na-sorbates yield only 75% of the inhibitory strength shown by sorbic acid.

For example:

$$0.13\,g\,100\,g^{-1}\,K \text{ or } Na\text{-sorbate} \cong 0.1\,g\,100\,g^{-1} \text{ sorbic acid} \cong 1000\,\mu g\,g^{-1}.$$

Sorbic acid is a mycostatic agent in that it does not reduce the actual number of yeasts and moulds in the product, but merely inhibits their activity, perhaps by interfering with their dehydrogenase systems. The effect of potassium sorbate on the activity of yoghurt starter cultures has been studied by Hamdan *et al.* (1971) and they reported a reduction in growth, acid development and acetaldehyde production. The dose rate of potassium sorbate was 0.05 and $0.1\,g\,100\,g^{-1}$, which would be

Table 2.12 Effect of potassium sorbate ($C_3H_7O_2K$) on pH values developed by three commercial yoghurt starter cultures (3% inoculation rate) incubated at 45°C

Time of incubation (h)	Starter culture R_1			Starter culture 403			Starter culture 405		
	A	B	C	A	B	C	A	B	C
					pH				
2	4.75	5.00	5.10	4.75	4.90	5.15	4.85	5.05	5.25
3	4.35	4.60	4.70	4.40	4.50	4.60	4.45	4.65	4.75
4	4.10	4.40	4.50	4.20	4.30	4.40	4.20	4.40	4.50

A, Control, no $C_6H_7O_2K$ added. B, Milk contains $0.05\,g\,100\,g^{-1}$ $C_6H_7O_2K$. C, Milk contains $0.1\,g\,100\,g^{-1}$ $C_6H_7O_2K$.

Adapted from Hamdan *et al.* (1971).

equivalent to 375 and $750\,\mu g\,g^{-1}$ of free sorbic acid, respectively. The rate of acid production by three different commercial starter cultures is illustrated in Table 2.12 and it can be observed that, at the lower concentration, the inhibition delayed the processing time by 1 hour.

Obentraut *et al.* (1982, 1984) reported that 72 and 92 samples of set- and stirred-type yoghurts, respectively, in Austria contained benzoic acid at $14–16\,\mu g\,g^{-1}$ and $10–19\,\mu g\,g^{-1}$, respectively. While in Japan two samples of dried yoghurt had benzoic acid contents between 190 and $282\,\mu g\,g^{-1}$ (i.e. equivalent to about 26 and $39\,\mu g\,g^{-1}$ of benzoate in fresh yoghurt), and only one sample contained $233\,\mu g\,g^{-1}$ of sorbic acid (i.e. about $32\,\mu g\,g^{-1}$ in fresh yoghurt) (Arimoto *et al.*, 1987; see also Serrano *et al.*, 1991).

In Turkey, ayran (i.e. a cultured milk beverage) containing $0.06\,g\,100\,g^{-1}$ of sorbic acid had an extended shelf life of up to 70 days under refrigerated storage (Oysun, 1988). However, although the use of Na-benzoate and K-sorbate at different rates extends the keeping quality of yoghurt and has a minimal effect on the sensory character, in some instances reduced starter culture counts at the end of the storage period have been noted (Sanyal *et al.*, 1990; Rajmohan and Prasad, 1994; Souad *et al.*, 1994). Reviews by Sieber *et al.* (1995) and Horak *et al.* (1997) highlighted the state-of-the-art view of benzoic acid as a naturally occurring preservative in cultured dairy products and cheese and illustrate the possible metabolic pathways for its formation.

An alternative approach to extend the keeping quality of yoghurt is the addition of Nisin which is a natural bacteriocin produced by certain species of *Lactococcus lactis* subsp. *lactis*. The sensitivity of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* has been studied by many researchers. It is safe to conclude that maximum inhibition of the yoghurt organisms occurs in milk containing $100–200\,RU\,ml^{-1}$ of Nisin (Naguib *et al.*, 1985a; Lee and Kim, 1985a, b; Gupta and Prasad, 1988, 1989a–c; Kebary and Kamaly, 1991). However, Bossi *et al.* (1989) reported that, while *S. thermophilus* was insensitive to Nisin (i.e. up to $5\,RU\,ml^{-1}$), the same concentration inhibited *L. delbrueckii* subsp. *bulgaricus*, a result that suggests strain sensitivity among these strains of lactobacilli. Nisin-producing organisms (i.e. $1000\,IU\,ml^{-1}$) were used to produce dahi in India, but the bacteriocin did not inhibit the proliferation of yeasts and moulds during the storage period (Rajmohan and Prasad, 1995a, b).

One preservative for yoghurt, which is likely to become important in the next few years, is natamycin or primaricin as it is often called. It was originally derived from *Streptomyces natalensis* and it is a polyene antibiotic that offers, in the present context, the following attractions:

- It is biocidal against yeasts and moulds, unlike sorbic acid which merely inhibits growth.
- It has no effect on the bacteria of the starter culture.
- It is sufficiently thermostable to withstand addition to the yoghurt milk prior to heating at 95°C for 7–10 min.
- It is sufficiently acid stable to withstand pH 4.0 for two/three weeks.

Delvocid (Gist-Brocades, Delft, Holland) is the commercial form of the fungicide, and each gram contains 500 mg of natamycin. Dosages of 10–20 mg kg^{-1} of natamycin have been shown to be effective in preventing microbial spoilage of yoghurt for up to 4 weeks and no interference in the fermentation process or the survival of the lactic acid bacteria was observed (Robinson, unpublished data).

As with sorbic acid, the optimum activity of natamycin lies in the acid range, but whereas the activity of potassium sorbate outside this range is limited, that of natamycin is less pH dependent. The activity of natamycin is based on its reaction with ergosterol, a compound in the cell wall membranes of yeasts and moulds. Due to this reaction, the cell membrane disrupts, leading to leakage of intracellular liquids and salts and eventually to death of the cell. As bacteria do not have these sterol-like compounds in their cell wall membranes, natamycin does not exert any antibacterial activity.

At present, natamycin can be used legally to prevent the growth of moulds on the rind of cheese, but it has not received regulatory approval for use in yoghurt. However, this situation may well change, since Brik (1981) states that natamycin is not absorbed from the human intestinal tract even if consumed at a rate of 500 mg day^{-1} and even an intake of 1000 mg day^{-1} only produced symptoms of nausea and diarrhoea. The LD$_{50}$ for the oral administration of natamycin to rats is 1500 mg kg^{-1} body weight. Obviously it is important that the recommended daily intake (RDI) is only 0.3 mg kg^{-1} body weight (FAO/WHO, 1990), but it is not clear why the figure is so conservative. Nevertheless, it should be noted that this figure only implies that a man of eleven stone should not consume more than 20 mg of natamycin day^{-1} and, with a proposed inclusion rate of 15 mg kg^{-1} of yoghurt, few consumers are likely to exceed the recommended RDI.

Yet other preservatives used in yoghurt making include (a) K-nitrite which inhibits the growth of lactococci and streptococci (Naguib *et al*., 1985b), (b) nitrite or nitrates reduce acid development and viscosity of yoghurt (Baranova *et al*., 1996, 1997; see also Steinka and Przybylowski, 1997), (c) lysozyme inhibits the growth of lactobacilli but not *S. thermophilus* (Kontova and Prekoppova, 1990) and (d) Microgard^{TM} can inhibit the growth of yeasts and moulds in yoghurt at concentrations ranging between 0.5 and 10 g 100 g^{-1} (Weber and Broich, 1986; Salih and Sandine, 1990). However, in some instances ethyl carbamate, which can exhibit carcinogenic activity in laboratory animals, may be present in yoghurt as a result of fermentation and/or the conversion reaction of diethyl pyrocarbonate to ethyl carbamate and levels reported ranged between <0.1 and 4.3 µg kg^{-1} (Canas *et al*., 1989; Hasegawa *et al*., 1990; Sen *et al*., 1993). Such levels of ethyl carbamate in yoghurt do not constitute any health risk to consumers.

Since all these types of preservative may be obtained in a powder form, they are added to the yoghurt milk with the rest of the dry ingredients; heat treatment of the milk does not affect their stability. However, in order to obtain maximum benefit from the preservative, the yoghurt must be of good quality and hence it is arguable whether its use is ever really justified.

2.7.3 Minerals, vitamins and/or fatty acids

2.7.3.1 Fluoridisation

Assali and White (1985) investigated fluoridisation (i.e. $4\,\mu g\,g^{-1}$) of yoghurt milk and no significant differences from the control were observed. Frank and Christen (1985) reported that the growth of lactic acid bacteria was not significantly affected in milk supplemented with Na-fluoride. This approach means that dairy products including yoghurt could be used as vehicles to provide children with additional fluoride in areas where water fluoridation is not practical. However, yoghurt producers would be well advised to note that the whole subject of fluoridation is extremely controversial. Thus, while few people object to the topical application of fluoride in toothpaste, the ingestion of fluoride in drinking water or in a food like yoghurt can, in some circumstances, be detrimental to health. For example, severe discoloration of teeth and damage to skeletal bones have been reported even with fluoride intakes within permitted guidelines and, given the healthy image of yoghurt, it would be most undesirable if the public perception of the product was damaged by a trendy gimmick.

2.7.3.2 Fatty acids

Caprioc or palmitic acids added at a rate of $0.01\,g\,100\,g^{-1}$ could not be detected as an off-flavour in pasteurised and homogenised milk, but caproic could be detected in dahi (Pantulu *et al.*, 1993). It has been noted also that modifying the feed of cows or the addition of soy oil to yoghurt milk did not affect the activity of the starter culture (Zbikowski *et al.*, 1982). The production of vegetable oil yoghurt is discussed in detail in Chapter 5.

2.7.3.3 Vitamins

Fortification of yoghurt by vitamins is targeted at children and has been marketed in some countries (Anon., 1983a). The stability of vitamins A and C in yoghurt was evaluated by Ilic and Ashoor (1988), Fiedlerova *et al*. (1993) and Noh *et al*. (1995). Both vitamins decreased during the storage period. This effect was minimised by using water-miscible beadlets of β-carotene (Parker *et al.*, 1992).

2.7.3.4 Low sodium

Low sodium yoghurt has become increasingly important for its nutritional properties and as a physiologically functional food. The milk base is processed in a cation exchange unit containing a strong acid resin (Nakazawa *et al.*, 1990). The quality of yoghurt made from low sodium milk was similar to the control and the reduced sodium level did not affect the activity of the starter culture. However, the sodium and potassium contents were reduced or increased, respectively, when compared with milk as follows: from 540 to $63\,\mu g\,g^{-1}$ and 1530 to $2360\,\mu g\,g^{-1}$, respectively. The

application of this approach to other cultured dairy products has been reviewed by Petik (1987).

2.7.3.5 Modified mineral content

Modification of the mineral content in yoghurt has been reported by many researchers. Some examples include the following processes on the milk base: (a) reducing the calcium content to 50% and enriching with magnesium up to $1\,g\,l^{-1}$ (Pechery, 1985), (b) increasing the iron level in yoghurt by using iron tanks to ferment the milk (Coutsoucos and Colli, 1995; Batilde-Lima *et al.*, 1995) or adding iron to the milk base (Hekmat and McMahon, 1997), (c) increasing the calcium content in yoghurt using Ca-gluconate (Flinger *et al.*, 1988; Hansen and Flinger, 1996) and (d) the growth of *S. thermophilus* was reduced in calcium fortified milk and by using a higher inoculum, the rate of acid development was restored (Yousef and Rusli, 1995).

2.8 Homogenisation

Homogenisation means, quite literally, the provision of a homogeneous emulsion between two immiscible liquids, for example, oil/fat and water. The types of emulsion that may exist in dairy products are divided into two categories:

- Oil-in-water emulsion where the oil droplets are dispersed in the aqueous phase – the majority of homogenised dairy products fall into this category.
- Water-in-oil emulsion where the water droplets are dispersed in the oil phase – a typical example is butter.

Yoghurt milk is a typical oil-in-water emulsion and, as a result, the fat has a tendency to separate upon standing (especially in the fermentation tanks during the incubation period). In order to prevent this, the milk base is subjected to high speed mixing or homogenisation, that is, forcing the milk under high pressure through a small orifice or annulus. The overall relevance of this process to the manufacture of yoghurt is illustrated in Table 2.13.

However, these general effects are a reflection of the impact of homogenisation on specific milk constituents and in particular the effects described in the following Sections 2.8.1 and 2.8.2.

2.8.1 Effects on milk constituents

It is well established that the diameter of the fat globules in milk ranges from 1 to $10\,\mu m$, with an average around $3.5\,\mu m$. This variation in globule size is directly dependent on the same factors that influence the chemical composition of milk (i.e. breed of the cow, stage of lactation, age and health of the cow, type of feed, etc.). The effect of homogenisation is to reduce the average diameter of the fat globules to $< 2\,\mu m$ (see Fig. 2.11), to prevent cluster formation and the tendency of the fat to rise to the surface and to decrease agglutination and elective bouyancy, due to the adsorption of casein micelles and submicelles.

The proteins in milk (casein and whey/serum protein) may undergo one or more of the following changes: (a) denaturation of some serum protein may occur, (b) casein/whey protein interactions may take place as result of denaturation of the

Table 2.13 Physical–chemical changes caused by homogenisation of milk used for yoghurt manufacture

Effect of homogenisation	Changes related to yoghurt
Increase	
Viscosity	Reduction in fat globule size and increased adsorption onto the casein micelle which increases the effective total volume of suspended matter.
Xanthin oxidase activity	Due to the disruption of the fat globule membrane which contains about half of the enzyme present in milk.
Colour (whiter)	Increase in number of fat globules which affects light reflectance and scattering.
Lipolysis	Increase in total fat surface area available to lipases; destruction of fat globule membrane which may enhance lypolysis by the starter culture.
Proper mixing	Especially if milk is fortified with milk powder.
Phospholipids in skimmed milk	As a result of the physical action, more fat globule membrane material is transferred to skimmed milk.
Foaming	As a result of increased phospholipids in the skimmed milk phase, pumping of yoghurt milk can cause foaming in the incubation tanks.
Decrease	
Fat globule size	Prevents 'cream line' formation in yoghurt, especially during incubation.
Oxidised flavour	Due to the migration of phospholipids to skimmed milk phase and formation of sulphydryl compounds which act as antioxidants; possibly through denaturation of whey proteins causing exposure of hidden SH groups.
Protein stability	Changes in protein–protein interaction as a result of some denaturation and shift in salt balance.
Agglutination and effective buoyancy	Decrease in fat clustering due to adsorption of casein micelles and submicelles to fat globules.
Casein in skim phase	Partial transfer to casein from skimmed milk to form a new membrane around newly formed small fat globules (see Fig. 2.11).
Syneresis	Increase in hydrophilicity and water-binding capacity due to casein fat globule membrane interaction and other protein–protein interactions.

Adapted from Brunner (1974), Mulder and Walstra (1974), Harper (1976).

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latter type of protein and/or a shift in the salt balance and (c) production of sulphydryl compounds from denatured whey proteins may be observed. However, the effects and/or changes on the miscellaneous milk constituents are documented in Table 2.13, and these desirable effects of homogenisation can only be achieved if certain processing conditions are observed, namely:

- correct level of fat in the process mix
- correct homogenisation pressure
- correct temperature of homogenisation.

Before processing any type of mammalian full fat milk, there are no interactions between the major milk components, that is, the proteins (β-lactoblobulin (β-Lg),

Fat globule

100 - 10,000 nm **Membrane** **10 nm**

Glycerides		water	100 mg
triglycerides	38 g	proteins	350 mg
diglycerides	0.10 g	phospholipids	200 mg
monoglycerides	10 mg	cerebrosides	30 mg
Fatty acids	60 mg	glycerides	>100 mg
Sterols	0.10 mg	fatty acids	15 mg
Carotenoids	0.30 mg	sterols	15 mg
Xanthophylls	1 mg	other lipids	
Vitamins A,D,E,K		enzymes	
Water	60 mg	alk. phosphatase	
Miscellaneous		xanthine oxidase	
lactones		some others	
coumarin		Cu	4 µg
others		Fe	100 µg
		Mo	+

Membrane (2-3% casein)

Fat globule (1-10 µm, diameter)

Homogenisation

Normal homogenised fat globule; casein 24% of membrane protein (0.1-3µm, diameter)

10-20% Fat interacts with casein to give high density complex

Partially hydrophobised fat globule, rarely found in well homogenised milk

Partial transfer of phospholipids

Partial transfer of casein

Skim milk

Fig. 2.11 Fat globule structure, composition and schematic representation of the effect of homogenisation on size
$1\,mm = 10^3\,\mu m = 10^6\,nm = 10^7\,Å.$

Adapted from Mulder and Walstra (1974), Harper (1976) and Harper and Sieberling (1976).

α-lactalbumin (α-La) and the caseins), the fat and lactose (Walstra and Jenness, 1984). The fat constituent in raw milk is encapsulated within a membrane made of protein, lipids and phospholipids (Mulder and Walstra, 1974). Heat-induced and high pressure-induced processes cause chemical and physical changes in the milk fat globules. The chemical changes involve the fatty acids residue, but the effect of homogenisation and heating results in complex interactions between the milk components. Mulder and Walstra (1974), Dalgleish and Sharma (1993), Sharma and Dalgleish (1994), McCrae and Muir (1991), McCrae (1994), McCrae *et al*. (1994), Tomas *et al*. (1994), van Boekel and Walstra (1995), Dalgleish *et al*. (1996) and Sharma *et al*. (1996) have reviewed the effect of these physical changes on the quality of many dairy products and possible changes applicable to homogenisation before heat treatment are:

- Break up of the fat globules (1–10 μm) to give particle sizes in the sub-micron range.
- Some of the casein micelles break up and bind with newly formed fat globules to stabilise them.
- Serum proteins have a relatively minor role, but some may interact with the fat globules in the absence of heating.
- The fat particles in the homogenised milk have a different structure from the native fat globule, and hence they have different properties with respect to coagulation of milk by enzymes or heat; the enzymes are relevant in cheesemaking.

However, when the milk is heated, the induced interactions are more significant (for details refer to Section 2.9) and can be summarised as follows:

- Denaturation of α-La and β-Lg takes place, with subsequent interactions, principally of the β-Lg whey/serum protein.
- β-Lg becomes more reactive after denaturation due to the presence of unpaired sulphydryl (SH) groups.
- Possible reactions of denatured β-Lg include interaction with other β-Lg, interaction with κ-casein on the surface of casein micelles and interaction with fat globule membrane which results in an approximate doubling of the amount of fat-bound protein.

Thus, heating of homogenised milk ≥70°C results in new structures being formed, mainly the denatured whey/serum proteins which may undergo further reactions such as:

- interaction with other denatured β-Lg to form a gel;
- interaction with κ-casein on the surface of micelles in suspension;
- interaction with κ-casein adsorbed onto the fat globules;
- interaction with residual fat globule membrane;
- adsorption onto the fat globule surface, i.e. displacing the adsorbed caseins.

2.8.2 Aspects of processing
The use of single stage or double stage homogenisation is only critical in products containing high levels of fat (e.g. cream) and since the fat in cream has a tendency to recluster, double stage homogenisation is recommended. However, yoghurt milk is usually processed through a single stage homogeniser at around 65–70°C and at

pressures ranging between 15 to 20 MPa. Pressures up to 30 MPa have been reported, but in practice they are not widely used. Kebary and Morris (1989) studied the effect of homogenisation (i.e. two stage up to 27.6 + 3.5 MPa) on fat clustering and the distribution of fat globules in recombined milks, and these effects increased as the homogenisation pressure and fat content increased. According to the review by Tamime and Marshall (1997), the effect of homogenisation of the yoghurt milk and subsequently the quality of the manufactured product are that: (a) the fat surface area is increased, the size of the globule is decreased and the composition of the membrane is different, (b) in part, the fat surface is coated with surface-active materials, mainly proteins, (c) the turbulent effect of homogenisation favours the adsorption of casein micelles over serum proteins (*c*. 5%), so covering 25% of the surface area of the fat globule, (d) in recombination (i.e. the milk fat is homogenised into the skimmed milk) the resulting fat globule membrane consists only of serum protein, (e) the homogenised fat globules act as large casein micelles (i.e. because the membrane consists mainly of caseins) which increase the effective casein concentration, and hence, participate in casein reactions such as acid precipitation, (f) the increased number of small fat globules enhances the ability of the milk to reflect light and, as a result, the fermented milk appears whiter and (g) the risk of syneresis (i.e. separation of free whey onto the surface of set fermented milk) is reduced, and the firmness of the end product is increased giving it a better mouthfeel.

In some instances, homogenisation of the yoghurt milk takes place after heat treatment of the milk base, but this approach carries with it the risk of contamination unless high standards of hygiene are observed and/or an aseptic homogeniser is used. Kulkarni *et al*. (1990c, d) reported that when using 30% WPC (about 25 g TS 100 g^{-1}) for the production of cream yoghurt (10 g fat 100 g^{-1}), the following process could be recommended: (a) improving the product by adding 2% SMP, (b) heating the milk to 95°C for 22 s to achieve 70% denaturation of β-Lg and (c) finally homogenising at 75°C and 20 MPa pressure. However, in separate reports, Plock *et al*. (1992) and Huss and Kessler (1991) evaluated different processing parameters (e.g. casein-to-whey ratio, one-stage homogenisation at pressures ranging between 5 and 30 MPa or homogenising the milk base many times before or after the heat treatment stage at 95°C for 80 s), and they concluded that maximum gel consistency and water-holding capacity of yoghurt was achieved with homogenisation at ≤25 MPa pressure after heat treatment. Whether or not the homogenisation of milk after heating is beneficial to product quality has, of course, to be verified further. Thus, when a batch of cow's milk with 16% TS (vacuum evaporation) was divided into two and homogenised either before or after heating, the textures (set yoghurts) and viscosities (stirred yoghurts) were identical (Robinson, unpublished data). Perhaps the method of fortification is critical and/or the precise temperature regime, but clearly the situation is in need of further clarification. Ozer (unpublished data) observed a similar pattern for both cow's and sheep's milks, but homogenisation after heat treatment did improve the textural properties of goat's milk yoghurt. Nevertheless, the improved viscosity of yoghurt that is reported to follow homogenisation of the milk is due primarily to:

- a change in the water-holding capacity of the milk proteins which tends to reduce syneresis (Grigorov, 1966a; Kessler and Kammerlehner, 1982; Kneifel and Seiler, 1993);

- the increased amount of milk fat globule membrane material, i.e. phospholipids and proteins in the skim phase, which may also improve the water-holding capacity of the coagulum (Samuelsson and Christiansen, 1978);
- the rate of acidification of the milk which was increased by increasing the pressure of homogenisation (i.e. 0–15 MPa) (Volkova and Radulov, 1986).
- the curd tension of the bio-yoghurt which was influenced significantly ($P \leq 0.01$) by the level of SNF (18 g 100 g^{-1}) and two-stage homogenisation (pressures of 14.6 and 3.5 MPa), while the fat content (4.5 g 100 g^{-1}) also affected the characteristics of the curd ($P \leq 0.05$); however, holding times of the heated milk at 90°C had no effect (Asgar and Thompkinson, 1994).
- improvements in the physical properties of yoghurt which were achieved by fortification of the milk using UF method, heating of the milk between 100 and 120°C for 4 or 16 s and two-stage homogenisation (14.2 and 3.5 MPa, respectively) at 55°C after heating the milk (Savello and Dargan, 1995).
- yoghurt milk homogenised at 0, 10.3 and 34.5 MPa pressures which showed differences only in syneresis and water-holding capacity (Schmidt and Bledsoe, 1995).
- replacement of the milk fat with vegetable oils or fat substitutes which affects the rheological, sensory characters and the microstructure of yoghurt (for more detail refer to Chapter 5).

In addition, the processing conditions (temperature and pressure) employed during the homogenisation of the milk base can affect the extent of any changes (Misra, 1992). Storgards (1964) produced an increase in the viscosity of sour milk by progressively increasing the pressure (i.e. from about 5 to 30 MPa) of homogenisation without heating the milk; a similar trend was also reported for milk subjected to heat treatment (Fig. 2.12). This effect was previously reported by Galesloot (1958) and a summary of his results is presented in Table 2.14. Abrahamsen and Holmen
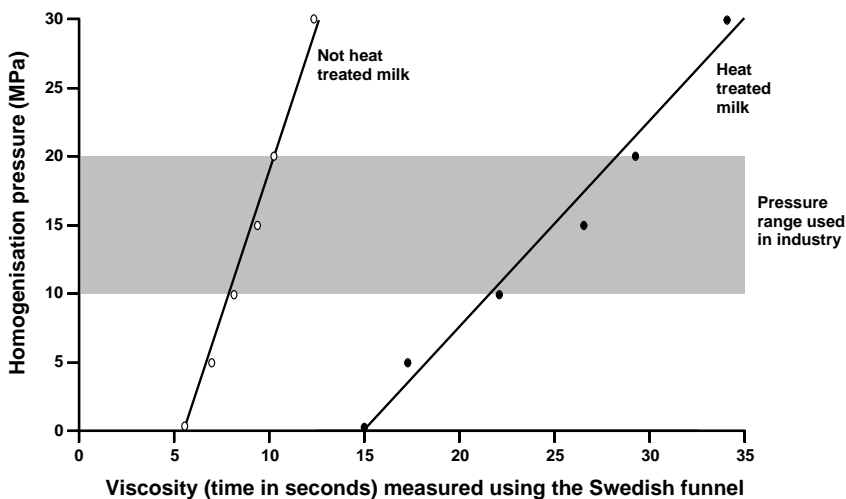
Fig. 2.12 Effect of different homogenisation pressures on the consistency/viscosity of sour milk

Data compiled from Storgards (1964).

Table 2.14 Effect of homogenisation and heat treatment on the consistency/viscosity of yoghurt

Measurement of consistency/viscosity of yoghurt	Heat treatment of milk for 30 min at							
	70°C		78°C		86°C		95°C	
	A	B	A	B	A	B	A	B
Falling sphere[a] (depth, cm)	3.0	>15.0	1.5	10.5	1.2	6.0	1.2	2.7
Posthumus funnel[b] (time, s)	9.0	5.0	14.0	7.5	17.0	8.5	18.7	9.0

A, Homogenised milk; B, non-homogenised milk.

[a] Falling sphere: the deeper the sphere sinks into the yoghurt, the thinner is the product. [b] Posthumus funnel: the longer the time required for the yoghurt to pass through the funnel, the more viscous is the product.

Data compiled from Galesloot (1958).
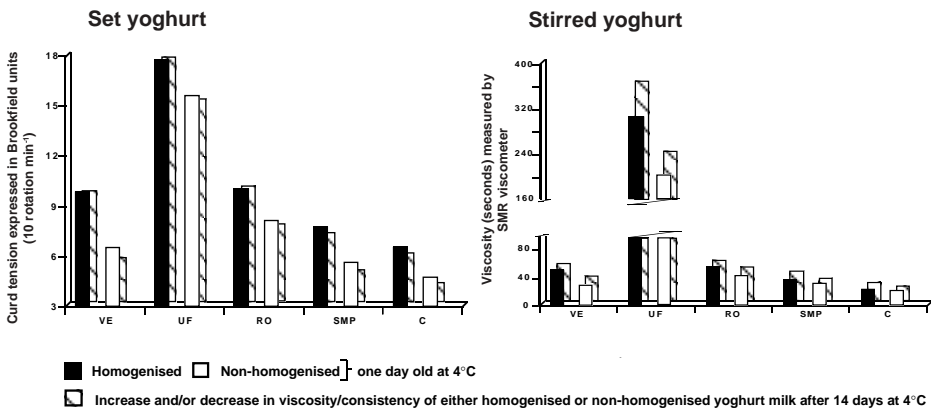
Fig. 2.13 Viscosity/consistency of goat's milk yoghurt (homogenised and non-homogenised) concentrated by different methods and stored for 1–14 days at 4°C

VE: Vacuum evaporation, UF: ultrafiltration, RO: reverse osmosis, SMP: skimmed milk powder, C: control.

Data compiled from Abrahamsen and Holmen (1981).

(1981) studied the quality of goat's milk yoghurt manufactured from homogenised and non-homogenised milks concentrated by different methods and they concluded that: (a) homogenisation of goat's milk was essential for yoghurt production (Fig. 2.13), (b) a reduction in the consistency of set yoghurt was reported after 14 days' storage and the best results were obtained when the goat's milk was concentrated using ultrafiltration and (c) goat's milk yoghurt had a lower viscosity than yoghurt made with cow's milk due to the low protein content of the goat's milk (Abrahamsen and Holmen, 1980, 1981). A similar observation was reported by Muir and Tamime (1993) on the firmness of sheep's milk yoghurt made from homogenised and non-homogenised milks without fortification. For more details refer to Chapter 5.

2.9   Heat treatment

Although the application of heat, that is, boiling of milk, has long been practised during the manufacture of yoghurt as a method of increasing the concentration of milk solids in the milk base, in the present context the effects of heat treatment can be broadly summarised as:

- destruction and/or elimination of pathogens and other undesirable micro-organisms;
- production of factors stimulatory/inhibitory to the yoghurt starter cultures;
- changes in the physicochemical properties of the milk constituents which are relevant in yoghurt making.

In commercial practice, heating of milk is the most widely used unit operation in the manufacture of a wide range of dairy products. The time/temperature combinations applied range from ≤65°C (thermisation) for a few seconds to 150°C for a few seconds for ultra high temperature (UHT) sterilisation. Milk for the manufacture of yoghurt is heated at different temperatures and the reported treatments, including the processing of liquid milk, are illustrated in Table 2.15. The choice of any one particular time–temperature combination is based on a number of factors, but assuming that there are no limitations imposed by the plant itself, those mentioned above tend to be the dominant considerations.

Table 2.15   Some time/temperature combinations used during the processing of liquid milk and yoghurt milk base

Time	Temperature (°C)	Process	Comments
Few s	≤65	Thermisation	Main purpose is to kill psychrotropic bacteria; it causes no other irreversible changes.
30 min 15 s	65 72	Batch pasteurisation Pasteurisation	Destruction of almost all pathogenic organisms present in milk, but not all vegetative cells of micro-organisms are killed; inactivation of some enzymes; flavour and whey proteins remain unchanged.
4–20 s 30 min[a] 5 min[a]	85 85 90–95	High pasteurisation	Destruction of all vegetative cells, but not bacterial spores; most enzymes are destroyed, but not milk and bacterial proteinases or bacterial lipases; denaturation of whey proteins.
40–20 min 20–2 s	110–120 135–150	In-container sterilisation and autoclaving UHT	Destruction of all micro-organisms and spores; some UHT treatment may not suffice to inactivate all enzymes; chemical changes, colour and flavour of milks are affected.

[a] Heat treatments that are widely used in the yoghurt industry.

Data compiled from Davis (1968), Ged and Alais (1976), Lyster (1979), Walstra and Jenness (1984) and Fox (1989, 1995).

Table 2.16 Chemical and physical effects of heat treatment of milk and their relevance in yoghurt manufacture

Milk constituent	Heat-induced changes	Relevance in yoghurt manufacture	Consequences for yoghurt
Nitrogenous			
Whey proteins	Denaturation and aggregation, inactivation of immunoglobulins	Almost complete	Destruction of lactenins, reduction in creaming ability
	Active –SH group production	Maximum at 90°C/10 min	Cooked flavour, lowering of Eh, formation of antioxidant properties
	α-La and β-Lg interaction	Occurs before and/or interaction with κ-casein	Contributes to gel stability
	β-Lg and κ-casein interaction	Very significant	Minimises syneresis, increases micelle size, stabilises gel
Casein	Partial hydrolysis, release of glycopeptide from κ-casein	Of limited significance	Slight increase in free amino acids and peptides
	Dephosphorylation	Very little	Slight redistribution of phosphorus
	Aggregation, disaggregration, interchain cross-linking, e.g. by isopeptide bonding	Occurs especially with smaller micelles	Increase in micelle size and formation of protein network
Enzymes	Inactivation	Destruction of lipases and proteases from milk and bacteria	Minimises rancid and bitter off-flavours
Other	Decomposition of amino acids to flavour compounds	Significant effect	Contributes to flavour
	Amino acid–lactose interaction, Maillard reaction, Schiff's base formation, reduction in available lysine	Occurs to only small degree, e.g. lysine loss *c.* 0.3%	Slight decrease in nutritive value, significant where yoghurt fortified with high-heat powders and concentrates
	Amino acid–amino acid interaction, e.g. formation of lysine–alanine	Occurs to a limited degree	Minimal

Carbohydrates			
Lactose	Decomposition to form organic acids, furfural and hydroxymethylfurfural	Occurs to small extent	Reduces pH and Eh, produces formic acid and affects growth of starter cultures, contributes to yoghurt flavour
	Reaction with amino acids (see above)		
Other	Decrease in sialic acid and hexosamines, increase in hexoses	Occurs at 85°C for 10 min	Unknown
Miscellaneous			
Fat	Formation of lactones, methyl ketones and other volative ketones	Occurs to small degree	Contributes to flavour
	Hydrolysis	Insignificant	Insignificant
Vitamins	Destruction of some water-soluble vitamins	C, B_1, B_6, B_{12}, folic acid, reduced	Reduction in nutritive value
Minerals	Redistribution of Ca, P, Mg between soluble and colloidal forms	Significant effect, modifies surface structure of casein micelle	Reduces pH, affects curd particles, decreases coagulation time
Micro-organism	Destruction	Elimination of pathogens and other organisms	Ensures public safety and minimises quality defects
Gases	Reduction in level of dissolved oxygen, nitrogen and carbon dioxide	Produces micro-aerophilic environment for starter culture	Ensures public safety and minimises quality defects

Compiled from Tamime and Deeth (1980). Reprinted with permission from *Journal of Food Protection*.

Thermal treatment of milk has been extensively studied in relation to many aspects such as heat-induced changes to milk constituents, changes in nutritional properties of milk, inactivation of enzymes (indigenous and/or of bacterial origin) and functional properties of dairy products (e.g. heat stability of UHT milk, evaporated milk and milk powder) (see Table 2.16); the reviews by Fox (1989, 1991, 1995) are recommended for further reading. During the manufacture of yoghurt, milk is heated at >70°C and the physical and chemical changes that can occur in the milk base are complex and multifunctional. The impact of thermal processing relevant to the functional properties of yoghurt is summarised below.

2.9.1 Destruction of micro-organisms/pathogens

The heat treatment of the yoghurt milk at 85–95°C (Table 2.15) is sufficient to kill the majority, if not all, of the vegetative cells of micro-organisms associated with raw milk (Gilmour and Rowe, 1990), but spore formers and some heat-stable enzymes will remain. This reduced competition ensures that the heated milk will provide a good growth medium for the yoghurt starter culture, but nevertheless, the bacteriological quality of the raw milk and any dry ingredients used in the milk base is of great importance.

Thus, a high level of psychotrophic bacteria can break down both β-casein and α_s-casein (DeBeukellar *et al.,* 1977) and the fat constituents in milk, and while the degradation of casein can lead to the formation of a weak coagulum and subsequent whey separation, hydrolytic rancidity can give rise to serious off-flavours (Cousin, 1977; Cousin and Marth, 1977a, b). It is also important that the enzymes (peptide hydrolases and lipases) of some *Pseudomonas* spp. are heat stable, and extremely high heat treatments (150°C) are required to inactivate them (Mayerhofer *et al.,* 1973; Kishonti, 1975; Adams *et al.,* 1975; Barach *et al.,* 1976, 1978; Hedlung, 1976; Adams and Brawley, 1981; Fairbairn and Law, 1986; Stead, 1986; McKellar, 1989; Driessen, 1989; Stepaniak and Sørhang, 1995).

Indigenous enzymes (*c*. 60) have been identified in raw milk and some of these enzymes are heat labile, while others can survive the UHT treatment of milk. The role of these enzymes in dairying has been critically reviewed by Fox (1991) and Farkye and Imafidon (1995). The activities of milk enzymes have been useful indicators of diseases or physiological changes in the udder of the mammal, of processing conditions applied to milk and of factors influencing the flavour and quality of dairy products. Fortunately, the survival of these enzymes has not been identified as a significant problem in the yoghurt industry (Cogan, 1977).

2.9.2 Production of stimulatory/inhibitory factors

The heating of milk can result in the release of certain factors that can either stimulate or inhibit the activity of lactic starter cultures. The work of Greene and Jezeski (1957a–c) summarises the overall events:

- stimulation of the starter culture in milk heated between 62°C for 30 min and 72°C for 40 min;
- inhibition of the starter culture in milk heated between 72°C for 45 min and 82°C for 10–120 min or 90°C for 1–45 min;
- stimulation of the starter culture in milk heated between 90°C for 60–180 min and autoclaving at 120°C for 15–30 min;

- inhibition of the starter culture in milk heated by autoclaving (120°C) for more than 30 min.

The apparent stimulation/inhibition/stimulation/inhibition cycle was due to changes in the serum or whey proteins and the above cycle could be simulated by the addition of denatured whey proteins or cysteine hydrochloride. The transition from one cycle to another, in response to different heat treatments, could well reflect the release of denatured nitrogenous compound(s) (e.g. at concentrations of 0.15–0.20 mg ml^{-1}) or from 10 to 20 μg ml^{-1} of cysteine, since when cysteine was added artificially, it augmented the sulphydryl groups made available by heating; cysteine became stimulatory in raw and low heated milks, but in highly heated milks the same concentration became inhibitory. Taking this idea further, the same workers offered the following explanation for the stimulation/inhibition cycles:

- The initial stimulation was attributed to the multitude of factors listed in Table 2.16.
- The release of cysteine, glutathione or thioglycolate and the expulsion of oxygen resulted in the stimulatory effect.
- The inhibition was due to an excess concentration of cysteine in the milk, accompanied by an increase in toxic volatile sulphides.
- The second cycle of stimulation was due to a reduction in the level of toxic sulphides as a result of further heating, or perhaps the formation of formic acid.

Consequently, Greene and Jezeski (1957a–c) recommended the use of high-heat powders, but such observations may not be applicable at the present time in view of (a) developments in powder manufacture technology and (b) improved selection of starter culture strains. However, refer to Section 2.4.2 for further detail.

Dutta *et al.* (1973) investigated the effect of different heat treatments on acid and flavour production by various single strains of lactic acid bacteria, including *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* and a summary of their work is given in Table 2.17. Overall the degree of heating had a rather variable effect on the activity of the yoghurt starter cultures, but the reasons for this behaviour were not discussed; however, it is most likely that changes to certain milk constituents (Table 2.16) promoted the observed variation in the activity of the starter cultures.

Table 2.17 Effect of heat treatment of milk on the activity of yoghurt starter cultures

Value	*S. thermophilus*			*L. delbrueckii* subsp. *bulgaricus*		
	63°C/ 30 min	85°C/ 30 min	Steaming/ 30 min	63°C/ 30 min	85°C/ 30 min	Steaming/ 30 min
Titratable acidity[a]	1.00	0.85	0.66	1.60	1.70	1.62
Volatile acidity[b]	9.00	9.00	7.00	40.00	34.50	31.00
Diacetyl (μg g^{-1})[c]	13.00	12.00	6.00	12.00	13.00	0.00
Proteolytic activity[d]	0.34	0.25	0.18	0.25	0.18	0.09

[a] Lactic acid (%); [b] ml of 0.01 N NaOH 50 g^{-1} of curd; [c] Level of diacetyl is abnormally high (see Chapter 7); [d] mg of tyrosine liberated g^{-1} of curd.

After Dutta *et al.* (1973). Reprinted with permission of *Milchwissenschaft.*

2.9.3   Changes in the physicochemical properties of milk

Fresh liquid milk is composed of around $87\,g\,100\,g^{-1}$ water and $13\,g\,100\,g^{-1}$ total solids, and the composition of yoghurt milk (after being standardised and/or fortified) is slightly altered to 84–$86\,g\,100\,g^{-1}$ water and 14–$16\,g\,100\,g^{-1}$ total solids. It may appear from such data that milk is simple in its composition, but on the contrary, milk has a very complex structure (see Fig. 2.2), even though its constituents are mainly water, carbohydrates, fat, proteins and minerals. These different components appear to be dispersed between two colloidal systems, that is, the fat globules and their membranes (Fig. 2.11), and the casein micelle complexes. In general, both colloidal systems are heat stable, and the effects of heat treatment on them and the relevance of these to yoghurt manufacture are summarised in Table 2.16. It is apparent from this data that yoghurt milk undergoes several changes during the heat treatment.

2.9.3.1   Effect on the proteins

Some detailed studies of the proteins in cow's milk have been reported by Cheeseman (1975), Whitney *et al*. (1976), Eigel *et al*. (1984), Banks and Dalgleish (1990), Walstra (1990), Dalgleish (1990a), Farrell *et al*. (1990), Creamer (1991), Fox (1992), Jakob (1994), Fox and Flynn (1994) and Holt and Horne (1996). In addition, the interactions of milk components including the basic chemistry and the action of milk proteins in different food systems have been reviewed by de Wit (1990a), Jost *et al*. (1990), Creamer *et al*. (1994) and Pearce (1994). The various constituents that go to make up the total protein content of milk are:

Casein		76–88% of total protein
α_{s1}-	45–55	
β-	25–35	% of fraction indicated
κ-	8–15	
γ-	3–7	
Whey proteins		15–22% of total protein
Serum albumin	0.7–1.3	
β-lactoglobulin	7–12	% of fraction indicated
α-lactalbumin	2–5	
Immunoglobulin	1.9–3.3	
Proteose/peptones		2–6% of total protein

The casein in milk constitutes the major group of bovine proteins which play an important role during the manufacture of certain dairy products, for example yoghurt and cheese. The structures of these proteins, based on some models that have been proposed, suggest that the caseins exist as micelles or aggregates of sub-micelles which are basically formed from α_s- and β-casein stabilised by κ-casein in association with calcium and calcium phosphates (Banks and Dalgleish, 1990).

The other types of protein (i.e. serum or whey proteins) appear to be in solution, and they have a more defined, compact, globular shape than the caseins. This structure is due to the formation of disulphide bonds (as a result of the cysteinyl residues present), the lack of phosphate groups and the fact that they do not react with calcium or aggregate together in the native state (Banks and Dalgleish, 1990). The functional properties of the whey proteins become more apparent after heating the milk, since at temperatures above 80°C, they are denatured and react/bind with κ-casein to form a more stable micelle.

Table 2.18 Changes (%) in nitrogenous fractions of milk from different species after heating

Nitrogen fraction	Cow			Goat			Sheep		
	63°C/ 30 min	80°C/ 10 min	120°C/ 15 min	63°C/ 30 min	80°C/ 10 min	120°C/ 15 min	63°C/ 30 min	80°C/ 10 min	120°C/ 15 min
Casein	+0.60	+14.95	+18.77	0	+24.43	+25.31	+3.98	+26.35	+2.35
Non-casein	−1.61	−45.63	−57.77	0	−35.90	−41.81	−10.50	−68.06	−76.05
Soluble protein	−2.23	−62.40	−89.38	0	−58.08	−70.58	−15.07	−79.39	−93.96
β-Lactoglobulin	−1.80	−59.56	−94.30	0	NR	−100.00	−34.65	−74.25	−100.00
Non-protein	0	0	+25.00	0	+4.76	+4.76	+11.36	+15.00	+15.00

NR: not reported.

Adapted from Ramos (1978).

A good example of this effect is observed when milk is heated to 90°C (fore-warmed) for a period of time that ensures complete reaction between the different types of proteins, since it can then be heated to 120–140°C to give a stable end product (e.g. UHT milk). Comparative data for the effect of heat on the milk proteins (including caseins) of different species are illustrated in Table 2.18.

Caseins, as mentioned elsewhere, are heat stable when compared with whey proteins. Thus, β-Lg and α-La are denatured at the temperatures employed for the processing of the milk base (Dannenberg and Kessler, 1988a, b; de Wit, 1990b; Pearce, 1994; Law, 1995), and while β-Lg reacts with other milk components when denatured, β-La undergoes heat-induced interactions only after severe heat treatment (Dalgleish and Sharma, 1993; Sharma and Dalgleish, 1994). The possible interactions are:

- The association of small aggregates of denatured β-Lg molecules to form larger aggregates (Xiong *et al.*, 1993), or as a function of pH and temperature (MacLeod *et al.*, 1995).
- The interactions between β-Lg and κ-casein as a result of heating the milk involve hydrophobic interactions of exposed SH groups (Haque and Kinsella, 1988; Noh and Richardson, 1989; Dalgleish, 1990b).
- During the heating of milk at ≤90°C, the interactions of β-Lg and α-La with the casein micelles have similar kinetics but when heating the milk in UHT system, α-La reacts more slowly than β-Lg due to the rate of heat transfer (Corredig and Dalgleish, 1996a, b).
- Heat treatment may extensively modify one of the fat globule membrane proteins, *c.* 49 kDa (Kim and Jimenez-Flores, 1995) and, as a consequence, the interactions between denatured whey proteins and the fat globule membrane proteins may not be explained solely by —SS linkages.
- The attachment of κ-casein onto the surface of the fat globule membranes results in losses of triacylglycerols and changes in lipid content upon heating milk at 80°C for 20 min (Houlihan *et al.*, 1992a, b; Singh, 1993; van Boekel and Walstra, 1995).
- Interaction of β-Lg with the homogenised milk fat globule surface may displace the adsorbed micellar caseins (Xiong and Kinsella, 1991a, b; Dalgleish and Sharma, 1993; Sharma and Dalgleish, 1993, 1994).

Table 2.19 Effect of heat treatment on the coagulation process during the manufacture of yoghurt

Item	Heat treatment of milk					
	85°C	85°C/30 min	90°C	90°C/30 min	95°C	95°C/30 min
Coagulation time (hour)	2.43	2.01	2.34	2.04	2.29	2.04
Acidity at coagulation (% lactic acid)	0.63	0.49	0.63	0.50	0.63	0.50
pH at coagulation	4.70	5.16	4.78	5.12	4.80	5.08

Adapted from Grigorov (1966b).

- Binding of colloidal calcium phosphates and other ions by the caseins; this shift in the ionic constituents is not critical in acid gel formation (Schmidt and Poll, 1986; Aoki *et al*. 1987a, b, 1988, 1990; Wahlgren *et al*., 1990; Holt, 1995; Zhang and Aoki, 1995).
- Aggregation of casein micelles into larger particles and also dissociation of casein micelles to form soluble caseins at 100°C or above (Singh, 1993; Law, 1996).

It is evident that heat-induced changes of the proteins in milk, and subsequently acid development (see Section 2.10.3 on gel formation), can affect other properties. For example, the optimum hydrophilic properties of the proteins are obtained when the milk is heated to 85°C for 30 min (Grigorov, 1966a–c). The effect of different heat treatments on the coagulation of cow's milk is shown in Table 2.19. The observed improvements in the rate of gel formation are possibly due to interactions between β-Lg and casein, since heating milk at 80°C for 30 min denatures more than 90% of the β-Lg compared with only 60% of the α-La (Larson and Rolleri, 1955). Maximal hydration of the protein, according to Grigorov (1966c), occurs when milk is heated at 85°C and decreases gradually as the temperature rises; this view is shared by many researchers, including Prodanski (1967) and Iyengar *et al*. (1967). This decrease in the hydrophilic properties of the casein/β-Lg complex can adversely affect the quality of the yoghurt, possibly increasing the tendency to syneresis, and hence, ignoring other considerations, the heat treatment of milk intended for the production of yoghurt should be between 85°C and 95°C.

The effect of heat on the proteins, according to Parry (1974), is a two-stage process: first, the structure is altered causing denaturation, and second, aggregation takes place followed by coagulation, depending on the level and duration of heating; β-Lg undergoes such a process when the —SH groups are reactivated as a result of heating (Walstra and Jenness, 1984). The aggregates are of two sizes, depending upon which reactive groups are involved, that is, small aggregates of β-Lg (3.7 S) with interlinking —SH groups, and larger aggregates of β-Lg (29 S) in which the formation of —SS bonds may be important (some early references have been cited by Sawyer, 1969; McKenzie, 1971; Lyster, 1979).

In the 1970s, the information published on the heat denaturation of β-Lg recognised the interaction between β-Lg and κ-casein, but reports by Elfagm and Wheelock (1977, 1978a, b) suggest α-La is also involved. In brief the interaction may be as follows:

$$\beta\text{-Lg} \quad \rightarrow \quad \beta\text{-Lg} \quad + \alpha\text{-La} \quad \rightarrow \quad \alpha\text{-La }(\beta\text{-Lg})$$

$$\text{(small} \qquad\qquad \text{(large} \qquad\qquad\qquad\quad +$$
$$\text{aggregates)} \qquad \text{aggregates)} \qquad\qquad\qquad \kappa\text{-casein}$$
$$\text{(casein micelle)}$$

$$\alpha\text{-La }(\beta\text{-Lg})\ \kappa\text{-casein}\ \ \raise2pt\hbox{$\hookleftarrow$}$$
$$\text{(serum protein/casein complex)}$$
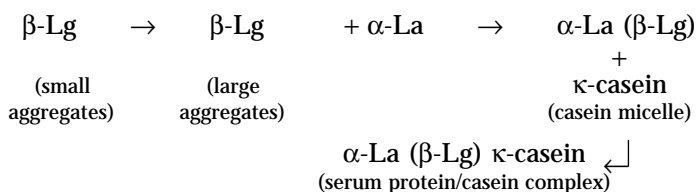
However, more recently Mottar *et al.* (1989) have proposed a slightly modified model in which the denatured β-Lg becomes associated with κ-casein of the casein micelles (i.e. phase 1). This results in the formation of appendages to the casein micelles which are irregular in structure and the surface becomes highly hydrophobic. When α-La starts to denature (i.e. phase 2), it interacts with β-Lg and fills the structural gaps formed in phase 1. The amount of α-La present to the micellar surface is dependent on the heating process and its intensity. This results in a smoother surface with decreased hydrophobicity and an increased water-holding capacity of the protein matrix (see also Hill, 1989). In a recent study, Calvo *et al.* (1993) concluded that thermally induced aggregation of α-La was dependent upon the concentration of free —SH groups present in other whey proteins; this appeared to function by inducing cleavage of the intramolecular —SS bonds in α-La leading to aggregation.

2.9.3.2 Effect on rate of denaturation of whey proteins

Law (1995, 1996) has quantitatively studied the relative rates of irreversible denaturation of whey proteins (i.e. immunoglobulins, serum albumin/lactoferrin, β-Lg and α-La) of different mammalian milks on heating at 70–90°C. The reported results suggest that (a) the concentrations of the individual whey proteins in cow's, goat's and sheep's milk are different, for example, the total whey protein contents were 0.65, 0.61 and 1.1 g 100 ml^{-1}, respectively, (b) upon heating, the order of denaturation of the milk of the three species of mammals was immunoglobulins > serum albumin/lactoferrin > β-Lg > α-La, and (c) at 90°C, the order of ease of denaturation of whey proteins was sheep > goat > cow. Other studies of the effect of heating on proteins have been reported by Law and Tziboula (1992, 1993), Law *et al.* (1993, 1994), Brown *et al.* (1995) and Tziboula (1997). The addition of a thermolabile variant of β-LgA to raw milk reduced syneresis of yoghurt when the milk base was processed at 70°C (Lee *et al.*, 1994; Batt *et al.*, 1994); this approach to yoghurt making could encourage future manipulation of the protein constituent(s) during their synthesis in the udder of the cow.

2.9.3.3 Effect on protein/fat interactions

Protein/fat interactions in recombined milks have been studied extensively by Singh and Creamer (1991) and Singh *et al.* (1993, 1996a). They concluded that these interactions are dependent on many factors, such as:

- Increasing the protein content in skimmed milk results in an increase in the protein load on the fat surface to reach a maximum of about 6 mg per m^2.
- Large fat globules have a lower protein load (i.e. mainly whey proteins).
- Altering the whey protein-to-casein ratio in the skimmed milk decreases the protein load adsorbed on the surface of the fat globules and can influence the composition of the protein layer.

- The extent of κ-casein dissociation increases when the SNF is increased from 10 to $20\,g\,100\,g^{-1}$ in recombined skimmed milk at pH 6.5–7.1 before heating, and/or when it is heated at 120°C for 2–11 min at pH 6.5 only.
- The rate of dissociation of κ-casein from the fat globule surface and the casein micelles was pH dependent.
- The protein load on the fat globule surface is decreased and the composition of the adsorbed protein is altered on disintegration of the casein micelles following the removal of colloidal calcium phosphate.

2.9.3.4 Effects on other milk constituents

It is evident that the component(s) in milk which are most dramatically modified by heat treatment at the temperatures practised in the yoghurt industry are the whey proteins, but other heat-induced changes can occur in milk and are of some significance. These include: (a) heating milk can affect the state of the milk salts, particularly calcium, phosphate, citrate and magnesium. Thus, these salts may exist in milk as soluble ions or in the colloidal phase as part of the casein micelle complex, and heating milk to 85°C for 30 min can change up to 16% of the soluble calcium into the colloidal phase (Kannan and Jenness, 1961). (b) Heating milk may reduce the amount of oxygen present, that is, lowering the redox potential, which encourages starter culture growth. (c) Undesirable flavours in milk are often removed by ordinary heat treatments, but severe heating can induce off-flavours, for example, the caramel flavour that results from the Maillard reaction between lactose and the amino groups of the proteins. (d) Vitamins in milk are subdivided into two main groups, the so-called fat-soluble vitamins (e.g. A, D, E and K) associated with the fat component of milk and the water-soluble vitamins (e.g. B group and C). The former vitamins are fairly heat stable, while vitamins B_6, B_{12} and C are heat labile. Table 2.20 illustrates the percentage losses of the heat sensitive vitamins in milk during different heat treatments. The relatively high heat treatments used in yoghurt

Table 2.20 Typical values of vitamin losses (%) from milk during different heat treatments[a] or processes

Vitamins	Pasteurisation		Sterilisation		Evaporated	Powders	
	Batch	Low	In-container[b]	UHT		Roller	Spray
Thiamine (B_1)	10	<10	20–35	10	20–60	20–30	10–15
Riboflavin (B_2)	N	N	<10	10	0	10–15	<10
Folic acid	0	0	40–50	15	–	–	–
Biotin	<10	<10	<10	<10	10–15	10–15	10–15
Pantothenic acid	N	N	<10	<10	<10	<10	<10
B_6	10	0	60–90	<10	90	20	35
B_{12}	20	10	40–50	10	60	30	20

[a] For further details refer to Table 2.15. [b] Heat treatments used: 110°C for 15 min and 115°C for 30 min; higher losses resulted during the latter treatment.

N, Data not available. –, Negligible.

The heat liability of vitamins A(+/–), carotene (–), D(–), E(+/–), K(?) and C(++) are not listed.

After Chapman *et al.* (1957), Porter and Thompson, (1972), Kon (1972), Ford and Thompson (1981), Renner (1983, 1989), Walstra and Jenness (1984), Burton (1988), Schaafsma (1989), Andersson and Öste (1995).

manufacture may, therefore, cause significant decreases in some vitamins and the presence of dissolved oxygen greatly enhances the sensitivity of the heat-labile vitamins (Hartman and Dryden, 1974; Renner, 1983, 1989; Walstra and Jenness, 1984; Burton, 1988; Andersson and Öste, 1995; Sharma and Lal, 1995). (e) Although heating the yoghurt milk base may destroy some vitamins and adversely affect the nutritional properties of yoghurt, the digestibility of proteins in the intestinal tract may be improved in comparison with unheated milk proteins (Puhan, 1988). (f) As a result of heat-induced changes in the milk constituents during heat treatment, fouling or formation of deposits on the surfaces of the processing equipment will occur. Thus, the operational time of the heat exchangers will be shortened and more cleaning is required. Studies on the different types of fouling of heating surfaces and the role of milk constituents have been reported by Kessler (1981), Dannenberg and Kessler (1988c), de Jong *et al.* (1992), Gotham *et al.* (1992), de Jong and van der Linden (1993), Hinrichs and Kessler (1995), Fryer *et al.* (1995) and de Rafael and Calvo (1996). The operational time of a plate heat exchanger processing fresh liquid milk is longer than when heating recombined milks at the same temperature.

It should be noted, however, that most of the technical data are collated from studies carried out on whole or skimmed milk and, although the various physico-chemical changes will occur in the yoghurt milk, the extent may be dependent on the composition of the milk base.

2.9.4 Processing effects on the physical properties of the gel

It is evident from Section 2.4 that the method of fortification of the milk solids can affect the firmness and syneresis of the yoghurt gel (see also Rohm, 1989, 1993a, b; Rohm and Schmid, 1993; Horne, 1993). Similarly, these same properties are influenced by the homogenisation pressure used (see Section 2.8). However, while the physicochemical changes in the protein components of milk could be considered to be one of the major changes influencing the quality of the manufactured yoghurt, the role of the starter culture in relation to acid development should not be overlooked.

Scanning electron microscopy (SEM) studies on the structure of gels derived from heated and unheated milks revealed some distinctive characteristics of the casein micelles. In heated milks, the gel is formed as the casein micelles gradually increase in size and form a chain matrix. This behaviour results in an even distribution of the protein throughout the yoghurt and the aqueous phase is immobilised within the network; the resultant coagulum is firm and less susceptible to syneresis. On the other hand, the casein micelles in the unheated milk form aggregates or clusters in which the protein is unevenly distributed and this heterogeneity impairs the immobilisation of the water; the coagulum is much weaker, by 50% compared with the previous coagulum (Kalab and Harwalkar, 1973, 1974; Kalab *et al.*, 1976, 1995; Kalab, 1979a, b, 1992; Harwalkar and Kalab, 1980; Modler and Kalab, 1983; Modler *et al.*, 1983). The contrast is well illustrated in Fig. 2.14. Also the casein particles (i.e. chains and clusters) in yoghurt containing $10\,g\,TS\,100\,g^{-1}$ were the largest observed and the dimensions of the particles decreased as the total solids content increased (Harwalkar and Kalab, 1986). In general, the larger the pores in the protein matrix, the easier the separation of whey, whilst the higher resistance to syneresis at pH 3.85 reflected increased gel rigidity compared with yoghurt which had a pH of 4.5.
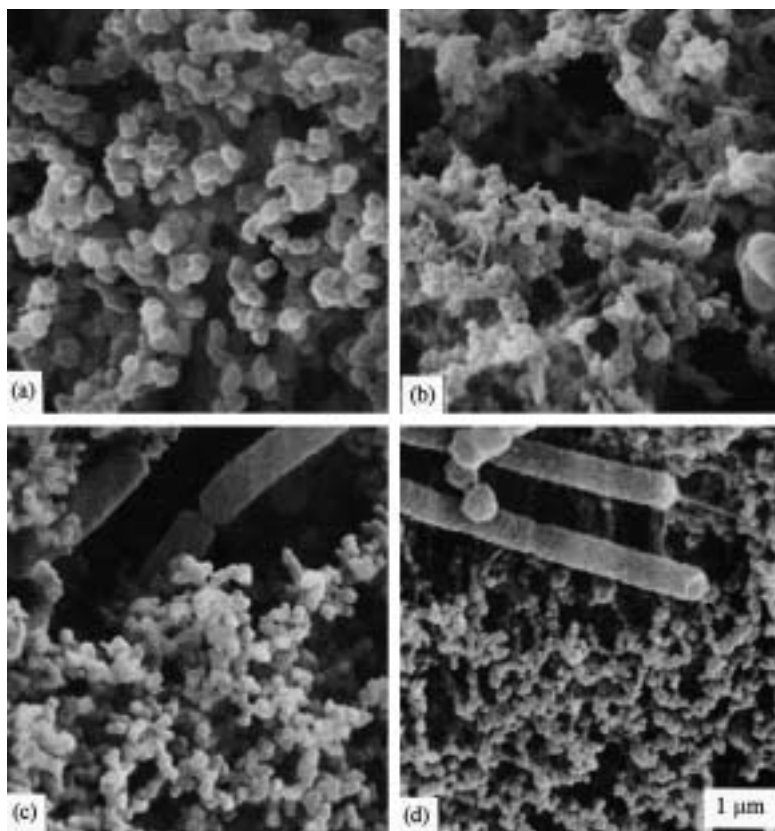
Fig. 2.14 Structural characteristics of the casein in different milks (10 and 20 g 100 g⁻¹ total solids) reconstituted from low skimmed milk powder and heated to 90°C, or unheated for 10 min

(a) Unheated milk (10 g TS 100 g⁻¹), (b) heated milk (10 g TS 100 g⁻¹), (c) unheated milk (20 g TS 100 g⁻¹), (d) Heated milk (20 g TS 100 g⁻¹).

Notice the differences in the structure of the coagulum in unheated and heated milks, (a) and (c) or (b) and (d), and the size of the casein micelle as the level of solids increases in the milk, (a) and (c) or (b) and (d).

After Kalab (1979a).

An investigation of milks subjected to heat (95°C for 10 min) revealed filamentous appendages composed of β-Lg/κ-casein and the interaction appears to involve —SS linkages and, possibly, the involvement of various salts, for example calcium phosphate and citrates (Davies *et al.*, 1978; Harwalkar and Kalab, 1981, 1988). These appendages tend to become "diffuse" after fermentation, but their presence in the coagulum of heated milks inhibited micellar coalescence, so giving rise to firmer curds with reduced tendencies to syneresis.

The microstructure of yoghurt consists of a protein matrix of micellar short or medium chains and micellar clusters, with the fat globules embedded in the matrix. Both the ratio of casein to non-casein protein in the milk and the method of fortification of the SNF can influence the porosity of the protein matrix. Ratios of 2.9:1 to 4.6:1 have been reported by Modler and Kalab (1983), Modler *et al.* (1983) and Tamime *et al.* (1984). The latter authors recommended a ratio of 3.3:1 because,
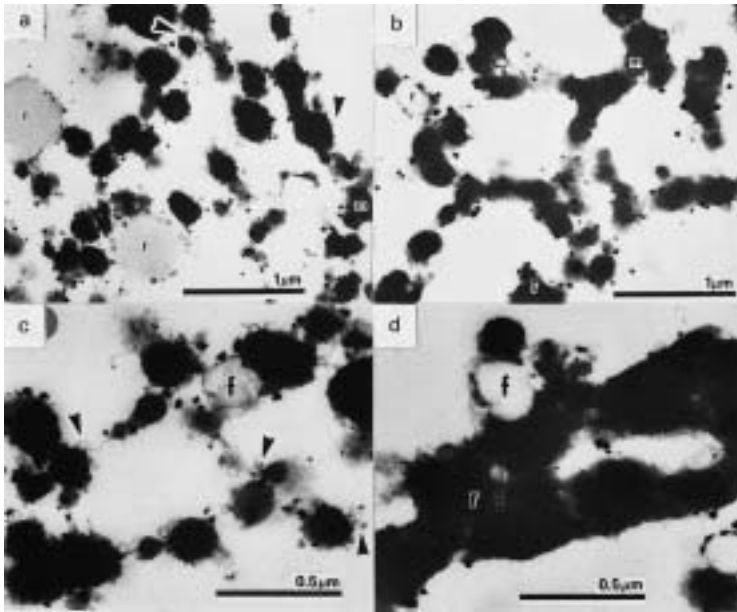
Fig. 2.15 Microstructure (TEM) of casein micelles chains and clusters in yoghurt prepared from skimmed milk fortified with SMP (a) and (c) and Na-caseinate (b) and (d)

Arrows point to spikes on casein micelle surfaces. f, Fat globules; m and r, simple and complex casein micelle chains, respectively.

After Tamime *et al.* (1984). Reproduced by permission of *Scanning Microscopy International.*

at higher ratios, fusion of the casein micelles occurred and this resulted in an unsatisfactory product because of textural problems (i.e. being rough and coarse). Figure 2.15 illustrates the microstructure of yoghurt (SEM and TEM) fortified with SMP and Na-caseinate. Incidentally, the yoghurts fortified by VE and RO had similar images to those of SMP, whilst UF yoghurt showed only limited fusion of casein micelles (Tamime *et al.*, 1984). However, using UF retentate powders (whole or skimmed – see Table 2.4) the original ratio of casein to non-casein protein is maintained in the milk base and hence a yoghurt with a firm body and minimal syneresis can be made.

Homogenisation and high-heat treatment of the milk base increases the hydrophilic properties of the coagulum and the stability of the yoghurt gel due to the denaturation of whey proteins and association with κ-casein. Labropoulos *et al.* (1981a, b, 1982, 1984) concluded that the physical properties of yoghurt manufactured from milk heated to 82°C for 30 min, compared with 149°C for 3.3 s, were best, and that the latter treatment is suitable only for the production of drinking yoghurt or yoghurt with thin consistency or low curd firmness. Similar observations were reported by Parnell-Clunies and Kakuda (1986), Parnell-Clunies *et al.* (1986a, b, 1988a) and Parnell-Clunies (1987) for whole milk heated at 85°C for 10–40 min (vat process), at 98°C for 30–112 s (high pasteurisation) and at 140°C for 2–8 s (UHT) for the manufacture of yoghurt. They concluded that:

- The order of yoghurt firmness and viscosity of the three heating methods was vat process > high pasteurisation > UHT treatments, whilst the highest water-holding capacity of the coagulum was observed with high pasteurisation treatment followed by UHT and vat process.
- Yoghurt made by the vat process exhibited syneresis and a grainy texture; UHT treatment resulted in weak texture of the yoghurt coagulum; the high pasteurisation process (i.e. 98°C for 1.87 min) represented the best process and was recommended for industrial production. However, other researchers have recommended 85°C for 30 min for maximum starter activity.

Nevertheless, Schmidt *et al*. (1985) improved the firmness, syneresis and texture of yoghurt by adopting the following method of milk processing. On the first day, separate whole milk at 37°C, fortify with SMP, warm to about 50°C, homogenise at 17.2 MPa (first stage) and 6.9 MPa (second stage) and cool to 4°C for 14 hours. On the second day, heat the milk at 138°C for a longer time than reported by Labropoulos *et al*. (1981a, b), cool to 42°C and inoculate with starter culture and after incubation, cool in ice bath at pH 4.3 and store for 1 week at 4°C. This method, if adopted for the manufacture of yoghurt, may be inconvenient for industrial application and the consensus in the reported literature suggests that high pasteurisation of the yoghurt milk is most suitable (Dannenberg and Kessler, 1988d, e; Mottar *et al*., 1989; Kessler *et al*., 1990; Kaytanli, 1993). It may also be important that, upon heating the milk base, the microstructure of the heated micelles showed large numbers of small particles of irregular shape attached to the micelle surface ([Fig. 2.16a](#)) and finely
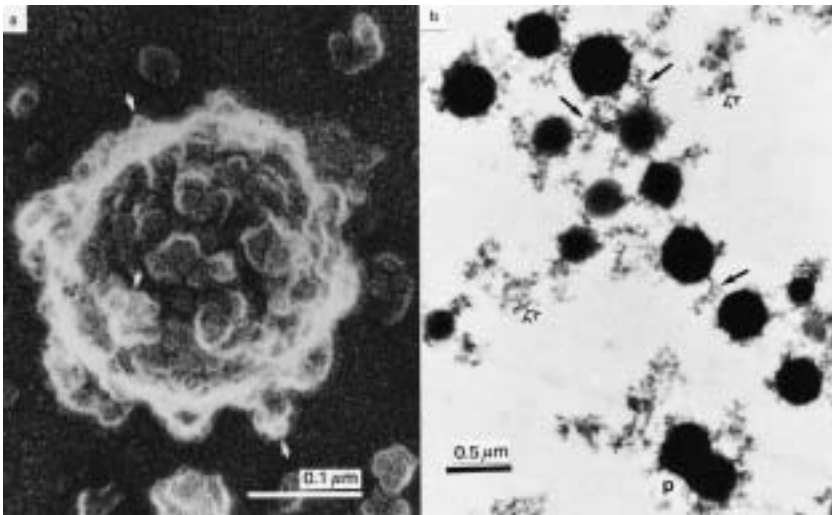
Fig. 2.16 Casein micelle (a) in heated bovine skimmed milk covered with corpuscular spikes (arrows) using rotary shadowing, and casein micelles (b) in milk fortified by UF-WPC appear as individual entities in a loose chain attached to finely flocculated protein (dark arrows) and some not associated with the micelles (light arrows). Occasionally some micelles (P) are tightly fused together

After Kalab *et al*. (1982) and Modler and Kalab (1983). Reproduced by permission of *Milchwissenschaft* and *Journal of Dairy Science*.

flocculated protein surrounding the casein micelles (or as separate entities) (Fig. 2.16b) in yoghurt milk fortified with UF-WPC (Kalab *et al*., 1982, 1983; Modler and Kalab, 1983; Kalab and Caric, 1990; Kalab, 1992). Recently, Hollar *et al*. (1995) dialysed a WPC mixture $(16\,g\,TS\,100\,g^{-1})$ against simulated UF milk containing calcium; on heating, the denaturation of the whey proteins was influenced by:

- the calcium content which, as it decreased, resulted in more soluble aggregates and less soluble precipitates being formed;
- the pH which, as it increased (5.8 to 7.0), caused more protein denaturation, fewer soluble aggregates and more soluble precipitates;
- α-La was denatured more extensively than β-Lg at 66°C and 71°C;
- the addition of low-heat SMP limited whey protein denaturation in WPC.

Another aspect, which was revealed by SEM studies (Kalab, 1979a), showed that *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* form "pockets" in the protein matrix of the yoghurt coagulum. These pockets were regarded by some workers in this field as artefacts caused by freeze drying of the sample, but both transmission electron microscopy (TEM) and the freeze fracturing of yoghurts, that is, sectioning while the aqueous phase is still present, confirmed the presence of the pockets (Kalab, personal communication). Figure 2.14 shows some lactic acid bacteria in a "void space". Furthermore, SEM micrographs (Kalab, 1979b, 1992, 1993) also revealed filaments of exopolysaccharide (EPS) produced by "slime" or "ropy" strains of yoghurt starter cultures (see also Brooker, 1979, 1987). Further detail regarding the chemical composition of these filaments is given in Chapter 7.

The dimensions of the casein particles in yoghurt milk are affected by the level of total solids in the milk base, and Kalab (1979b) observed that the size of the casein particles decreased with increasing levels of solids in the milk (see Fig. 2.14); the reason for this behaviour is not well established.

Image analysis using TEM was used by Skriver *et al*. (1997) to study the microstructure of yoghurt and they observed that (a) casein aggregates were larger in the yoghurt made from milk heated at high temperature, so confirming the observations reported by Kalab *et al*. (1976), (b) the star volume, which gave a measure of the average size of "pores" in the yoghurt gel, was influenced by the level of heat supplied to milk and also affected the structure of the casein matrix and (c) the covariance function was able to differentiate between yoghurts made from the same heated milks, but held for 10 min or 30 min.

Normal creaming in cold milk is influenced by the action of the globulins, which assist in the formation of clusters among the rising fat globules (Mulder and Walstra, 1974). Therefore, the denaturation of the globulin fractions in milk, as a result of heat treatment, causes a reduction in the cream layer (Walstra and Jenness, 1984). This action could work in favour of the small yoghurt producers whose production lines do not include an homogeniser. Furthermore, milk becomes whiter in colour on heat treatment, before the appearance of browning and according to Burton (1954) this could be due to:

- flocculation of the whey proteins;
- changes in the casein aggregates;
- calcium being converted from the soluble state to a colloidal or insoluble form.

2.10   Fermentation process

2.10.1   Introduction

During the manufacture of yoghurt, the heat-treated milk is cooled to the incubation temperature of the starter culture *(S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus)* and, in general, the milk is fermented at 40–45°C, that is, the optimum growth condition for the mixed culture – the short incubation method. In some cases the incubation period can be as short as $2\frac{1}{2}$ hours, assuming that the starter culture (3%) is an active one and the ratio between the rods and the cocci is well balanced. However, the longer incubation method, (i.e. overnight) can be used and the incubation conditions are 30°C for around 16–18 hours, or until the desired acidity is reached (Hrabova and Hylmar, 1987). Recently, zabadi (i.e. Egyptian equivalent of yoghurt) has been manufactured at 30°C or 35°C with minimum syneresis, improved firmness and smoothness and with a pleasant flavour (Mehanna, 1991). However, Cho-Ah-Ying *et al.* (1990) produced yoghurt using different strains of thermophilic starter cultures (i.e. EPS (exopolysaccharide) producer and non EPS-producer), and fermenting the milk at 38° and 43°C; they concluded that the temperature of incubation had significantly affected only one sensory character (e.g. texture), and overall the yoghurts made at 38°C had the tendency to score higher.

While the cooled milk is being pumped to the fermentation tanks, the starter culture is normally metered directly into the milk, or alternatively, if a multipurpose tank is being used, the starter culture is added either manually or, if the volume of the tank is large, the desired quantity of starter is pumped into the tank. As can be seen later, the actual fermentation stage can take place either in the retail container for the production of set yoghurt, or the milk is incubated in bulk for the manufacture of stirred yoghurt. However, no matter what type of yoghurt is being produced, the biochemical reactions responsible for the formation of the gel/coagulum are exactly the same. The intricacies of the fermentation processes are discussed in detail in Chapter 7. Thus, the only real differences between set and stirred yoghurt are the rheological properties of the coagulum, since in the former type the milk is left undisturbed during the incubation period and the resultant gel is in the form of a continuous semi-solid mass, while stirred yoghurts are, by contrast, the result of breaking the gel structure at the end of the incubation period and prior to cooling and further processing (refer to Chapter 5 for more detail).

2.10.2   Starter organisms

The commercial process of yoghurt making uses a defined mixture of lactic acid bacteria, for example *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, but other products may require a different blend. For example, Bulgarian buttermilk is produced using *L. delbrueckii* subsp. *bulgaricus* alone, whilst dahi in India is produced using a mixed starter culture containing *S. thermophilus, Lactococcus lactis* biovar *diacetylactis* and *Lactococcus lactis* subsp. *cremoris* (Tamime and Marshall, 1997). Bio-yoghurts are made with different and defined starter cultures containing the yoghurt organisms (single or mixed) and/or *Lactobacillus*, *Bifidobacterium* and *Enterococcus* species; for further detail refer to Chapter 5 and Tamime and Marshall (1997).

The reason(s) for selecting the combinations of starter cultures used during the manufacture of yoghurt and related fermented milk products are to achieve the

desired flavour characteristics of the product, mainly lactate, aroma compounds (acetaldehyde, acetoin and diacetyl) and EPS and to provide the consumer with a wide choice of therapeutic products. The former aspect is very important and, hence, careful selection of different strains of the yoghurt organisms may provide the manufacturer with the following broad options of flavour intensity and EPS production by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*:

Flavour	*EPS*
High	Low
Medium	Medium
Low	High

Although the low flavour producing strains tend to be categorised as high EPS producers, the same pattern may emerge organoleptically because the presence of EPS in a yoghurt may mask the flavour attribute of the product; such products will also have a different mouthfeel (Wacher-Rodarte *et al.*, 1993). Rohm (1992, 1993a, b), Rohm and Kovac (1994, 1995), Rohm *et al.* (1994) and van Marle and Zoon (1995) studied the textural and organoleptic characteristics imparted by a number of commercially available yoghurt starter cultures strains and they concluded that:

- Significant differences between the yoghurts were found for each sensory attribute except gel firmness.
- Multiple regression analysis of the sensory scores obtained using the hedonic scale were mainly influenced by the ropiness and flavour attributes showing negative and positive weightings, respectively.
- The apparent viscosity of stirred yoghurt was increased by the EPS cultures, but no correlation was observed between viscosity and the amount of EPS produced; the permeabilities of glucone-δ-lactone (GDL), EPS and non-EPS gels, as measured by using glass tubes (van Marle and Zoon, 1995), were significantly different, and the lowest value was shown by the EPS gel; thus, the permeability and apparent viscosity of stirred gels are inversely related.
- The relationship between shear stress and long relaxation time was more evident in the viscoelastic properties of products made with EPS cultures.

This latter aspect could be influenced by the attachment of ropy bacterial cells to the protein matrix, which thus decreased the firmness of the yoghurt gel. Figure 2.17 illustrates some examples of the microstructure of yoghurt made with EPS-producing starter cultures. Incidentally, a similar microstructure has been reported by Toba *et al.* (1990) for viili, a Finnish fermented milk, made with a ropy strain of *Lactococcus* species.

Schellaass (1983) and Schellaass and Morris (1985) observed that yoghurt made with EPS cultures exhibited a decreased susceptibility to syneresis and greater viscosity when compared with non-EPS producer strains (Robinson, 1988); however, excessive EPS production was obtained when the milk was fermented at 32°C. Such physical characteristics of the EPS yoghurt are attributed to the filamentous network between the bacterial cells and the casein matrix, but this interaction was disrupted when the yoghurt was subjected to shear stress at $220\,s^{-1}$ (Teggatz and Morris, 1990; Skriver, 1995; Skriver *et al.*, 1995). Hassan *et al.* (1995a, b) have used confocal scanning laser microscopy (CSLM) to observe the structure of yoghurt in its natural state and they observed that: (a) an envelope of EPS produced by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was evident surrounding the cells, (b) the diameter of
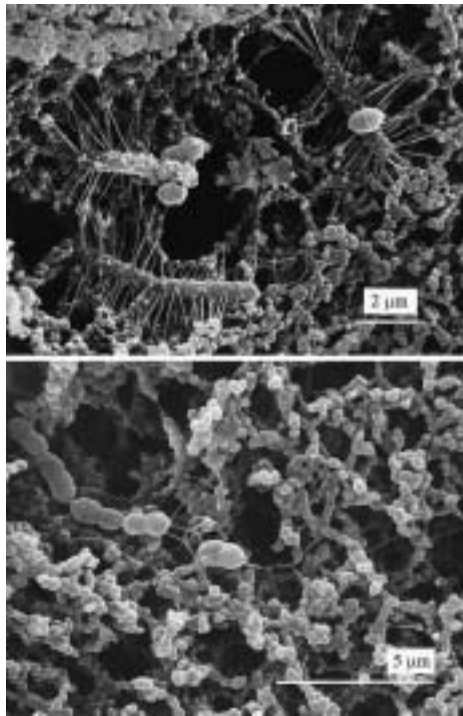
Fig. 2.17 Microstructure of yoghurt made with an exopolysaccharide (EPS) strain of
S. thermophilus and *L. delbrueckii* subsp. *bulgaricus*

After M. Kalab (personal communication).

the envelope was different depending on the bacterial species and (c) gelation of the milk, as determined by casein micelle aggregation and the cessation of bacterial cell movement, was initiated at pH 5.35; as the pH continued to drop, the EPS organisms caused the formation of non-reflective zones in the gel possibly due to the contraction of the casein matrix. The textural and rheological properties of yoghurt made with these cultures have been reported by Hassan *et al*. (1996a, b).

It is relevant to point out that very simple techniques can also be employed to study the behaviour of EPS yoghurt, such as monitoring the syneresis of yoghurt on an inclined heated black glass (Tamime, 1977) and measuring the length of an EPS "thread" formed at the end of an acrylic rod when withdrawn from yoghurt (Watanabe, 1987).

Advance(s) in yoghurt technology may include the interactive fermentation of milk by means of a membrane dialysis fermenter. Such a fermenter has been developed in The Netherlands (Klaver *et al*., 1992a) to obtain a yoghurt with a smooth structure, mild acidity and lower post-fermentation acidification during storage. The same researchers have also used the technology in buttermilk making (Klaver *et al*., 1992b). An alternative approach to limiting acid development and bitter taste in yoghurt during storage has been reported by Klaver *et al*. (1991), and the system could be described as follows: (i) the processed milk is cooled to the incubation temperature and fermented by *Lactobacillus* sp., followed by a heat treatment to inactivate the starter cultures, and (ii) the partially fermented milk is cooled and inoculated with streptococci; sweet or unfermented milk could be added as optional

extra. In Egypt, El-Kenany *et al.* (1996) inoculated milk with a yoghurt starter culture at 60°C or 70°C with a holding time of 5 min, before cooling to 45°C; this method of fermentation improved the shelf life of the product.

2.10.3 Gel formation

The formation of gels during the manufacture of certain dairy products is basically due to destabilisation of the casein complex. These gels are irreversible and are classified into different groups: (a) enzymic gels, which are formed as the result of coagulant action which destabilises the κ-casein allowing aggregation of the casein in the presence of calcium ions, (b) heat-induced gels, which can arise as a fault where gelation occurs in UHT milk or evaporated milk if the protein fraction is not well stabilised, (c) acid gels formed by the acid fermentation of milk, for example yoghurt, and (d) salt/heat-induced gels, which are normally produced during the manufacture of Ricotta cheese. It could be argued, however, that, although the production of yoghurt does not involve the addition of a proteolytic coagulant enzyme, proteinases originating from the yoghurt starter cultures may have a role. Hence, it should be understood that yoghurt may not be simply an acid-induced gel and that proteinases may contribute to the denatured protein matrix which could be relevant to the gel properties of yoghurt (Tamime and Marshall, 1997).

The main difference(s) between acid- and enzymic-induced milk gels have been reported by Walstra and van Vliet (1986) and van Vliet *et al.* (1989) and could be summarised as follows: (a) The permeability of the acid-induced gel does not change during the first 24 hour after gelation, whilst in an enzymic-induced gel, it increases continuously during the same period, and (b) a milk gel formed by coagulant enzymes is more robust than an acid-induced gel; the latter type of gel is fragile and shatters very easily. Nevertheless, Muir and Hunter (1992) have profiled the sensory attributes (i.e. *odour*: intensity, sour, fruity, buttery, yeasty, creamy, sweet, other; *flavour*: intensity, sour/acid, fruity, buttery, rancid, creamy, salty, bitter, lemon, sweet, chemical, other; *aftertaste*: intensity, bitter, sour/acid, other; *texture*: firmness, creaminess, viscosity, sliminess, curdy character, mouthcoating, chalky, serum separation) of fermented milks, and also identified these attributes as being important for consumer acceptability. The microstructure of yoghurt has been well studied, but few data have been published on the mechanism(s) of the acid induction of gels in milk by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* at 30–45°C. However, the casein micelles are composed of different protein fractions (see Section 2.9.3), and are associated with one another via Ca-phosphate bridges. During the fermentation of milk, the micellar or colloidal Ca^{2+} content (and possibly to a lesser extent magnesium and citrate) increases in the serum as the pH is lowered due to the solubilisation of micellar Ca-phosphate (Pouliot *et al.*, 1989; LeGraet and Brulé, 1993). Alteration of the physical nature of the casein micelles will play a major role in acid-induced milk gels.

The mechanism(s) of dissociation and aggregation of casein micelles in acid-induced gels has been reviewed by Tamime and Marshall (1997) and they reported that:

"direct acidification of milk using HCl or glucono-δ-lactone (GDL) and the addition of calcium chelating agents are different techniques used to study the gelation of milk under controlled conditions without the metabolic interference of the starter

cultures (Roefs *et al.*, 1985; Holt *et al.*, 1986; van Hooydonk *et al.*, 1986; Visser *et al.*, 1986; Roefs, 1987; Bremer *et al.*, 1990; Bringe and Kinsella, 1990, 1991; Banon and Hardy, 1991, 1992; Horne and Davidson, 1993).

Studies of casein micelle dissociation and aggregation during the acid-induced gelation of milk suggest that the mechanisms involved are pH, -ion concentration- and temperature-dependent (Aoki *et al.*, 1986, 1987a, b, 1988; Holt *et al.*, 1986; Singh *et al.*, 1996b; Teo *et al.*, 1996, 1997). Predominantly, β-casein dissociates from the casein micelles at low pH (Hooydonk *et al.*, 1986); however, dissociation of other casein fractions (κ-, α_{s1}-, α_{s2}) from the micelles has been reported by Roefs *et al.* (1985), Roefs (1987), Dalgleish and Law (1988) and Ward *et al.* (1996). All these authors also observed that the amounts and proportions of the dissociated caseins in the serum were pH- and temperature-dependent. At pH 5.6, all the major caseins were prone to dissociation and the dissociation occurred at the outer rather than the inner layers of the submicelles (van Hooydonk *et al.*, 1986). Solubilisation of the micellar Ca-phosphate occurs at pH $\leq$ 5.3, and there is a linear relationship between $Ca^{2+} + Mg^{2+}$ and inorganic phosphate (P_i) + citrate (cit.). The binding of ionic calcium and magnesium to casein appears to be independent of pH between 5.6 to 6.7. Calcium binding may involve carboxyl groups; however, a decrease in pH also affects the spatial properties because of electrostatic interactions between positively and negatively charged groups (van Hooydonk *et al.*, 1986). Dalgleish and Law (1989) observed a similar pattern of mineral solubilisation due to pH- and temperature-induced conditions, but could not suggest a universal relationship to describe the dissociation of salt ions and the caseins from the micelles. Lowering the pH reduces the repulsive forces and allows for hydrophobic interactions causing the casein micelles to coagulate. However, preheating the skimmed milk to 90°C followed by acidification at 30°C using slow hydrolysis of GDL shifted the coagulation pH to a value higher than 5.5, and shortened the coagulation time (Horne and Davidson, 1993; Cobos *et al.*, 1995a, b).

Quiescent heating of casein solutions made with reconstituted SMP or Na-caseinate, and acidified at 0–2°C produced physically stable suspensions of casein particles (Roefs *et al.*, 1990a, b). Gelation occurred above 10°C and lowering the temperature to 4°C after gel formation had the following effects: (a) the casein particles formed a complex irreversible structure, (b) the acid-induced gel was formed subject to an activation Gibbs energy which decreased on increasing the temperature, (c) if the gel was good at >10°C, the dynamic moduli, G' and G'', linearly increased with the logarithm of time over at least a week, and (d) the gel network consisted of large and small agglomerates of casein particle aggregates in the form of strands and nodes, with void spaces around 1–10 μm; this suggests that the strands and nodes are made of concentrated protein (about 25%) with a modulus of about $10^5 N^{-1}$ (Roefs *et al.*, 1990a, b). Similar gel characterisation of commercial fractions of β-Lg and α-La have been recently reported by Rojas *et al.* (1997).

The dissociation of casein micelles in milk has been induced by other means such as salt solutions ($CaCl_2$, $MgCl_2$ or NaCl) or calcium-chelating agents like EDTA, hexametaphosphate, oxalate, citrate or othophosphate (Holt *et al.*, 1986; Aoki *et al.*, 1988; Rollema and Brinkhuis, 1989; Bringe and Kinsella, 1991; Johnston and Murphy, 1992; Goddard and Augustin, 1995; Goddard, 1996). Dialysis against phosphate-free and Ca-phosphate buffers decreased both the colloidal Ca phosphate and P_i (depending on the type of buffer used) before casein dissociation occurred. Holt *et al.* (1986) reported that dissociation resulted from the breakup of

linkages between the casein and the inorganic components. However, the dissociation of casein micelles in simulated milk ultrafiltrate dialysed against imidazole buffer was dependent on the ester phosphate content (Aoki *et al*., 1988). The addition of Ca^{2+}, Mg^{2+} and Na^+ ions, which are associated with casein phosphates and carboxyl groups, tends to increase the hydrogen ion concentration due to reduced repulsive hydration forces between micelles. Hence, the attractive hydration forces cause coagulation because hydrogen ions displace bound Ca^{2+}, Mg^{2+} and Na^+ ions in the casein micelles. Ions (Cl, NO_3, Br and SCN) binding to lysine, arginine and histidine groups also decrease the repulsive hydration forces between colloidal ions of the casein micelles (Bringe and Kinsella, 1991).

Current published research on the mechanism(s) of gelation induced by direct acid addition to milk provides some limited information; however, since the milk base for the manufacture of fermented milks is prepared in a different way and subjected to homogenisation and high heat treatment, the properties of the fermentation-induced gel may differ. Roefs *et al*. (1985) concluded that, because low-heat SMP was used in their study: 'because of the dependency of results on the history of samples, both in terms of pH and temperature, it will take painstaking studies to determine precisely what changes occur'."

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It is evident that the formation of yoghurt gel is the result of both biological and physical action on the milk, such as the fortification, homogenisation and heat treatment of the milk base and the catabolism of lactose in the milk by the starter culture for its energy requirements and, as a result, the production of lactic acid and other compounds. These effects bring about the gelation of milk. Heertje *et al*. (1985) reported that, during the acidification of skimmed milk with GDL at 30°C, the casein micelles may undergo the following changes at different pHs (see also Mulvihill and Grufferty, 1995):

- 6.6–5.9, no evidence of change in the casein micelles, size about $0.1\,\mu m$ and homogenously distributed in milk.
- 5.5–5.2, partial micellar disintegration occurs and at ≤ 5.2, casein particles aggregate to form structures with empty spaces between them; however, when such interaction(s) between micelles take place, the milk gel should not be disturbed.
- 5.2–4.8, contraction of casein aggregates take place, and these particles are larger in size than the native micelles.
- ≤ 4.5, rearrangement and aggregation of casein particles occurs leading to the formation of a protein matrix consisting of micellar chains and clusters.

Parnell-Clunies *et al*. (1988b) concluded that acid-gel formation of milk was a multistage process consisting of an initial lag period of low viscosity, a period of rapid viscosity change and a stage of high viscosity. However, the same authors reported that dissociation of casein micelles occurred at pH 5.1 and was thought to be influenced by the conversion of colloidal Ca to Ca^{2+}. At pH 4.8 these casein subparticles reassociate to form larger casein aggregates bearing no specific shape and dimensions.

Overall it is reasonable to conclude and/or suggest that the α-La/β-Lg interaction with the κ-casein (linked by —SH and —SS bridges) partially protects the micelles; however, as the pH in milks is lowered, destabilisation or disruption of the

micelles starts to occur. As a result, the gel network or protein matrix consists of micellar chains and/or micellar clusters and entraps within it all the other constituents of the milk base, including the water phase.

2.11   Cooling

Yoghurt production is a biological process and cooling is one of the popular methods used to control the metabolic activity of the starter culture and its enzymes. Cooling of the coagulum commences directly after the product reaches the desired acidity, for example, around pH 4.6 or 0.9% lactic acid depending on the type of yoghurt produced, the method of cooling used and/or the efficiency of heat transfer.

Since the yoghurt organisms show limited growth activity around 10°C, the primary objective of cooling is to drop the temperature of the coagulum from 30–45°C to <10°C (best at around 5°C) as quickly as possible so as to control the final acidity of the product. The process of cooling yoghurt may be carried out using one-phase or two-phase cooling.

2.11.1   One-phase cooling

In this process the coagulum is cooled directly from the incubation temperature to <10°C prior to the addition of flavouring materials and packaging. This approach is based on the assumption that a cold coagulum is more stable than one at about 20°C, and hence less damage will occur during the subsequent stages (e.g. mechanical handling while introducing the fruit/flavours, and filling the retail cartons). In actual fact, the coagulum at about 20°C is less viscous and, as a consequence, the product can be transferred from one section of the processing equipment to another with minimal structural damage. Thus, one-phase cooling is not widely used in the industrial situation.

2.11.2   Two-phase cooling

The first phase of the cooling stage reduces the temperature of the coagulum from 30–45°C to about 20°C prior to addition of the flavouring materials and filling. The second phase of cooling takes place in the refrigerated cold store where the yoghurt is cooled to <10°C. The final cooling of yoghurt takes place, therefore, in the retail container and as the coagulum is left undisturbed, the viscosity of the yoghurt improves after 1–2 days' storage.

This latter approach to cooling is widely used in the industry for the production of acceptable viscous yoghurts. However, the influence of cooling rate on the physical characteristics of stirred yoghurt was evaluated at the Danish Dairy Research Institute (Anon., 1977) and they gave the following recommendations:

- The quality of stirred yoghurt may be greatly improved by packaging yoghurt at 24°C, followed by final cooling of the product in the container.
- To achieve the maximum effect on yoghurt quality, the second-phase of cooling must be carried out as slowly as possible over a 12 hour period.

- Concentration of the yoghurt milk, i.e. by evaporation and removal of about 10% water, was identified as the factor that most improved the quality of yoghurt.
- The recommended procedure was as follows: (a) before cooling commences, stir the yoghurt in the incubation tank until mixture is homogeneous, (b) cool the yoghurt (primary cooling) to 24°C and package, (c) cool the packed yoghurt in a cold store controlled by a two-step temperature regulator, i.e. the first 5–6 hours at an air temperature of 7–10°C, and then at an air temperature of 1–2°C for the remainder of the cooling period, (d) forced air circulation in the cold store is highly recommended to obtain uniform cooling of the packaged yoghurt and (e) the design and construction of the crate and the material(s) used for packaging can affect the cooling rate of the packed yoghurt.

However, the industry does not appear to have adopted these proposals to any marked extent. The general practice in large installations is to subject the packaged yoghurt to an intermediate shock cooling in a chill tunnel before reaching the refrigerated cold store (Bylund, 1995). White (1995) has described a multistage cooling process for yoghurt which entails the following basic phases:

- Shock cooling from 42°C to 30°C.
- Dysgentical stage from 30°C to 20°C.
- Lact-less phase to 14.5°C.
- Holding phase at 2–4°C.

This approach could be considered as a slight modification of the system described by Bylund (1995), but it may be difficult to adapt to industrial situations unless some of the stages are combined before the product is packaged.

In addition, it should be noted that the cooling of yoghurt starts at a relatively high pH value, and hence the rate of cooling (slow or fast) determines the final acidity in the product and the rate of cooling can affect the structure of the milk gel. Very rapid cooling may lead to whey separation, possibly due to a too rapid contraction of the protein matrix which, in turn, affects the hydrophilic properties (Rasic and Kurmann, 1978).

2.12   Addition of fruit/flavouring/colouring ingredients

The increase in the per capita annual consumption of yoghurt in the majority of countries (see Table 1.2) has been attributed both to the ever-increasing availability of fruit and/or flavoured yoghurts and to the diversity of presentation of the product. Thus, in the United Kingdom, for example, the retail economic value of yoghurt increased from £103 m in 1981 to £401 m in 1990, reflecting a growth of 3.9-fold (Anon., 1984a, 1991a); 90% of these sales are fruit and flavoured yoghurts.

A variety of different flavouring ingredients (fruits, natural flavours and/or synthetic flavours) are currently added to yoghurt and Table 2.21 indicates a range of available fruit additives. It can be observed that the fruit flavours, which are in regular demand, are surprisingly few in number, and the rest are introduced by the yoghurt manufacturers merely to encourage wider popularity for the product. The types of fruit/flavouring material used in the yoghurt industry are given below.

Table 2.21 Fruits and fruit flavours currently used in production of yoghurt

Regular demand	Average demand	Poor demand
I. Single		
Apricot	Banana	Apple
Black cherry	Bilberry	Bramble (artic)
Blackcurrant	Blackberry	Cranberry
Mandarin	Gooseberry	Damson
Peach	Grapefruit	Elderberry
Pineapple	Lemon	Grape
Raspberry	Melon	Guanabana
Strawberry	Orange	Guava
	Plum	Kiwi
	Prune	Kokum
	Rhubarb	Lime
	Tangerine	Loganberry
	Toffee	Mango
		Papaya
		Passion fruit
		Pear
		Pina Colada
		Quince
		Redcurrant
		Sapota
		Wortleberry
II. Mixed		
Fruit cocktail	Apple/Raisin	Apple/Wortleberry
Fruit of the Forest	Apple/Orange	Cherry/Elderberry
Peach/Raspberry	Cherry/Orange	Grape/Figs
Peach/Apricot	Cherry/Pineapple	Kiwi/Gooseberry
Raspberry/Redcurrant	Mixed Citrus	Peach/Passion fruit
	Pear/Banana	Pineapple/Coconut
	Strawberry/Blackberry	Raspberry/Coconut
		Strawberry/Kiwi
		Strawberry/Coconut

III. Miscellaneous flavours

Almond paste/Low-starch nuts	Mint
Banoffee	Mirabella/Vanilla
Buckwheat Honey	Mississippi Mud Pie
Butterscotch	Mocca
Champagne	Muesli
Chocolate	Muesli/(ω)-3 fatty acids
Chocolate covered crisps	Nuts/Cocoa, Caramel or Nougat
Coconut	Paprika/Celery
Coffee	Pear/Vanilla
Cucumber	Raspberry/Vanilla
Grape Mist	Tomato
Hazel nuts	Vanilla
Honey	Walnut
Lemon Meringue Pie	Wine
Maple syrup	

The classification of the above fruit/flavoured yoghurt is only applicable to the U.K. market as suggested by Tamime and Hamilton (unpublished data).

Data compiled from Brown and Kosikowski (1970), Swanson *et al.* (1972), Robinson and Tamime (1975), Duthie *et al.* (1977), Muller (1977), Bray *et al.* (1979), Chase (1981), Lang (1981a, b), Anon. (1981a, 1984b, 1986a, b, 1987a), Lehmann (1983), Kammerlehner (1986), Matsunobu *et al.* (1987), Schei and Abrahamsen (1987), Herner (1987a, b), Payne (1988), Kaufman *et al.* (1990), Balasubramanyam and Kulkarni (1991), Jarmarova and Hrabe (1992), Hoang (1992), Raiski (1993), Desai *et al.* (1994), Calvo *et al.* (1995) and Mortensen (1996).

2.12.1 Fruits

Fresh fruits can be used to flavour yoghurt, but due to the seasonal availability of such materials and their variable quality, their use in the industry is very limited. Processed fruits are, therefore, more widely employed, particularly as the desired fruit mixture can be standardised by the fruit processor to meet the specifications required by the customer. In general, fruit preparations for the yoghurt industry consist of fruit, sugar (syrup and/or artificial sweetners), stabilisers, flavours, colouring matter and food grade acids or pH adjusters (see Hegenbart, 1990; Mogensen, 1995). These types of fruits are classified as fruit preserves, canned fruit, frozen fruits or miscellaneous fruit products.

2.12.1.1 Fruit preserves

Fruit preserves are processed in a small quantity of sugar syrup to give an end product consisting of $(g\,100\,g^{-1})$ 70 fruit, 30 water, and this product may be referred to as pure or natural, since no colouring matter or preservatives are added. Depending on the processing technique, the product may become highly aromatic, but the natural colours of any fruit become dull due to the effect of heat treatment. It is also relevant that such products are expensive, so that overall demand from the yoghurt industry is limited.

2.12.1.2 Canned fruit

Canned fruit is similar to the product mentioned above, except that canned fruits are permitted to contain certain additives, such as (a) colouring ingredients which help to mask the loss of the natural colours of the fruit, (b) stabilisers which assist in protecting the structure of the processed fruit and improve the viscosity of the fruit product, and (c) flavouring agents which help to enhance the consumer appeal of the finished yoghurt.

Canned fruit is packaged in special lacquered tin cans, plastic drums with polyliners, laminated plastic bags (Mora, 1996) or stainless steel tanks. The level of sugar is maintained at $30–35\,g\,100\,g^{-1}$ and the pH is adjusted to <3, and although this latter factor helps to protect the product against spoilage, it may lead to minor problems of whey separation. Different time/temperature conditions are used for the heat treatment of the various fruits and the microbiological specifications of such products can either be "sterile" or to standards proposed by the fruit processors (see Chapter 10). Although the processing of fruit is sometimes carried out by large dairy organisations, the majority of yoghurt manufacturers rely on specialist fruit processors.

2.12.1.3 Frozen fruits

Frozen fruits are stored at around $-20°C$ for use whenever required. The product is then thawed, sweetened and finally heat treated and, depending on the acidity of the fruit, the temperature of the heat treatment can vary from as low as $60°C$ to as high as $95°C$. Since the freezing process can damage the structure of the fruit, care must be exercised to minimise injury, that is, by harvesting the fruit at a certain degree of ripeness, quick freezing and/or the addition of stabilisers during the heating stage. Colouring matter is sometimes added during processing to offset the browning reactions (enzymatic or oxidative) that can occur during thawing/subsequent heating. The final processing of frozen fruit can be carried out at the dairy, an approach which may be attractive in large-scale factories.

A recent development in fruit processing is the use of the 'osmodehydrofrozen' process which consists of osmotic treatment in sugar solution, limited air dehydration to reduce A_w and freezing and storage (Torreggiani *et al.*, 1988). Fruits processed using this technique require no preservatives, maintain their natural flavour and colour and have an acceptable texture (Erba *et al.*, 1994). Furthermore, when such fruit(s) or dried pieces (Mastrocola *et al.*, 1997) are added to yoghurt, they have the tendency to absorb some of the free or unbound water from the yoghurt gel and hence help to reduce whey separation of the product during storage. Giangiacomo *et al.* (1994) reported that the sensory properties of yoghurt with added osmodehydrofrozen apricot or peach cubes of high solids content significantly improved the consistency of the product; enhanced rehydration properties of osmodehydrofrozen fruits have been achieved in the presence of sorbitol in the syrup (Erba *et al.*, 1994).

2.12.1.4 Miscellaneous fruit products

These may include (a) fruit puree which is homogenised to give an end product in the form of a paste; the shape of the fruit is lost altogether and the fibrous material may also be removed, (b) fruit syrup which is a clear product devoid of solid contents but with a sweetening agent added to it and used during the manufacture of flavoured set yoghurt or drinking yoghurt; in set yoghurt, the syrup is added to the inoculated milk before the packaging and the incubation stages, but for drinking yoghurt, the syrup could be added to the cold natural yoghurt, and (c) jam which is used only during the manufacture of certain types of set yoghurt or in the absence of other processed fruit sources. It is not advisable to add jam to stirred yoghurt since the high viscosity of jam may make it difficult to mix properly with the natural yoghurt; prolonged mixing can result in whey separation or a reduction in the viscosity of yoghurt. However, if jam is used to flavour set yoghurt, a special metering device must be installed on the filling machine so that the required amount of jam is deposited in the carton before it is filled with the inoculated milk.

Low sugar jams can be made with a combination of artificial sweeteners; however, xylitol and sorbitol affect the texture and the use of maltodextrin as a bulking agent can affect the appearance and taste of the product (Hyvonen and Torma, 1983a). The keeping quality of such jams has been studied by Hyvonen and Torma (1983b) and they reported that: (a) jams made with sorbitol and xylitol were of good quality and similar to sucrose-based jam, (b) there was a deterioration in colour, taste and preference during storage of fructose and high fructose jams but, in presence of xylitol, these defects were minimised, and (c) crystallisation and deterioration in the quality of the xylitol–maltodextrin jams was observed during storage.

Bulk fruits (i.e. fresh, sulphited or frozen) have already been cleaned from foreign matter (vegetable matter, insects, stones, metal or sticks) and mouldy, blemished or unripe fruit removed before processing. However, pesticide residues, general microbiological standards and the presence of undesirable additives have to be considered before accepting fruit for processing. A survey of 252 samples of fruits to be used in yoghurt for insect fragments was reported by Locatelli (1988) who found that 15% of large fruits and 58% of berries had been contaminated with insects due to post-harvest contamination.

The heat treatment of the fruits is carried out either in a batch process at 85°C for 10 min or a continuous process at 100°C (flash) (G. Spinks, personal communication; Spinks and Davey, 1970; Szemplenski, 1981). It is important that the tank for

batch processing is designed to minimise fruit damage during heating, whilst scraped-surface heat exchangers are used in the continuous method (see also Sommi, 1996). Some future developments in the processing of fruit may include ohmic heating, high pressure or microwave heating (Langley-Danysz, 1996) and irradiation (Kiss, 1975) but their use is limited at present. However, hot filling of the processed fruit into sterile containers can extend the shelf life of the product without the addition of preservatives (Kivi, 1981; Eller, 1988; Ehrhardt, 1991; Anon., 1993c), as can the use of nitrogen for gas flushing/modified atmosphere packaging (Anon., 1993b).

Some recommended processes for fruits may include (a) stabilisation of the product using apple pectin (Weiss, 1983), or a mixture of low-methoxy pectin and xanthan (Leipold, 1983), or hydroxypropylated starches (Walter, 1996) or amidiated pectin that has been standardised with Ca^{2+} salt and carob seed meal (optional) (Kratz and Dengler, 1995a–c), (b) treatment of cereal(s) and/or museli with a water-in-oil emulsion before mixing with yoghurt maintains the crispy texture (Kaufman et al., 1990), (c) soaking peach slices in a solution of calcium chloride ($0.3\,g\,100\,g^{-1}$) before the heat treatment stage retains flesh firmness (Kim and Choi, 1983), and (d) addition of chopped raisins at a rate of $10\,g\,100\,g^{-1}$ in yoghurt was highly rated by consumers in Chile, but depended on the use of a special variety rather than any type of raisin (Nicholls et al., 1984).

2.12.2 Flavouring agents

The heat treatment of fruit preparations can result in a reduction in their flavour intensity and hence it is the practice to add flavouring agents to compensate for such losses (Nursten, 1982; Werry, 1982; Heath, 1983; Cowle, 1985; Hudson, 1986; Hodrien, 1990; Jaubert, 1992; Fisher and Scott, 1997). Flavouring agents are divided into three categories depending on their source:

- natural flavours and flavouring substances (botanical origin),
- nature-identical flavouring substances (botanical origin),
- artificial/synthetic substances (chemical origin).

Although the above classification may seem simple, in actual fact the list of possible agents can run into thousands. Flavouring compounds of chemical/synthetic origin are sometimes used due to their provision of a flavour similar to that of a natural ingredient (see Table 2.22), but the list of permitted compounds varies from one country to another. In the United Kingdom, the SI (1995) contains a list of flavouring agents which could be added to food; however, according to the FAO/WHO (1990), no list needs to be provided regarding the artificial flavouring substances that are permitted for use in fruit and flavoured yoghurts and related products. Instead, the maximum level of use is limited by good manufacturing practice (GMP), which means that the additive in question is self-limiting in food products including yoghurt with respect to technological aspects, sensory properties or for other reasons. Thus, GMP also means that the amount of the substance added to food products during the manufacturing stages shall not exceed the amount required to accomplish the purpose for which the additive is permitted to be added to food (FAO/WHO, 1990). These compounds are also used during the manufacture of flavoured (set or stirred), drinking, frozen and, possibly, dried yoghurt.

Table 2.22 Some popular varieties of yoghurt and an indication of the additives (nature-identical and synthetic) that can enhance the flavour of a fruit base[a]

| Retail flavour | Compounds naturally present | | Important synthetic compounds |
	Character-impact compound[b]	Important contributory flavour compounds	
Apricot		γ-Decalactone γ-Octalactone Linalool 3-Methylbutyric acid β-Ionone	γ-Undecalactone
Banana	3-Methylbutyl acetate	Pentyl acetate Pentyl propionate Eugenol	
Bilberry		Ethyl 2- and 3-methylbutyrate Ethyl 3-hydroxy-3-methylbutyrate *trans*-2-Hexenal	
Blackcurrant		Methyl butyrate Ethyl butyrate 1,8-Cineole Diacetyl	*trans*- and *cis-p*-Methane-8-thiol-3-one
Grape, Concord	Methyl anthranilate		
Grapefruit	Nootkatone 1-*p*-Menthene-8-thiol	Limonene Decanal Methyl butyrate Ethyl butyrate Ethyl acetate Acetaldehyde	
Lemon	Citral		15 compounds
Orange		Ethyl butyrate Ethyl 2-methylbutyrate Linalool Octanal (+)-Limonene Acetaldehyde β-Sinensal	
Melon (honeydew)	*cis*-6-Nonenyl acetate	Ethyl 2-methylbutyrate	
Melon (musk)	*cis*-6-Nonenal	2-Methylbutyl acetate Dimethyl disulfide	
Peach	γ-Decalactone	γ-Octalactone δ-Decalactone γ-Dodecalactone Linalool	γ-Undecalactone
Pear	Methyl and ethyl *trans*-2, *cis*-4-decadienoate	Hexyl acetate	
Pineapple		Furaneol Methyl hexanoate Methyl 2-methylbutyrate Methyl and ethyl 3-(methylthio)-propionate	Allyl hexanoate

Table 2.22 *Continued*

| Retail flavour | Compounds naturally present | | Important synthetic compounds |
	Character-impact compound[b]	Important contributory flavour compounds	
Plum		Ethyl nonanoate γ-Decalactone Benzaldehyde Linalool Methyl cinnamate	
Raspberry	1-*p*-Hydroxyphenyl-3-butanone	*cis*-3-Hexenol Damascenone α- and β-Ionone	
Strawberry		Mesifuran Furaneol Methyl and ethyl hexanoate Methyl and ethyl butyrate *trans*-2-Hexenal	Ethyl 3-methyl-3-phenylglycidate
Tangerine		Methyl *N*-methylanthranilate Thymol α-Sinensal Acetaldehyde	

[a] Concentration is an important variable. Flavour houses are very skilled in providing concentrates of approved components in the appropriate proportions in an appropriate base. Whether and which compounds may be added differs by country, and is usually legally controlled. [b] A character-impact compound is one where the odour by itself is already strongly characteristic of the named food.

Data compiled from Nursten (1977), Buttery (1981), Maarse (1991) and Winterhalter and Schreier (1993).

Different food products, including alcoholic drinks, have been used to flavour yoghurt and some examples of these are:

- Sweet products (honey, maple syrup, butterscotch),
- Nuts (coconut, hazel, brazil, walnut),
- Cereals (muesli),
- Vegetables (cucumber, tomato, celery),
- Miscellaneous (coffee, mocca, spices, paprika, vanilla).

Flavour is an important aspect of food quality and it is caused by chemicals in food, possibly arising during processing, interactions between chemical components and/or the activity of the starter cultures and their enzymes. The latter will be discussed in Chapter 7, but the following sources are recommended for further reading regarding the science of flavours (Birch and Lindley, 1986; Heath and Reineccius, 1986; Bauer *et al.*, 1990; Acree and Teranishi, 1993; Ashurst, 1994). Nevertheless, some specific compounds have been suggested as flavour additives for yoghurt: (a) odourants and/or flavouring compositions containing a substituted tetralin or indan to modify or enhance berry flavours; the recommended rate of addition was 0.1–2.0 μg g^{-1} of flavoured yoghurt (Gonzenbach and Ochsner, 1983), (b) the use of humulon, which is obtained from hops, as a flavour enhancer in yoghurt about 10 μg g^{-1}; this compound tended to suppress the sweetness of the fruit slightly whilst the flavour was intensified (Klusters and Paul, 1987) and (c) the use of unusual

Table 2.23 Permitted food colouring matter arising exclusively from flavouring substances as a result of carry-over

Name of colour	Maximum level (mg kg^{-1})
Indigotine	6
Brilliant black PN	12
Sunset yellow FCF	12
Tartrazine	18
Cochineal	20
Carminic acid	20
Erythrosine	27
Red 2G	30
Ponceau	48
Caramel[a]	150
Brilliant blue FCF	200

[a] Ammonia or ammonia sulphite process.

Data compiled from FAO/WHO (1990).

flavours in yoghurt such as herbs (Anon., 1993a) or geranium, elderflower, apple blossom and rosehip (Winwood, 1987).

2.12.3 Colouring matter

Colour is added to fruit and flavoured yoghurts to make the products more attractive (Pasch *et al.*, 1975; Ulberth *et al.*, 1993). The active agents may be naturally derived, nature identical, caramel or artificial (Collins and Timberlake, 1993). The list of colours which may be used as food additives differs from one country to another, but it should be noted that the colouring agents permitted in one country may not be identical to those allowed in another. However, the FAO/WHO (1990) have offered some guidance about which colour compounds should be permitted and at what concentrations in yoghurt, assuming that the agents come entirely from the fruit/flavouring ingredients (see Table 2.23). A list of natural colours that can be used in food including the E-number has been provided (Anon., 1993d), whilst Hod (1995) has listed kosher food colouring ingredients.

Fourteen permitted food colourants in South Africa have been evaluated in yoghurt making and were added at 75% of the permitted level (Venter *et al.*, 1988); heating and fermentation of the milk were identified as causing colour bleaching. Otte (1988) reported that by increasing the fat content in the milk base, the colour intensity decreased whilst the colour shade and saturation were only slightly affected. Flavonoids from sandalwood (*Pterocarpus santalinus*) and roselle (*Hibiscus sabdariffa*) have been used to intensify natural colours of fruit purees for yoghurt (Labatut, 1989).

2.13 Packaging

2.13.1 Introduction

Packaging is an important step during the production of yoghurt and Paine (1967) has defined the objective of packaging food as: "Packaging is a means of ensuring

the safe delivery of product to the ultimate consumer in sound condition at minimum overall cost."

In general, the specifications of any food packaging material should include information for the following:

- Toxicity of the materials,
- Levels of contamination,
- Moisture resistance and/or permeability to water vapour,
- Gas permeability for N_2, CO_2 and O_2 (the former gases are important in modified atmosphere packaging),
- Permeability to volatile flavour and aroma compounds and/or chemicals in the environment,
- Transparency to visible or UV light,
- Permeability to dirt and/or to micro-organisms, and
- Migration of molecules from the packaging material to the product.

It is evident that most, if not all, of the above mentioned specifications for packaging material are applicable to yoghurt packaging. However, as can be observed from the subsequent sections, there are many different types of packaging materials and some selected publications on the theory and practice of food packaging have been reported by Paine (1969), Sacharow and Griffin (1970), Paine and Paine (1983), Griffin *et al*. (1980), Peleg (1985), Mathlouthi (1986), Briston (1989), Jenkins and Harrington (1991), Stöllman *et al*. (1994) and Soroka (1995). Periodically the International Dairy Federation publishes monographs updating the technical information available on the packaging of milk and milk products and specific aspects dealing with fermented milks have been reported by Fluckiger (1976, 1980, 1982) and Odet (1984, 1988, 1995).

2.13.2   Functions of packages

If yoghurt is to reach the consumer in a sound condition, the packaging material will play an important role, and the retail package should be designed to meet the requirements, to provide protection, to be easy to handle, to provide a vehicle for a message and so on.

2.13.2.1   *Provide protection*

Yoghurt is a highly perishable product and the purpose of the container is to protect it from the environment, that is: (a) dirt or other foreign bodies, (b) micro-organisms (bacteria, yeast and moulds) which can affect the keeping quality of yoghurt, (c) gases (e.g. oxygen) which can help the yeasts and moulds to grow and spoil the product, and (d) light which may cause discoloration of fruit/flavoured yoghurts or possibly oxidation of the fat.

Product protection also seeks to avoid spillage, pilferage or loss by evaporation. The latter aspect is doubly important, since loss of moisture can not only affect the chemical composition of the product, but may also lead to deviations from the declared weight on the package and possible problems with the weights and measures authorities. In addition, the package must prevent the loss of flavour volatiles or the absorption of undesirable odours. However, a detailed study by Bosset and Fluckiger (1986a, b, 1987) and Bosset *et al*. (1986a, b, 1995) evaluated the impact of environmental aspects, such as light and temperature on the quality of yoghurt

packaged in different containers (e.g. glass jars (coloured and uncoloured), polystyrene cups (transparent coloured or uncoloured) and non-transparent overwrapped with a cardboard sleeve), and suggested the following:

- The protective effects differed with the type of packaging material used and protection from light was identified as affecting the quality of the product more than protection from oxygen, though these factors can have synergistic effects; however the rate of O_2 permeability across the packaging material should be very low for long life or pasteurised yoghurt.
- Transparent uncoloured glass jars or polystyrene cups provided least light protection; the latter type of package also had high gas permeability.
- Depending how the containers were stored (on its side, upright or upside down), the aluminium foil cover and polyethylene lids gave less effective protection of the product.
- Less damage to the product could be achieved during storage by applying the following protective measures. In the absence of aseptic facilities during production, filling and storage at 8°C should not exceed 16–18 days. Use of fluorescent lighting caused less damage, e.g. Philips TL82.
- It was noted also that the sensitivity of the analytical methods used to measure the degree of photodegradation in the product varied.

2.13.2.2   Ease of handling
Yoghurt and related products usually exist in the form of viscous liquids. The retail container must provide a convenient means of handling the product in the factory, during storage and transport, and throughout the sale period in supermarkets and shops.

2.13.2.3   Provide a message
The printing and other graphic work on the exterior of the package will serve to provide the product with a "brand image" and/or display a message to persuade a potential buyer to purchase, and will contain the information proposed in the guidelines for food labelling such as:

- identity of the product,
- name and address of the manufacturer,
- approximate chemical composition or nutritional data of the product, or the ingredients listed in descending order by weight,
- best-before-date,
- possible suggestions of recipes or other instructions for use.

2.13.2.4   Miscellaneous functions
In general a packaging material which is in direct contact with a foodstuff must be non-toxic and no chemical reactions should take place between the material and the food product (refer to Crosby, 1981; Jensen, 1972; and Section 2.13.1 for further information). For these reasons, plastics are widely used in the dairy industry, and due to the acidic nature of yoghurt, aluminium foil is used for lids, unless plastic "push-on" lids are more suitable.

It is against this general background that the following approaches to marketing yoghurt have evolved.

2.13.3   Types of packaging materials
Packaging materials for yoghurt are basically divided into two main categories: in the first instance, the unit container is the vessel which comes into actual contact with the yoghurt and the specifications mentioned above regarding the "ideal" package are applicable to such containers, and in the second instance, the outer or shipping container does not come into contact with the yoghurt, but is used to facilitate handling and dispensing of the unit containers along the retail chain.

Different types of unit container are available on the market, and these packs may be classified into three main types depending on the physical strength of the container.

2.13.3.1   Rigid unit containers
Glass bottles are still used in some countries, for example, France and Eastern Europe and some parts of the Middle East to package yoghurt (Fluckiger, 1980), and although glass is an excellent packaging material, its use is limited by the high cost of manufacture and the current market trend in favour of "single-trip" containers. Nevertheless, the glass bottle was very popular and, even today, wide mouth glass bottles are the most attractive form of packaging for flavoured yoghurts; closure is by a metal "pull-ring" or screw-on metal cap. A closure system involving heat sealing aluminium foil laminates onto glass bottles is available in different markets.

The system of recycling or returning the empties on a voluntary basis could affect the use of glass bottles and some studies have been carried out in Switzerland and Germany (Anon., 1983b; Berndt, 1984; Regez, 1984). It may be that the increasing pressure to improve the environment and to educate the consumer will increase use of returnable glass jars for yoghurt packaging.

Earthenware vessels are produced from clay and the part of the container which comes in contact with the yoghurt is normally glazed. They are returnable and are used in the Middle East and India to package set yoghurt and dahi, respectively. During the incubation period the pots are left uncovered so that a crust is formed on the surface and, before the cooling stage, the pots are covered with parchment held firmly in position using a rubber band. These containers are not widely used due to problems of achieving a high standard of hygiene and the cost of manufacture. Thus, Singh (1978) evaluated the microflora of earthenware pots used for dahi and reported that high total counts, as well as coliforms, *Staphylococcus* spp. and yeast and moulds, were normally present. Improvements in the microbiological standards of these pots could be achieved if the pots were immersed in boiling water for at least 2 min or water containing $250–500\,\mu g\,g^{-1}$ chlorine. However, the clay used to manufacture this earthenware may contain $30–100\,\mu g\,g^{-1}$ of lead and, as a consequence, the lead content of set-type dahi made in these pots was $1.743\,\mu g\,g^{-1}$. This level of lead is still below the permitted limit $(7\,\mu g\,g^{-1})$ proposed by the Food and Drug Administration in the US (Nagaraja and Vishweshwaraiah, 1986), but it is another factor that operates against the use of earthenware.

Other rigid containers that are recommended for the packaging of some types of yoghurt-based products, for example, dried yoghurt, are metal cans or aluminium foil laminated pouches. The keeping quality of the product is improved by gas flushing (nitrogen or carbon dioxide), nitrogen being more widely used. These metal containers are similar to those used for packaging of whole milk powder. Rigid,

semi-rigid or flexible plastic containers could also be used (see later), as could composite containers coated internally with a layer of plastic.

2.13.3.2   Semi-rigid unit containers

These types of container are normally manufactured from plastics and some technical properties of the different types of plastic material (i.e. water vapour transmission rate and gas transmission rate of O_2, CO_2 and N_2) that can be used for the manufacture of containers for yoghurt have been reported by Kumar (1989), Cuq *et al.* (1995) and Guilbert and Gontard (1995). The actual plastic materials (i.e. the polymers) are relatively inert, but the chemicals and monomers used during the fabrication stages can be deposited in the finished material. Although such compounds may be harmless *per se,* they can react with the food and give rise to off-flavours, and hence great care has to be exercised to ensure that such compounds are absent.

In the case of yoghurt, the container must be acid resistant, prevent the loss of flavour volatiles and be impermeable to oxygen, since the presence of the latter can encourage yeast and moulds to grow. Examples of materials which could be used for the manufacture of the yoghurt containers are: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polyvinylidene chloride (PVDC). In the United Kingdom, the majority of the containers are manufactured from PS, although the use of PP is becoming popular. However, irrespective of the material, the containers can be either rigid, semi-rigid or flexible, and while the former categories are normally used to package set and stirred yoghurt, concentrated yoghurt and/or frozen yoghurt, the flexible type (i.e. film – see later) can only be used to package dry yoghurt-based products.

The finished containers are referred to as cartons, tubs or cups and can be manufactured in any shape or design that appears to possess consumer appeal; some typical examples are illustrated in Fig. 2.18. Basically, there are two different techniques that can be used for the manufacture of plastic cups. Firstly, the injection moulding process in which the material is softened in a heated cylinder prior to injection under high pressure into a cooler/mould where it hardens. After the cup is formed, it is ejected from the mould. This type of container is characterised by having a relatively thick wall, that is, it is a rigid cup (Astrom, 1989; de Groof, 1993). These preformed cups are then delivered to the dairy nested in rows inside a thin PE bag (i.e. of 25 μm thickness). Usually the bags are sealed to prevent the ingress of dust or other contaminants and are overwrapped with a fibreboard box to ensure safe transit and prevent crushing. At the dairy the rows of cups are fitted onto the filler and the process of packaging is then referred to as a fill/seal operation. Second is the thermoforming process, in which the plastic material is delivered to the dairy in the form of a continuous roll, one end of which is fed into the first section of the yoghurt filling/packaging machine. The sheet of plastic is heat softened and formed into or around a mould, so that the unit container is formed immediately prior to filling with the yoghurt. This system of packaging is referred to as a form/fill/seal operation. In the thermoforming process, the yoghurt cups have a relatively lower wall thickness than those produced by the injection moulding system, and the containers could, therefore, be classified as semi-rigid. Incidentally, the reels must be delivered to the dairy well overwrapped so that they do not get damaged in transit or storage.

Irrespective of what types of cup are used, closure of the container is usually achieved using aluminium foil (i.e. capping/crimping or heat-sealing) or plastic

Fig. 2.18 Illustrations of some containers for the packaging of yoghurt

Top row: Illustrates multipack containers and different packs for drinking yoghurt (e.g. rigid, plastic bottles and cups, and a ring-pull metal can). Middle row: Yoghurt containers consist of glass jars (left hand side) and twin chamber packs (right hand side). Bottom row: Laminated paper board cartons (Pure-Pak and Tetra Brik); some have recloseable lids or special hole for a straw (left hand side). Containers on the right hand side illustrate thermoformed and preformed plastic containers.

(press-on lids or heat sealing). Heat-sealed caps are more popular, since the cups are then watertight and subsequent contamination and seepage are prevented. Aluminium foil is used because its permeability to gases and odours is negligible, and, in addition, it is greaseproof, opaque, "brilliant" in appearance and can be easily decorated. Because of the acidic nature of yoghurt, it is recommended that the foil should be lacquered to prevent corrosion and, to provide cohesion during heat sealing, the foil should be coated with PE, ethylene vinyl acetate copolymer (EVA), PS or PVC (see later for further detail). Heat-sealed plastic lids are sometimes used.

Plastic packaging materials are also used in the yoghurt industry to provide so-called fresh crunch products for the consumer. An example of one such innovation was reported by Colangelo (1980) and Anon. (1983c, 1991b) in which the yoghurt was packaged in what was referred to as a "piggy-back" configuration. In this system the flavoured yoghurt is filled and sealed into a plastic cup. Either on the same machine, or on a different unit, nuts, raisins and carob chips (known as granola) are filled into another transparent cup which is also heat sealed. Then the yoghurt tub and the cup containing the granola slot together so that the latter container completely covers the yoghurt tub prior to the two packages being heat-sealed or crimped together. In theory, the freshness of the fruit/nut mixture is retained until the consumer mixes the two components together just before consumption, but there is little evidence to date that the additional labour is really justified.

Another type of semi-rigid cup (i.e. 2–4 cm in depth) has been patented in France (Verdier, 1987) and is suitable for packaging yoghurt to enable consumers to eat the yoghurt using their tongues without the aid of a spoon. Two pots can be attached

together to provide an adequate portion of the product, but this type of package has not been used yet by the industry.

As a saving on the cost of rigid plastic beakers, a thin polystyrene beaker was suggested, surrounded by an envelope of recycled paper (Poldervaart, 1994). This type of packaging is known as the K-3 system and allows easy separation of the paper from the plastic by flattening the container when empty. Nevertheless, the current interest of plastic manufacturers is to replace PS with PP because (a) there are cost savings (Recaldin, 1990), (b) there are residual effects of the styrene monomer in the product (see Section 2.13.5) which could affect the flavour and (c) burning PS at <1200°C yields large quantities of soot that pose an environmental problem (Løkkeberg, 1993). However, for the purposes of recycling mixtures of plastic consisting of PS and PP, the two plastics can be separated, after washing to remove organic material and grinding, on the basis of specific gravity, using a hydro-cyclone or swim-sink process (Wirths, 1991).

2.13.3.3 Flexible unit containers

Flexible unit containers are either in the form of plastic sachets or paper cartons. The former type are made from laminates, (e.g. PE/aluminium foil/PE or PE/paper/aluminium foil/PE) and are only used to package dehydrated yoghurt. The most popular method of filling is the form-fill-seal approach. The container must be impermeable to gases and water vapour.

Paperboard cartons became a popular container for dairy products in the 1950s with the introduction of the waxed cartons. These containers were used in the past for the packaging of yoghurt, but their popularity has diminished in favour of plastic cups and/or laminated paper cartons. One disadvantage associated with the waxed carton is its tendency to leak and despite improvements in manufacture (i.e. the application of a multilayer coat of wax and EVA copolymer), their use as yoghurt containers in the United Kingdom has remained limited.

However, the use of cartons to package liquid milk is widely practised in North America, Europe and the United Kingdom and such containers could be easily used to package yoghurt (Fig. 2.18). Two types of carton are normally available, a simple type where ordinary paper board is coated on both sides with a plastic material, (e.g. PE) and a multilayer type which consists of the following layers: PE/paper board/aluminium foil/PE. The latter type of carton is normally used for packaging UHT milk, since the aluminium foil layer not only renders the carton impermeable, but also helps to improve the rigidity of the container.

Depending on the exact system employed, paperboard cartons are delivered to the dairy either as collapsed preformed cartons (e.g. the Pure Pak, Elopak or Tetra Rex methods) or in the form of a reel (Tetra Brik). The sequence of packaging followed for preformed cartons is:

- A bank of collapsed cartons is fed into a special sleeve of the filling machine.
- A single carton is automatically removed from the sleeve, opened and the bottom is sealed.
- The carton is filled with yoghurt and the top sealed.
- The packaged product is ready for dispatch.

Alternatively, the cartons can be formed from a reel using the technique of form-fill-seal and an illustration of the sequences involved in the formation of one such

container, prior to filling it with yoghurt, is shown in Chapter 3, Section 3.3.11. One common feature of these packaging systems (e.g. Pure Pak, Tetra Rex or Elopak) is that the carton has a gable end. In some instances this gable structure may prove to be useful for pouring the product, but one disadvantage is the large storage area required compared with that needed for yoghurt packed in flat-top cartons. However, the recent development of the "flat top" Tetra Rex and Pure Pak cartons, which are relatively square in shape, combines the desirable features of a gable (i.e. excellent pouring characteristics) with efficient utilisation of the space in refrigerated cabinets.

Improvements in packaging materials for cartons used for fermented milk products, including the effect on the quality of the product, are (a) the CO_2 concentration of kefir and similar cultured milks packaged in double-layered PE was reduced by 75% and whey drainage occurred, whilst with aluminium foil-lined cartons, the CO_2 concentration was reduced only slightly and the flavour and stability of the products were significantly improved (Anon., 1986c; Castberg *et al.*, 1986; Gjengedal and Oterholm, 1988), (b) the introduction of a specially designed filling valve into a gable-top carton using a Cherry Burrell QL-9 machine makes it possible to package a drinkable type of yoghurt (Anon., 1986d), (c) Ensobarr® is a newly developed paper board gable-top carton from Finland which is suitable for packaging yoghurt; the aluminium foil layer has been replaced by a Chemi Thermo Mechanical® pulp, which provides a given stiffness using less weight of paperboard, and has better folding and recycling properties (Holmström, 1996), and (d) the provision of a straw for 250 g Pure Pak, or a screw cap for 1 l cartons increased the packaging options for drinking yoghurt (Schlicht, 1996).

Finally, the laminated paper cup is used in the U.S. and some other countries where the cups are preformed and delivered to the dairy nested in cardboard boxes. These cups are sealed using press-on lids or possibly heat-sealed foil lids (Anon., 1984b, c).

The size of the above containers is divided into two main groups, "single serve" cartons, with content ranges from 150 to 200 ml (in some cases it may be less), and "family size" cartons where the capacity of the container ranges from 250 to 1000 ml (Herner, 1988). In the latter sizes, press-on lids are also provided, since not all the yoghurt may be consumed at the same time and it is necessary to provide, for reasons of hygiene, a lid which can be reclosed. Incidentally, a type of intermediate container which is becoming very popular is the special purpose multipack (see Fig. 2.18), where four, six or more yoghurt cartons are packaged together. These multipacks were introduced into the market in the 1970s (Lang, 1972; Chaussadas, 1986; Anon., 1989; Keck, 1983, 1991a; Hilliam, 1992; Hartman, 1995), and are sometimes used when launching a new fruit yoghurt onto the market, or alternatively they are used as a family pack. A similar type of family pack is now widely produced on form-fill-seal machines (i.e. thermoformed), where four or more yoghurt cartons (each pair is a different flavour) are formed as one composite unit. Over the past decade a twin chamber or tub pack has been introduced and in this pack natural yoghurt is filled into the larger compartment whilst the fruit flavour is packaged in the smaller chamber. The main advantage of this pack is that the yoghurt manufacturer need not stop and clean the equipment when changing from one product flavour to another (Ackermann and Guays, 1984; Zott, 1989). The normal practice is to start by filling the light coloured fruits and progressively changing to the darker types. Although expensive, this system of packaging is popular with

the consumer who can mix the fruit with the yoghurt prior to consumption in a quantity to suit his/her palate.

It is evident that a wide range of packaging containers are being used in the yoghurt industry and the ultimate choice could be influenced by the following considerations:

- Cost per unit container, speed of filling and cost of packaging machine,
- Nature of the yoghurt products (e.g. liquid, viscous, concentrated or powder),
- Provision of product protection during storage, distribution and retailing,
- Capacity of the unit container,
- Returnable or non-returnable package and, in the case of the former type, whether the container can be cleaned and sanitised,
- Requirements for a specific duration of shelf life, including the barrier properties (e.g. O_2 and light permeability) of the material,
- Marketing concepts and consumer acceptability (Odet, 1988).

One aspect, which has always been debated in the dairy industry, is the scenario regarding glass versus non-returnable containers including cartons and plastic cups or bottles. Some reports have been published by Anon. (1983b, 1988, 1994b), Bojkow (1986), Keck (1991b), Robinson (1991) and Thalmann and Schmid (1996). Cost and environmental aspects are the major factors which have to be considered before choosing any one type of packaging material, but according to Robinson (1991), the solution of waste problems is difficult and, in the case of plastics, may have serious consequences in the future.

2.13.4   Comparative studies on the permeability of different yoghurt packages

As mentioned earlier, the work of Bosset and Fluckiger (1986a, b, 1987) and Bosset et al. (1986a, b) has highlighted the effect of light and O_2 on the quality of yoghurt held in a wide range of containers. However, other studies which have shown a direct correlation between the permeability of the packaging material and the quality of yoghurt can be summarised as follows:

- The O_2 permeability (kPa day^{-1}) through a Pure Pak carton (i.e. PE/paper board/PE) was 0.77 mg and 1.79 mg at 7°C and 25°C, respectively; these permeability values were about half the theoretical values calculated for the O_2 permeability of PE layers of 0.03 mm and 0.015 mm, respectively (Langeveld et al., 1984).
- Cultured buttermilk packaged in high density PE bottles was stored at 1°C and exposed to white fluorescent lighting for 96 hour, and these conditions induced an off-flavour and reduced the riboflavin concentration by 0.3 μg ml^{-1}. A taste panel could not identify the light-induced off-flavour in the experimental samples (Hoskin, 1989); Bosset et al. (1995) also confirmed that yoghurt is a light-sensitive product.
- Yeast and mould counts (>10 colony forming units (cfu) g^{-1}) were detected in 30 out of 60 containers of yoghurt in preformed containers, whilst none of these organisms were detected in the same yoghurt packaged in form-fill-seal containers (Jordano, 1987); this may reflect differences in O_2 permeability and/or the sterility of the container before filling (McKay, 1992).
- Brown glass bottles prevented photo-oxidation of yoghurt (natural, strawberry, chocolate or mocca), whilst only natural yoghurt was susceptible to light when

packaged in transparent PS containers (Dieffenbacher and Trisconi, 1989); a marked decrease in the green and yellow hues due to loss of riboflavin was noted (Desarzens, 1989) and pentanal was identified as the carbonyl compound produced during photo-oxidation (Daget, 1989).

2.13.5 Migration of monomers and other compounds

In the U.K., according to the Ministry of Agriculture, Fisheries and Food (MAFF, 1983, 1987), there were until 1974 insufficient toxicological data available on styrene to assess the long term safety of food in contact with it. Since then there have been numerous analytical studies and the results of surveys of styrene levels in yoghurt in different countries are shown in Table 2.24. It is evident, however, that the residual styrene in yoghurt is well below the tolerable daily intake ($0.6 \mu g\, g^{-1}$) proposed by the EU Scientific Committee for Food (Hammarling *et al.*, 1995).

Factors that can influence the migration of many compounds from packaging materials, including PS and PP, include product filling temperature, fat and moisture contents and pH (Thomsen and Stena, 1987). However, the migration of monomers does not affect the colour of yoghurt and the rate of migration after 2 days at 45°C was not more than $1.1\,mg\,dm^{-2}$ (Macias Matos *et al.*, 1988). Nevertheless, caution is essential and three out of 20 yoghurt pots made in Cuba exceeded the migration limits set by the Hungarian Standards (Garcia Melian *et al.*, 1988). Both yakult (a Japanese fermented beverage) and yogo (a drinking yoghurt) sampled in Hong Kong contained no styrene monomers, despite the fact that the styrene content of the yogo plastic cup was about $150 \mu g\, g^{-1}$ (Lau *et al.*, 1995). Another monomer that has been detected in yoghurt is ethylbenzene at a level ranged between 2 and $4 \mu g\, kg^{-1}$ (Ehret-Henry *et al.*, 1994).

Cultured skimmed milk from three factories packed in Tetra Pak and Elopak cartons contained different levels of aluminium after storage at 4°C for 20 and 40 day, respectively; the results suggested a slight increase ($8-18 \mu g\, kg^{-1}$), while some samples showed a reduction ($3-42 \mu g\, kg^{-1}$) in the aluminium content after storage (Eklund and Brenne, 1990). These results may indicate that the increase or decrease in aluminium content was influenced by the type of cartons or milk utilised for processing, although the level was satisfactory according to Eklund and Brenne. Further

Table 2.24 Styrene content in yoghurt made in different countries

Country	Number of samples	Fat[a]	Residual styrene (range)
U.K.	34	NR	$<1-200 \mu g\, kg^{-1}$
Chile	16	NR	$0.08-0.19 \mu g\, kg^{-1}$
Holland	8	0.1–0.5	$3-4 \mu g\, kg^{-1}$
	16	1.5	$5-11 \mu g\, kg^{-1}$
	16	3–3.5	$2-5 \mu g\, kg^{-1}$
Sweden	11	NR	$\sim 0.01\, mg\, kg^{-1}$
France	4	0	$10-12 \mu g\, kg^{-1}$
	6	3.5	$8-11 \mu g\, kg^{-1}$

[a] Fat content expressed as $g\,100\,g^{-1}$.

NR, Not reported.

Data compiled from MAFF (1983), Gilbert and Startin (1983), Carmi and Santa Maria (1984), Linssen *et al.* (1993), Ehret-Henry *et al.* (1994) and Hammarling *et al.* (1995).

Table 2.25 Taste recognition threshold concentrations of styrene in yoghurt

Product	Fat (g 100 g^{-1})	TRTC (μg g^{-1})
Yoghurt		
Natural	0.1	36
Natural	1.5	99
Natural	3.0	171
Yoghurt drinks		
Natural	0.1	82
Strawberry	0.1	92
Peach	0.1	94

migration studies from paperboard packaging material have been detailed by Castle *et al.* (1997a, b), whilst Linssen *et al.* (1992) reported that volatile compounds (i.e. >8 carbon atoms) and highly branched components in artificially flavoured drinking yoghurt tended to be absorbed by PE bottles. Incidentally, these PE bottles were composed of three layers: a PE layer with 2% carbon sandwiched between two PE layers with 5% TiO$_2$ to create white inner and outer layers.

Although the styrene monomer can only, in part, be removed from the polymer by extrusion of the packaging material (Linssen *et al.*, 1995), the level of residual monomer can cause off-flavours in the product. Thus, flavour threshold values of such monomers are important parameters with respect to detecting off-flavour perception in food products. Jensen (1972) reported the following flavour threshold for monomers from PS (μg g^{-1}) in yoghurt: 0.2 styrene, 0.9 ethylbenzene, 7.0 *o*-xylene and 1.0 cumene. However, the taste recognition threshold concentration (TRTC) of styrene in yoghurt is influenced by the presence of sugar and flavouring material and the level of fat (Table 2.25; Linssen *et al.*, 1993, 1995).

2.13.6 Tamper-evident packaging

Since the 1980s, the general consensus in the food industry has been in favour of tamper-evident packaging that enables food retailers and consumers to identify opened packages easily. This approach has become universal in the yoghurt industry, because the older systems of closure (e.g. snap-on plastic lids or crimped foil caps on glass jars) are not acceptable safeguards of public safety.

Many different types of tamper-evident packs have been developed for the food industry and the systems available for yoghurt include the following:

- Heat sealing of foil laminates to a plastic container should ensure a secure seal so that tampering with the container shows as visible damage to the foil.
- Sealed cartons (e.g. Pure Pak or Elopak) have flaps that are securely sealed and tampering shows as visible damage.
- Shrink-wrap film around yoghurt pots sealed with press-on plastic lids or around a set of pots provides another method of packaging that is tamper-evident.
- Hot filling of yoghurt causes the foil laminate heat-sealed lids to adopt a concave shape after cooling; opening the plastic cup causes the lids to change shape and a similar effect occurs with metal caps if yoghurt is packed in glass jars.

- Heat sealing of press-on plastic lids (see Johansen and Buer, 1991) is a newly developed tamper-evident system.
- Breakage of the aluminium ring-pull, which is sometimes used to seal glass bottles, indicates tampering.
- In some instances, a narrow paper strip is sealed over the metal cap used for glass jars and the seal must be broken to open the jar.
- The use of security closures, such as "pull-up" plastic strips or pilferage proof screw caps on wide mouth plastic jars or rigid plastic bottles and cartons, respectively, provide alternative tamper-evident systems which have been used in the yoghurt industry; such systems are sometimes known as breakable caps.
- Tear away closure systems include a "pull-tab" attached to a tamper-evident device consisting of horizontal and vertical ribs; these devices are ruptured easily (i.e. removal of the central portion of the lid) due to force applied on pull-tab (Anon., 1995b).

It is evident, however, that different tampering-evident systems can be used with all the yoghurt packaging containers mentioned in Section 2.13.3 and although these systems can increase the cost of production, product safety can satisfy consumer concerns. More details of tamper-evident systems for foods, including fermented milk products, have been reported by Herner (1987c), MacDonald and Cox (1988) and Freeman (1992).

2.13.7   Aluminium foil lids

Aluminium foil is widely used to seal yoghurt containers (e.g. plastic cups) and due to the acidic nature of yoghurt and the necessity of heat sealing, the aluminium foil is normally coated with a layer of plastic. If the preformed type of plastic cup is used, the aluminium foil lids are usually precut and around 2500–3000 lids are packed into a special magazine to minimise mechanical damage. The diameter of these lids is <100mm, and they usually have a pull-tab for easy opening.

The gauge of the foil is around 40μm, and each lid is normally embossed. The embossing pattern can be varied to suit the customer's requirements and the impression can be up to 100μm in depth. The embossing is essential to facilitate easy pick-up of single lids from the magazine assembly prior to placing over the filled cup and heat sealing.

For yoghurt packaged using the form-fill-seal technique, the aluminium foil is delivered to the dairy as a reel, with the width of the reel being varied in relation to the number of filling heads (abreast) on the packaging machine. The aluminium foil (gauge around 40μm) is similar to that of the precut type, but the embossing process is omitted since it would serve no function.

As can be observed from packaged yoghurt on the market, both types of lid (precut or from a reel) can be printed with different information and attractive designs. The technique of printing could be the flexograph or the gravure; the latter method is normally used where more than five different printing colours are required. The reverse side of the lids is coated with heat-sealable material and the thickness of the laminate ranges between 6 and $10\,g\,m^{-2}$. The variation in the thickness of the lacquer is directly dependent on the type of heat-sealing material used and, for example, $6–8\,g\,m^{-2}$ of EVA is applied to foil intended for heat sealing to polystyrene or polypropylene. For the latter type of plastic cup, a modified version

of EVA is used, that is, it has a stronger solvent intended for higher temperature sealing purposes. On the printed side of the aluminium foil, the EVA lacquer is coated with a layer of high heat varnish in order to protect the graphic design during the heat-sealing stage at the dairy (J.R. Englehart, personal communication). Elms (1989) has reviewed the latest applications of ethylene acrylic acid (EAA) as a sealant for flexible packaging.

Some further types of lids for plastic pots may include: (a) a breathing membrane which consists of a three-ply lidding system that is suitable for packaging kefir in order to minimise the pressure buildup (due to considerable CO_2 production) that would ultimately lead to heavy bulging of the container (Fluckiger, 1986), (b) a specially designed pot and metal foil sealing system for packaging hot yoghurt that eliminates the vacuum generated inside the pot during the cooling of the product (Padovani, 1987), (c) a sealing system for pots containing drinking yoghurt that consists of a lightweight paper and thin foil laminate with a weakened zone for insertion of a straw (Huet, 1986), (d) a cap with a peripheral rim that is strong and easily peeled off the container without undergoing distortion (Kretz, 1987), (e) a new type of foil, which has been developed in Hungary, consisting of a laminate of PP/PE (Stark, 1986), (f) paperboard flexible foil laminates interacting with lactic acid were observed not to adhere during storage (Olafsson and Hildingsson, 1995); one reason for this could be the polarity and chemical stucture of the acid, and (g) a welded seal plastic lid which can be replaced on a partially empty container once the seal has been broken; this development makes the lid tamper-evident (Johansen and Buer, 1991).

2.13.8   Sterilisation of packaging materials

Sterilisation of packaging materials (i.e. plastic cups and lids, foil laminates or paperboard cartons) in the yoghurt industry ensures that possible post-production contamination of the product is minimised and meets the criteria required when using an aseptic processing system. These criteria include that the yoghurt must be sterile, the packaging container and/or materials in which the product is packed must be sterile and the environment/chamber where the sterile product and containers are brought together must be also sterile (Ito and Stevenson, 1984). Illustrations and descriptions of aseptic yoghurt machines will be given in detail in Chapter 3. Little published data are available on the microbiological quality of packaging containers; however, Asperger (1983) reported that the total count (i.e. mainly sporeformers) of plastic cups was < 10 cfu $100 \, ml^{-1}$ rinse and coliforms or moulds were absent. Data on microbial properties of other packaging materials were also reported by Asperger and in view of such information, it is clear that the sterilisation of yoghurt packaging material is necessary during the manufacture of "long-life" yoghurt.

It is safe to assume that form-fill-seal yoghurt plastic cups are commercially sterile due to the high temperature required to make these containers. However, preformed plastic cups and/or collapsed cartons may require sterilisation before filling them with yoghurt in order to minimise postproduction contamination. Astrom (1989) has reviewed the different systems available to sterilise the packaging material for aseptic process and these may include the following methods:

- hydrogen peroxide (H_2O_2) spray, dip or vapour,
- steam,

- hot air impregnated with H_2O_2,
- thermoforming containers using form-fill-seal system, perhaps plus H_2O_2,
- dry hot air,
- ethylene oxide,
- combination of H_2O_2 plus UV light,
- gama radiation (see also Ito and Stevenson, 1984; Neijssen, 1993).

Radiation and H_2O_2 sterilisation of dairy packaging materials are the most commonly used methods; however, in the latter method, the sterilant is removed by heat and its residue on the surfaces may have delayed its acceptability in some countries. Nevertheless, the effects of H_2O_2 sterilisation on the migration of monomers of PP and PE to the food are negligible (Castle *et al.*, 1995) and the only slight change made to these plastics was a superficial modification of the polymer surface.

The use of UV-C lamps to sterilise yoghurt plastic cups and foil laminates was first reported in the early 1980s in an aseptic Hamba filling maching (E. Möller, personal communication). The filling/closure operations take place in a cabinet which is kept free of contaminants by a stream of sterile air at 30–40°C; this air temperature is recommended to prevent condensation. The components of the filling machine are cleaned and sterilised using cleaning-in-place (CIP). The development of UV-C lamps for Hamba machines was well documented by Möller in a lecture in the Department of Milk and Dairy Industry at Hanover University in Germany (see also Hansen, 1980; Möller, 1982). The intensity of the UV-C lamp is in the range of 100–200 mW cm^{-2}, and the distance between UV-C lamp and the packaging material is adjusted in such a way that the cups are at a distance of 10.5 cm; the total exposure time is around 7.5 s and three emitters are used to sterilise the entire inner surface of the cup. The aluminium foil lids are exposed for 2 s at a distance of 4 cm. The efficiency of UV-C lamps against different bacterial species is illustrated in Fig. 2.19 and according to Möller (1982), the shelf life of fruit yoghurt packaged in containers sterilised by UV-C lamps was extended to 42 days at 5–7°C.

In some instances yoghurt may be aseptically filled in bags or bag-in-box containers (see Prahlad, 1989). Martin (1982) details the facilities required for such methods of packaging, and these comprised a laminar flow cabinet fitted with air sterilisation, decapping, filling and recapping devices. Along the side wall of the cabinet, an opening provided access through which the prewrapped sterile bags were introduced to be unwrapped, filled and capped. A UV sterilisation system was provided and the method of packaging could be either fully or semi-automated.

2.13.9   Outer or shipping container

These types of packaging material do not come into contact with the yoghurt, but their importance in the industry is to facilitate easy handling and stacking of the cups during storage, transport and display in supermarkets. Different types of container can be used, divided into two groups, returnable and single-trip.

The returnable containers (or crates) are made of metal or rigid plastic, but since the crates require collection, they are not widely used. However, metal crates are popular where set yoghurt is produced in glass bottles and the fermentation process takes place in a water bath. This method of processing is not widely used.
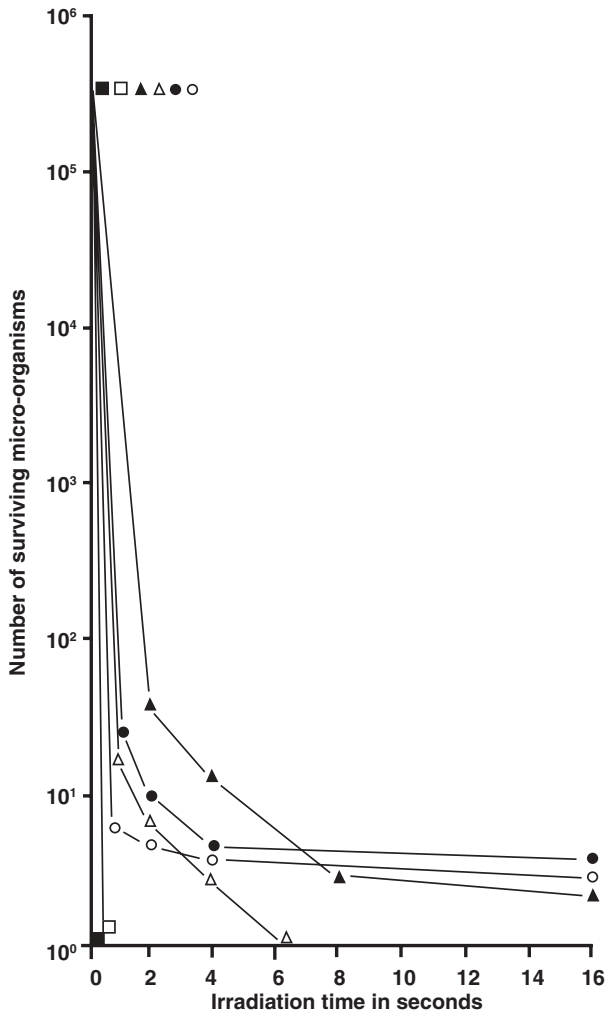
Fig. 2.19   Inactivation of bacteria, yeast and moulds by UV-C radiation

Total microbial load is 5×10^5 cfu ml^{-1} and test area is 36 cm^2.

●, *Bacillus stearothermophilus*; ○, *Bacillus subtilis*; ▲, *Aspergillus niger*; △, *Penicillium frequentans*;
■, *Saccharomyces cerevisiae*; □, *Rhodotorula graminis*.

After Möller (1982, personal communication).

Single-trip containers are more widely used than the returnable type and some of the different types available on the market are:

- semi-rigid plastic crates
- nest trays (flexible plastic or any similar material – see Anon., 1980b)
- cardboard trays.

The latter types of tray (or paperboard cartons) can be overwrapped with a heat-shrink material or alternatively the nested trays can be piled on top of each other (4–6 trays high) inside a cardboard box.

The choice of any one particular system and/or type of outer container is governed primarily by such factors as:

- cost,
- degree of mechanisation,
- ease of dispensing and marketing,
- stackability and ease of cold air circulation in the refrigerated store.

This latter aspect is important if the yoghurt is filled at 20°C and final cooling takes place in the cold store.

In large organisations, the trays (overwrapped with heat-shrink material) or cardboard boxes of packaged yoghurt are usually stacked on a wooden pallet which is later shifted using a forklift truck, that is, from production area → cold store → transport vehicle. Alternatively, metal trollies could be used, for example the Tetra-tainer type produced by the Tetra group. The packaged yoghurt in its single-trip shipping container is stacked onto these trollies. The advantage of this system is the ease of movement of the product from cold store to transport vehicle and from transport vehicle to supermarket and/or refrigerated cabinet. Such methods of handling mean that the retail cartons are not handled at all from the time that they leave the dairy until they can be picked up by the consumer.

Handling the outer or shipping container(s) can be integrated, in part, with the packaging and/or filling machines and the degree of automation is primarily governed by the throughput of the filler and the cost of labour. Manual handling of these containers is very common where the labour costs and the daily scale of production are low. Some of the devices for moving the yoghurt cups or cartons into shipping containers include: (a) a specially designed nested packaging tray (made from cardboard or plastic) holds the yoghurt pots in place and a folding flap on the upper part of the tray secures the pots in position (Galiegue and Thiry, 1990), (b) an improvement for the in-line palletising of trays of yoghurt suitable for use in situations where only manual palletising appear to be possible was described by Anon. (1987b); incidentally, this mechanical system is known as the "Somic Paletta", (c) a system for handling blanks of rectangular cardboard which folds to form a tray-like receptacle suitable for carrying yoghurt pots in rows; this has been patented in Germany (Anon., 1987c), and (d) U-shaped tray for 250 g yoghurts packs or a cardboard wrap-around for larger packs can be secured for palletising using a 2 mm plastic band and antislip hot glue to replace stretch or shrink wrapping (Schlicht, 1996). Rosti (1995) has reviewed the latest trends and developments in multipacks and wrap-around assemblies, including the economics, materials and different closure systems.

2.14   Refrigerated cold storage, transport and distribution

Cooling the yoghurt to <10°C, and maintaining this low temperature until the product reaches the consumer, helps to slow down the biological and biochemical reaction(s) that are taking place in the yoghurt. The former reactions result from the metabolic activity of the yoghurt starter culture and possibly any microbial contaminants that resisted heat treatment and survived the fermentation process or were introduced as post-production contaminants (e.g. yeast and moulds). Possible biochemical reactions are:

- fat oxidation in the presence of oxygen,
- hydration of the protein constituent in yoghurt,
- changes in the colour of the fruit additive (e.g. becomes dull and pale), can take place due to acidic condition of the product,
- slight dehydration may take place and the exposed surface of the yoghurt may change its physical appearance,
- presence of added hydrocolloids (stabilisers) and/or pectins from the fruit improves the viscosity/consistency of yoghurt during storage.

In order to minimise some of these reactions, the refrigeration of yoghurt is essential and, with this proviso, the keeping quality of the product could well be up to three weeks from the date of production. However, during the first 24–48 hours of cold storage an improvement in the physical characteristics of the coagulum is observed, mainly due to the hydration and/or stabilisation of the casein micelles, and hence it may be desirable to delay the sale/distribution of the yoghurt accordingly.

Since the quality of yoghurt is dependent on a multitude of factors after production, the following recommendations may help to ensure that the product reaches the consumer in a satisfactory condition. Notably, recommendations for the hygienic manufacture of milk and other dairy products including yoghurt encompass the implementation of hazard analysis critical control point (HACCP) and/or other similar systems, and for this reason dairy products have been classified in three categories according to their temperature requirements during storage → transport and distribution → retailing (Anon., 1994c, 1995c): (a) products stored at <−18°C such as ice cream and related frozen products, (b) short shelf life perishable products (e.g. pasteurised liquid milk, cream, yoghurt, fermented milks, fresh and soft cheeses, butter and retail portions of hard and semi-hard cheeses) to be stored between 0°C and 10°C and (c), products such as UHT milk, powders, canned products and processed cheese may be stored at ambient temperature but <30°C.

Therefore, it has been recommended (Anon., 1994c, 1995c) that yoghurt should be stored at 0–10°C (±1°C temperature tolerance) and in the same temperature range during transport, but with a ± 2.5°C temperature tolerance. However, to safeguard the quality of the product, most large manufacturers tend to store and transport yoghurt at <10°C (Hinsperger, 1990; Farquhar and Symons, 1992).

2.14.1 The cold store
- Reduce, as far as possible, rough mechanical handling of the packaged yoghurt.
- Maintain the storage temperature as low as possible (i.e. <5°C) and avoid any fluctuations.
- Provide good cold air circulation in the store, especially if the yoghurt is filled at 20°C and final cooling takes place in the cold store.
- Avoid losses of cold air through the use of a poorly designed insulated store.
- If the yoghurt is packaged in a transparent container, protect the product using special lighting to reduce decolorisation or oxidation.
- Always retain the packaged yoghurt for at least 48 hours before dispatch, so that the final stability of the coagulum is achieved.

2.14.2 During transport

- Refrigerated transport is required during the summer months in the temperate zones of the northern or southern hemispheres; during the winter months insulated lorries can be used.
- In tropical and subtropical areas, refrigeration of the transport vehicle is necessary.
- During transport, shaking the yoghurt can lead to a reduction in viscosity and whey syneresis; it is difficult to overcome this defect, especially during long road journeys.

The packaged yoghurt (i.e. unit and shipping containers) is subjected to vibratory motions during shipping and distribution and the potential damage to the product may include broken or damaged structure of set yoghurt gel, whey separation, disruption of the coagulum of stirred yoghurt and formation of a narrow skin of yoghurt between the foil laminate and the tip of the plastic cup. Richmond *et al.* (1985) studied the physical damage to set yoghurt packaged in waxed paper cartons using a vibratory table. In order to simulate the conditions during transport, 12 yoghurt cartons were placed in a cardboard nested tray, with or without stretch wrapping, and stacked 10 high. The results suggest the following:

- Yoghurts made without the addition of stabilisers had high levels of syneresis.
- Stretch wrapping minimised the effect of whey separation.
- Most damage to the yoghurt occurred in the top layer of the stack.
- Incubation and cold storage of packaging materials (i.e. unit and shipping) caused changes in physical structure which resulted in loss of stackability and product loss.

The same authors concluded that similar "challenge" tests should be conducted on yoghurt packed in different plastic cups of different designs, because the shape of the container may affect the coefficient of friction. Nonetheless, no more studies have been carried out to date.

It is worthwhile pointing out that vehicles used for transporting yoghurt should comply with special recommendations (UN, 1991) which include the installation of an automatic temperature probe (ATP) device and the requirement that trucks should have smooth internal surfaces that can be easily cleaned, be fitted with suitable shelvings, if any, and that door openings should be fitted with plastic strips to minimise heat loss.

2.14.3 The retail shop and the consumer

- The yoghurt must be displayed in refrigerated cabinets until it is purchased.
- Yoghurt should be consumed directly or otherwise stored in a domestic refrigerator until required.
- Yoghurt should be consumed around 10°C, as below this temperature the flavour profile is not appreciated due to the coldness, and above 10°C the product loses its freshness and may undergo a reduction in viscosity.

2.15   Conclusion

The quality of yoghurt (set or stirred types) is influenced by a multitude of factors during the preparation of the milk base, processing stages, packaging, storage and distribution. The following summary of points to be considered may help to ensure that a quality product reaches the consumer:

- level of protein content in the milk base
- process parameters such as homogenisation and heat treatment and on very rare occasions the addition of coagulants
- addition of stabilisers
- exopolysaccharide production by the starter cultures
- development of acidity and/or rate of acid development
- presence of inhibitory agents in milk
- post-fermentation acidification
- post-production heat treatment (refer to Chapter 5)
- vibratory motion during distribution and retailing
- mechanical handling of the coagulum (refer to Chapter 3)
- miscellaneous treatments such as the use of oils, fat substitutes or postfermentation concentration (refer to Chapter 5).

2.16   References

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3

Processing plants and equipment

The process of yoghurt production has evolved through the ages from a simple preparation carried out in the home on a very small scale to medium and large-scale production centres handling many thousands of litres per day. The utensils and equipment required vary in relation to the type of yoghurt produced, scale of production and the level of technology adopted. Hence, it would seem logical to review the available equipment and plant against a scale of yoghurt produced per day:

- Home or small-scale production
- Medium-scale or manufacture by a small producer/retailer
- Large-scale production

3.1   Home or small-scale production

Traditionally, yoghurt is prepared at home and ordinary kitchen utensils are used. The milk is heated in a cooking pot and the production of the coagulum takes place in the same container; Fig. 2.1 described briefly the overall process. However, one factor which is critical during the incubation period is the maintenance of a uniform temperature. This is achieved by wrapping the pot in a woollen blanket and placing it in a warm place, for example, near a cooker. Although the traditional process could still be recommended to individuals producing their own yoghurt, a simplified recipe is illustrated in Fig. 3.1.

The linen airing cupboard (i.e. area beside the hot water cylinder in a modern house) is sometimes used during the fermentation period, although yoghurt "makers" (Fig. 3.2) have become available for enthusiasts to produce yoghurt under controlled conditions (see also Taylor, 1981; Light, 1993; Hyman *et al.*, 1996). Alternatively, warm milk inoculated with the starter culture (or natural yoghurt) is placed in a wide mouth Thermos flask and left undisturbed, allowing the milk to ferment and coagulate. Cooling is carried out directly after coagulation has taken place and fruit and/or sugar are normally added to the cold yoghurt.

```
┌─────────────────────────────────────────────────────┐
│ Place 1l of whole milk in a saucepan and heat to     │
│ near boiling (for the production of thick viscous    │
│ yoghurt add 20-40g of skimmed milk powder, ≙ to      │
│ 2.5-5 tablespoons); the addition of sugar is optional.│
└─────────────────────────────────────────────────────┘
                          │
                          ▼
┌─────────────────────────────────────────────────────┐
│ Cool to 45˚C (or just above blood temperature) and   │
│ add 1 level tablespoon of plain unsweetened yoghurt. │
└─────────────────────────────────────────────────────┘
                          │
                          ▼
┌─────────────────────────────────────────────────────┐
│ Pour into the containers of a yoghurt "maker" and    │
│ seal with snap on lids, alternatively, wrap the pot  │
│ with a blanket, or pour the inoculated milk into     │
│ clean, wide mouth thermos flask.                     │
└─────────────────────────────────────────────────────┘
                          │
                          ▼
┌─────────────────────────────────────────────────────┐
│ Depending on the activity of the yoghurt organisms   │
│ and/or temperature of incubation, the milk should    │
│ coagulate in 3-18h.                                  │
└─────────────────────────────────────────────────────┘
                          │
                          ▼
┌─────────────────────────────────────────────────────┐
│ Cool the yoghurt as quickly as possible - preferably │
│ overnight in the refrigerator.                       │
└─────────────────────────────────────────────────────┘
                          │
                          ▼
┌─────────────────────────────────────────────────────┐
│ Blend in fruit and sugar (fresh fruit, puree or jam) │
│ in accordance with personal preference and stir gently.│
└─────────────────────────────────────────────────────┘
                          │
                          ▼
┌─────────────────────────────────────────────────────┐
│ Maintain product under refrigeration until consumed. │
└─────────────────────────────────────────────────────┘
```

Fig. 3.1   Production of yoghurt at home

Note the following: (a) one pot of the natural yoghurt produced could be used as a starter culture to inoculate the following batch, (b) excessive subculturing can lead to a prolonged incubation period, and hence it is recommended that a fresh yoghurt should be introduced weekly, and (c) short incubation periods are obtained using fresh, active starter cultures, an approach which is highly recommended.

3.1.1   Miscellaneous systems

The processing steps involved, including the equipment required, in the manufacture of set or stirred yoghurt by this simple procedure are summarised here:

- Milk base is prepared in cans/churns.
- The cans are immersed in a water bath which is required for the heat treatment of the milk; the heat source could be steam or electrical. At the cooling stage, the hot water is replaced by cold water from the mains.

Fig. 3.2 Yoghurt maker where glass jars with screw-on plastic caps are used

- At 45°C milk is inoculated with starter culture and incubated in bulk (stirred yoghurt), or for set yoghurt the milk is dispensed into cups prior to incubation; special cabinets can be used for the fermentation, or alternatively the temperature in the water bath can be maintained at 42–45°C to ferment the milk in bulk.
- At the desired acidity the cans/churns are removed from the incubator unit(s) and stored overnight in the cold store.
- Fruit is added separately to each can/churn and mixed gently using a milk/cream plunger.
- Filling and packaging is carried out using hand-operated units (see below).

3.1.2 Packaging system

For this scale of yoghurt production, it is inappropriate to install a proper packaging machine due to the high capital investment required. Subsequently, the yoghurt is packaged using hand-operated unit(s), but extreme care should be exercised in order to minimise contamination of the product. Figure 3.3A shows how yoghurt can be produced in a 10 litre stainless steel churn, followed by the addition of fruit on top of the cold yoghurt and mixing. The fruit flavoured yoghurt is dispensed into plastic cups manually using a stainless steel jug, and finally the aluminium foil lids are crimped in place (Fig. 3.3B–E). Incidentally, an improved method of closure of the yoghurt cups uses a hand-operated heat sealer.

An alternative method of packaging very small volumes of yoghurt per day involves use of a small-scale cup filler. A typical example is the CD 500/1000 machine (see Fig. 3.4). This unit is capable of filling yoghurt cold or hot, and the filling head is fitted with an antifoam nozzle. The capacity of filling ranges between 85 and 600 ml or g, and the piston used for filling the yoghurt has an easy measure adjustment with a fine setting.

The sequence of operations could be described as follows: place the yoghurt cup on the tray and press the foot pedal; the machine will dose out the set measure of

Fig. 3.3   Hand filling of yoghurt cups

product. The filling head automatically resets when the cup filling sequence is complete and the filled cups are then heat sealed using a separate unit (Fig. 3.4B). The speed of filling depends on the cup capacity and the speed of the operator but, in general, the cup filling speed ranges between 10 and 20 containers min^{-1}.

Alternatively, paperboard cartons could be used for the packaging of yoghurt using a hand-operated cartoning and filling machine (Fig. 3.5). This method of filling yoghurt could be referred to as a hand form/fill/seal operation. The hand-operated bottom carton sealer (Fig. 3.5A) preforms, crimps, heats, folds and bottom seals all sizes of carton, and pre-breaks the tops in preparation for the "top sealer"; incidentally, a similar unit was illustrated in the previous edition of this book and the design has been changed to include an air-operated base sealing plate.

The hand filler/sealer is basically designed for liquid milk but, by slightly modifying the filling head, it becomes feasible to fill a viscous product such as yoghurt (see Fig. 3.5B). The preformed cartons are placed under the filler and a microswitch operates the time fill. Then, the carton is pushed under the sealer and the handle is pulled to seal it. The speed of both the hand carton/sealer and the filling/sealing machine is about 10 units min^{-1}.

Fig. 3.4 Small filling machine (A) and a thermostatically controlled heat sealer for aluminium foil lids (B)

Reproduced by courtesy of C.K.X. Engineering, Sudbury, U.K.

(A)   (B)

Fig. 3.5 Hand-operated packaging equipment for filling yoghurt into cartons

A, Carton maker/sealer; B, hand filler sealer.

Reproduced by courtesy of C.K.X. Engineering, Sudbury, U.K.

3.2 Medium-scale production

The volume of yoghurt production in this category is rather low, perhaps in the region of a few hundred litres per day, and such small producer/retailers aim to market their yoghurt within a limited area. The different types of equipment which could be used at this level are described below.

3.2.1 Hand-operated vat

In some parts of the world, equipment manufacturers may produce specially designed small processing vessels (i.e. hand-operated, multipurpose tanks) where the agitation of the milk base during heating and cooling is done manually. The different steps involved during the production of yoghurt can be summarised as follows:

- Sanitise the equipment directly before using chemical sterilising agents, drain and rinse with clean water.
- Pour the milk into the vat, add the required amount of dried ingredients (milk powder) and mix with the aid of a stainless steel wire whisk.
- Start the heating cycle using an electric element to heat the insulated water jacket and hand agitate the milk.
- After reaching the desired temperature, the heating element is switched off and the milk is held for 10–30 min (depending on temperature), prior to cooling.
- During cooling, the water in the jacket is replaced by circulating mains water. At 40–45°C the milk is inoculated with starter culture and left undisturbed during the fermentation period.
- After a few hours, or at the desired acidity, mains water is circulated through the jacket to cool the coagulum, a process that may be assisted by gentle agitation.
- At around 15–20°C, a known volume of yoghurt is drained out, mixed with fruit/flavouring additives and hand-filled into plastic cups.

3.2.2 Multipurpose vat

This type of vat is really a batch pasteuriser which is slightly modified to meet the requirements of yoghurt manufacture and it is widely used for the production of viscous yoghurt (Fig. 3.6). These vats are usually made of stainless steel and insulated with a water jacket. The capacity may be in the region of 50–2250 l. When this type of vat is used, the processing stages of stirred yoghurt production usually follow two alternative patterns. In the first approach the vat is utilised for all the different steps necessary for the preparation and production of yoghurt (Fig. 3.7, process A). However, in the second approach the vat is merely used for the preparation of milk, that is, mixing the dried ingredients with milk, heat treatment and cooling to incubation temperature (Fig. 3.7, process B).

Processes A and B described in Fig. 3.7 illustrate clearly the steps necessary to produce stirred yoghurt, but for the manufacture of set yoghurt, process C (Fig. 3.7) should be followed. Processes B and C are similar except that in process B the milk is fermented in bulk, while in process C the milk is incubated in the retail container. The major differences between set and stirred yoghurt are illustrated elsewhere (see Chapters 2 and 5).

The multipurpose vat (Fig. 3.6) can be heated using different sources of energy (e.g. electrical, steam or gas) and this versatility makes this type of processing equipment very popular with the small producer. During the cooling stages, mains water

Fig. 3.6 Typical batch pasteuriser which can be used as a multipurpose vat for the production of yoghurt

Gusti-steam, electric or gas heated "Pastomix" – "Pastolux" vat for heat treatment of the milk base or cold storage of yoghurt (see text).

Reproduced by courtesy of T. Gusti, Wellingborough, U.K.

can be used or a closed-circuit cooling system circulating chilled water may be employed. However, if in-tank cooling is used for cooling the yoghurt, a slow speed agitator (i.e. <45 rpm) is operated to mix the coagulum gently and assist cooling but, at the same time, inflict minimum reduction in viscosity on the product. The diameter of the outlet valve must be ≥5 cm in order to facilitate ease of drainage of the yoghurt. On such a small scale of production, the stages of fruit mixing and filling can be carried out manually, but great care must be taken to minimise post-production contamination. Figure 3.7, process B, illustrates this approach. The fruit is added to each can/churn and gently mixed with the yoghurt by means of a milk/cream plunger.

3.2.3 Mini dairy

The "mini dairy" is a small compact processing plant which was developed in the late 1970s by Alfa-Laval A/B, Lund in Sweden – a project sponsored by the Swedish
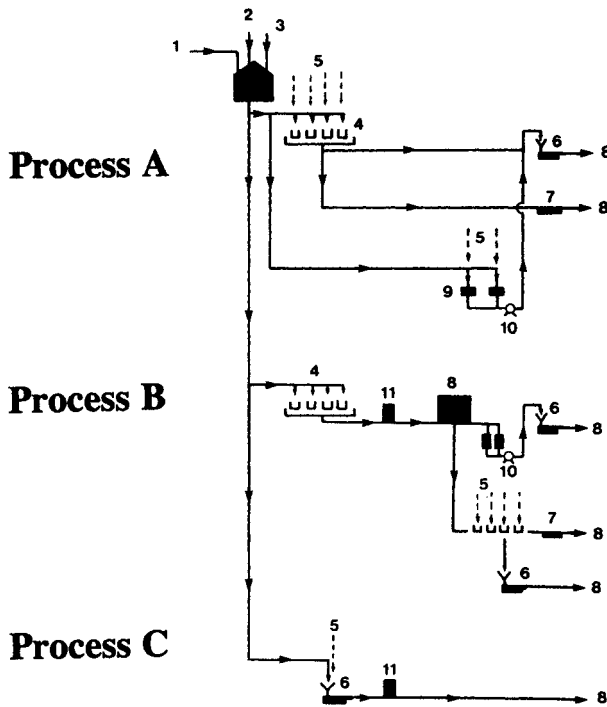
Fig 3.7 Small-scale production of yoghurt using a multipurpose tank

1, Inlet for liquid milk; 2, dried ingredients (milk powder(s) and sugar) added manually; 3, starter culture added manually; 4, stainless steel churns (process A – contain cold yoghurt or process B – processed milk base inoculated with starter culture); 5, fruit added manually; 6, small-scale filling machine; 7, hand filling machine; 8, cold store; 9, two small tanks (in parallel) used for the addition of fruit with yoghurt so that filling can be continuous; 10, positive pump; 11, incubation cabinet for set yoghurt (process C).

government to establish small-scale milk processing units in the developing countries. At present, Tetra Pak and Alfa-Laval Agri are responsible for marketing of these units in different parts of the world. The mini dairy unit is basically designed for processing market milks, cheese and fermented milks. For yoghurt, for example set, stirred and/or drinking type, the unit is capable of producing 1000l per batch over an extended 8 hour shift. All such units are pre-assembled and tested to give a short and efficient installation and start up time. The energy required for heating and cooling is provided by mains electricity or a diesel powered electric generator and hot water is generated by an oil- or wood-fired furnace. Figure 3.8 illustrates a unit for processing milk for the manufacture of the products mentioned above (Gandhi, 1986; Caviezel, 1987; Briem, 1992; Olivetti, 1993; see also Capogna *et al.*, 1997).

3.2.4 Small-scale packaging machines

Although hand filling has been adopted by many small dairies, the use of a proper filling machine does offer some advantages. A wide range of fillers is available on the market, and these filling machines are equipped with a diversity of sealing mech-
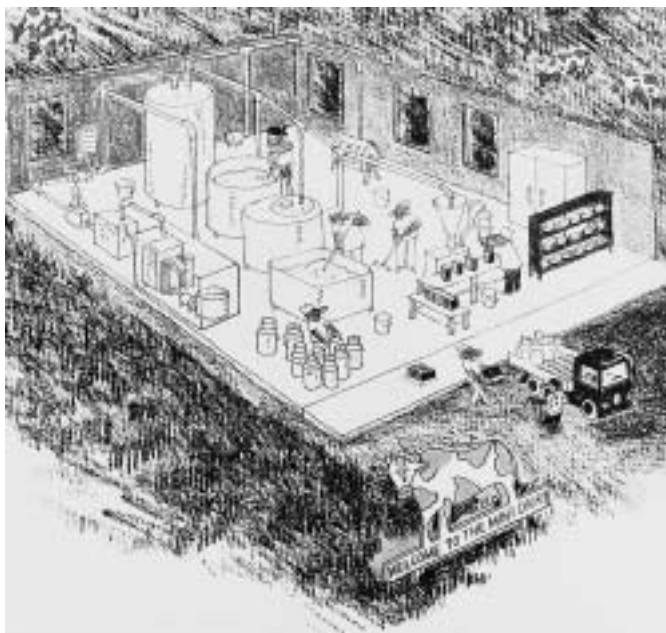
Fig. 3.8 General view of a mini dairy processing plant

Reproduced by courtesy of Tetra Pak (Processing Systems Division) A/B, Lund, Sweden.

anisms, for example the ability to heat seal foil lids, crimp foil lids or snap-on plastic lids. The ultimate selection of a particular type is largely a matter of personal preference (see Platt, 1990). Most manufacturers of packaging machines also produce small-scale equipment to meet the demand from small dairies. Some examples follow.

Regal RP/SA2 – This machine is semi-automatic and consists of:

- stainless steel hoppers that hold the yoghurt and the fruit base
- stainless steel rotary table
- a foil dispense assembly with a spot sealer
- heat sealing assembly.

An illustration of this machine is shown in Fig. 3.9. The sequence of operation is as follows: (a) the preformed containers are loaded into the machine by hand and a photo-electric cell (PEC) detects their presence, (b) the operator indexes the rotary table clockwise to the filling assemblies, (c) when the container is filled (i.e. with fruit flavoured yoghurt or, in a two-step sequence, with fruit and the yoghurt base, separately), the operator indexes the rotary table clockwise to the foil dispense assembly where a foil is placed automatically and spot sealed in position and (d) the operator then indexes the table to the heater assembly where the aluminium foil lid is heat sealed automatically.

As the operator indexes the rotary table once more, this allows removal of the filled yoghurt containers. However, every time the table is indexed, another container should be loaded to repeat the cycle. The volume of the fruit dispensing unit ranges from 10 to 80 ml, and for yoghurt 60 to 300 ml. Incidentally, the machine is
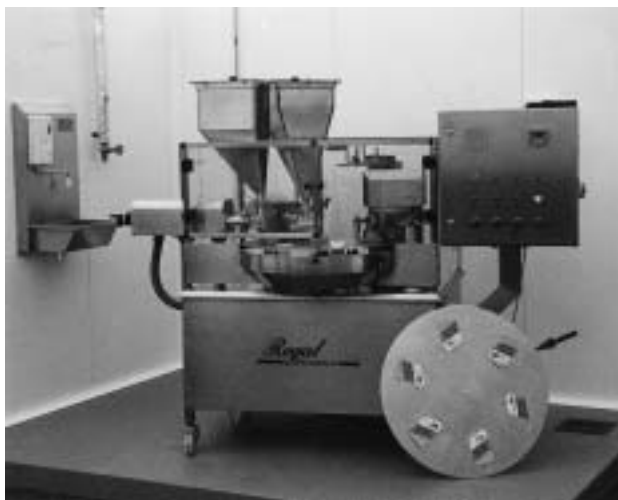
Fig. 3.9   General view of the Regal semi-automatic

Note: Arrow indicates stainless steel table specially designed for filling twin chamber container.

fitted with a fully interlocked stainless steel mesh safety guard. The same manufacturer produces fully automatic filling machines up to 12 000 cups hour^{-1}.

Waldner Dosomat 1 Eco, 1, 2 & 10 – These are rotary cup filling and closing machines that cover capacities ranging from 1000 to 20 000 cups hour^{-1}. These machines are fully automatic with the dosing unit mechanically driven; this unit operates on the piston principle that ensures filling with absolute care and accuracy. For viscous products like yoghurt, product aspiration is realised by direct feed via equalising pistons and the dosing range is regulated by handwheel. A range of containers (e.g. cartons, plastic pots or glass bottles) can be used on this machine for packaging yoghurt. Figure 3.10 illustrates one example operated within a laminar flow cabinet hood. All models of the rotary Dosomat machines are fitted with a coding system of one of the following types:

- coding with quick drying ink or hot stamping with ink ribbon on the lid or cup bottom
- heat or cold embossing into cup bottom
- labelling
- ink-jet

The closure of the container (i.e. heat sealing with a snap-on lid) can be achieved by heat, ultrasonic or high frequency sealing. All models are suitable for clean-in-place (CIP). Incidentally, the number of filling lanes on the rotary table ranges from one up to eight depending on the model and throughput.

GEI Turbo Rotafil – This is a multifunctional compact system of filling. The machine is available with different sizes of interchangeable indexing table for packaging into a wide range of container sizes. It can be supplied with many optional features such as:

Fig. 3.10　Waldner Dosomat rotary-type yoghurt filling machine
Reproduced by courtesy of Ultrapak, Aldershot, U.K.

- automatic container dispenser and discharge systems
- multistation or filling head facilities
- automatic closure, heat sealing and securing of antitampering devices
- date and price coding system.

The filling speed is around 8400 pots hour^{-1} on a four-head production system. However, the specially designed filling head (see Fig. 3.11) ensures that there is a regulated speed of filling, capacity to deliver fruit pieces intact into fruit flavoured yoghurt, and (c) virtually drip-free cut-off between the fills.

Cockx R 4000 – The machine is a 16 pocket, eight station unit with options of prefill and overlid (Fig. 3.12). In general, it is fitted with cup magazines, mechanical main piston fill, lid appliers, heat sealers, date coders and cup ejection onto a conveyor with an extended collection table; the filling speed is about 4000 cups hour^{-1}.

The machine has been designed to allow, if required, two different products to be filled at the same time as the starwheel indexes two pockets at a time. The filling valves are independent and, as an extra, two hoppers can be fitted as an alternative to the single unit. If the pre-fill extra is used, then larger capacity cups can be filled faster with a pre-dose prior to the main fill. The nozzles can be changed for different products and have a positive cut-off. The measure adjustment is inside the main frame of the machine, easily accessible through the interlocked doors. The lid magazines can be switched independently and can be changed for containers of different rim size. The heat seal heads have easily changeable seal plates and the date coders can be quickly adjusted for height and position. The filled and sealed cups are raised out of the pockets and swept onto a deadplate prior to being pushed onto a small conveyor where they are guided onto a collection table for packing.

Fig. 3.11 Filling heads on Turbo Rotafil packaging machine
Reproduced by courtesy of GEI International, Woburn Sands, U.K.

Fig. 3.12 Cockx rotary cup filler and sealer
Reproduced by courtesy of C.K.X. Engineering, Sudbury, U.K.

The fill, lid application and heat seal systems are all controlled by sensors and all doors are fully interlocked for safety. There are no process controllers fitted to the machine and the mechanical variable speed drive is connected to the piston fill drive system by a chain and is also connected to a camshaft. This camshaft has a series of roller operated valves operated by individual cams which are easy to set up or adjust. In this way, it is easy for the customer fully to understand the working of the machine at each station. Lubrication ports are on one panel with the feeds through copper tubes to the bearings.

3.3 Large-scale production

In this category, the equipment employed for the manufacture of yoghurt is specially designed to handle thousands of litres per day and a highly sophisticated technology has evolved which offers a dairy both improved mechanisation and automation. Since the publication of the first edition of this book, few technical developments have occurred with respect to yoghurt technology and the latest technological progress in this field has been reviewed in two International Dairy Federation monographs (IDF, 1988, 1992). Driessen and Loones (1992) presented a comprehensive chart summarising the new developments in technology including products with special micro-organisms and these are:

- Membrane techniques which make it possible to utilise the required properties and avoid the unwanted properties of microbial metabolites.
- Separate cultivation which makes it possible to combine micro-organisms that need differing conditions for their proliferation, for example, mesophilic and thermophilic strains.
- Applying automatic pH control to end the fermentation process and achieve a more consistent product.
- Mounting the cooler on top of the filler, to achieve better viscosity in stirred fermented milks.
- Applying in-line inoculation which makes manufacture of set fermented milks more flexible.
- Overpressure of sterile air which has proved to be effective in protecting starters against contamination with other micro-organisms and bacteriophages.

The topic has been extensively reviewed elsewhere (Anon., 1981a, b, 1983a; Salji *et al.*, 1985; Evavoll, 1985; Nicolaus, 1987; Bianchi-Salvadori, 1989; Driessen and Loones, 1990, 1992; Nilsson and Hallström, 1990; Robinson and Tamime, 1990, 1993; Puhan *et al.*, 1994a, b; Nilsson, 1994; Strahm and Eberhard, 1994; Bylund, 1995; Karagozlu and Gonc, 1996; Gardini *et al.*, 1996). As a consequence, it was decided that only up-to-date information will be provided here.

The diversity of these technologies can be discussed most easily in relation to:

- type of yoghurt produced (e.g. set or stirred)
- effect of mechanisation on the quality of the yoghurt
- application of automation to the manufacture of yoghurt.

There are several approaches that can be employed for the production of yoghurt and, as each yoghurt manufacturer has his own specific requirements, each plant is supplied, in effect, tailor made. It is evident that plants which produce set and stirred
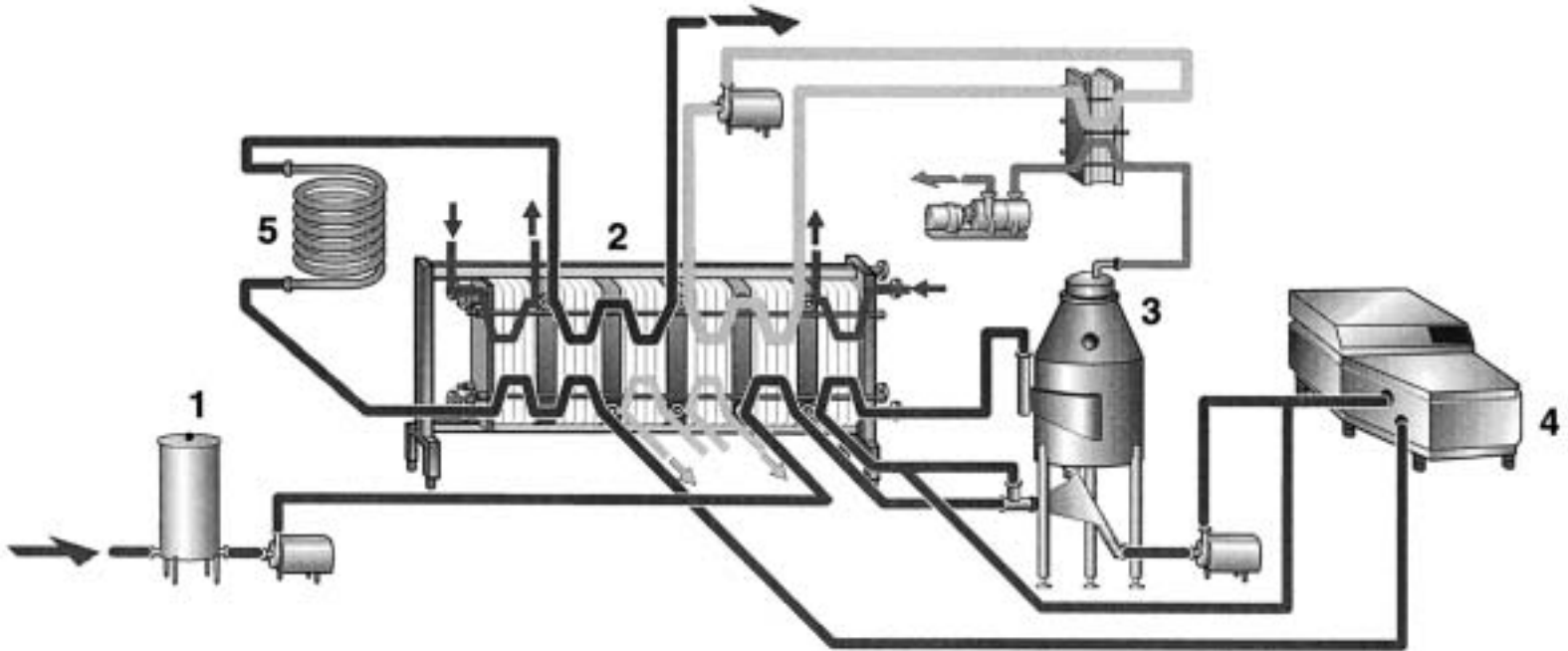
Fig. 3.13 Flow diagram of general pretreatment of milk for the manufacture of set and stirred yoghurts
1, Balance tank; 2, plate heat exchanger (PHE); 3, evaporator; 4, homogeniser; 5, holding tube.
Reproduced by courtesy of Tetra Pak (Processing Systems Division) A/B, Lund, Sweden.

yoghurt (or a combined processing plant) have some stages in common (see Fig. 3.13), for example, milk reception and handling, preparation of the milk base, homogenisation of the yoghurt milk and heat treatment, and hence it is appropriate to review the relevant equipment in relation to the different stages of manufacture; more specialised units are discussed separately.

3.3.1 Milk reception, handling and storage

At present, milk collection from farms in the United Kingdom, developing and industrialised countries is carried out in bulk using a road tanker, although in some instances, rail tankers or churns could be used. The facilities provided at a typical dairy for reception of this bulk milk have been described by Tamime and Kirkegaard (1991) and Bylund (1995) (see Fig. 3.14). The milk intake can be either metered using a metering pump, or weighed (e.g. at a weighbridge for road tankers or in a duplex weighbowl for churns). When milk is accepted, and after a sample for chemical and microbiological analysis has been taken, the general practice for handling the milk may include: (a) filtering the milk to remove contaminants (e.g. straw, hairs, soil) with the most universal system used being a stainless steel filter; however, an optional treatment to clean the milk is clarification using a separator; and (b) cooling the milk to <5°C using a plate cooler prior to storing in a silo.

The reception of milk in churns is somewhat different from that from a road tanker. Normally the churns are unloaded in the reception area and the lids removed. The freshness of the product is quickly determined by sniffing the churns and if any unusual smells are noted, the milk is rejected; a composite sample of milk from each farm is further analysed chemically and for bacteriological quality.

As already discussed elsewhere (see Chapter 2), the milk is subjected to a number of preliminary treatments before it becomes yoghurt. These processes are standardisation of the fat content, fortification of the solids-not-fat (SNF) and homogenisation and heat treatment of the milk base. These treatments will be discussed separately.

3.3.2 Standardisation of fat content in milk

The fat content of milk can vary according to source and season, but in yoghurt the level is prescribed by consumer taste or the Statutory Instruments of the countries concerned, so that standardisation becomes essential.

The theoretical approach to milk standardisation can best be visualised as follows:

Whole milk → Separator

Surplus cream ← Cream   Skimmed milk

Blend
(standardised milk)
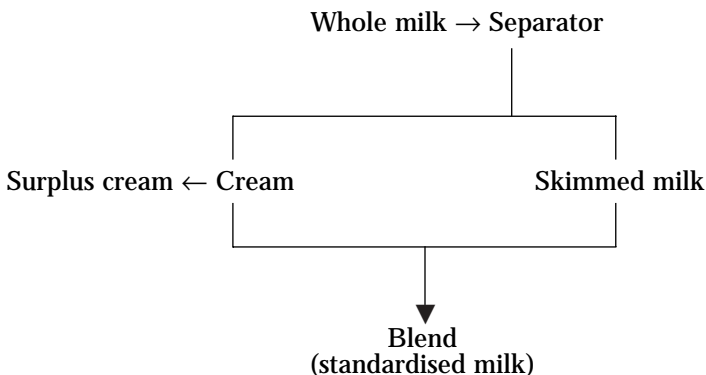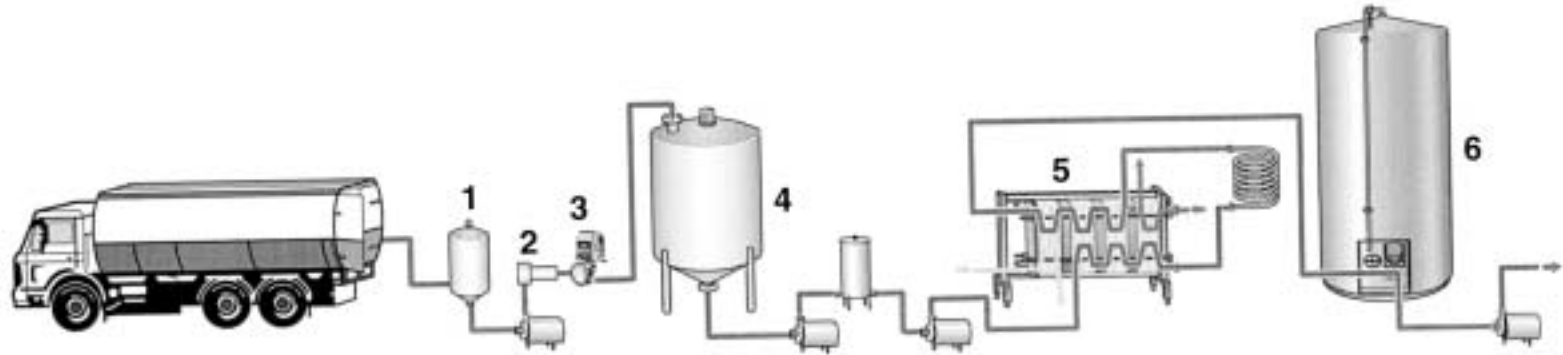
Fig. 3.14 Milk reception, handling and storage at a large factory

1, Air eliminator; 2, filter; 3, milk meter; 4, intermediate storage tank; 5, thermisation and cooling or cooling only; 6, silo tank.

Reproduced by courtesy of Tetra Pak (Processing Systems Division) A/B, Lund, Sweden.

and the accuracy of the process is dependent on such factors as:

- type of equipment used and the efficiency of fat separation obtained
- control system used.

The skimming efficiency of the available plant has greatly improved over the years, so that residual fat in skimmed milk usually falls between 0.05 and $0.07\,g\,100\,g^{-1}$; the skimming efficiency of the separators is thus referred to as 0.05–0.07, respectively. The control system employed in milk standardisation lines can be either manual or automatic, and while the former may be recommended for small/medium size producers, the automatic system is essential for dairies handling large volumes of milk per day.

A number of different systems can be used for milk standardisation (Hellström, 1986; Anon., 1992, 1996a; Bird, 1993). The efficacy of any one particular system depends on its ability to ensure that:

- The pressure of the skimmed milk at the outlet pipe is lower than the pressure in the tank where the skimmed milk and cream are remixed.
- The fat content in the cream remains constant; the proportion of cream remixing with skimmed milk can be stabilised, i.e. there are proportional mixing controls.
- The final fat content of the process milk is within preset limits.

Compomaster KCC – This is an automatic system for standardisation of the fat content in the milk and surplus cream (Fig. 3.15). This unit is directly connected to a separator; however, when liquids are mixed continuously in volumetric proportions, the Compomaster can be used without a separator. In this system of standardisation, combined mass flow meters, density meters and temperature transmitters are used to measure the cream and skimmed milk, respectively (Hansen, 1996). Thus, by knowing the density and temperature of both skimmed milk and cream, it is then possible to calculate the fat content of the cream. The unit automatically adjusts to the set points for the fat content in both standardised skimmed milk $(1-5\,g\,100\,g^{-1})$ and cream $(18-50\,g\,100\,g^{-1})$.
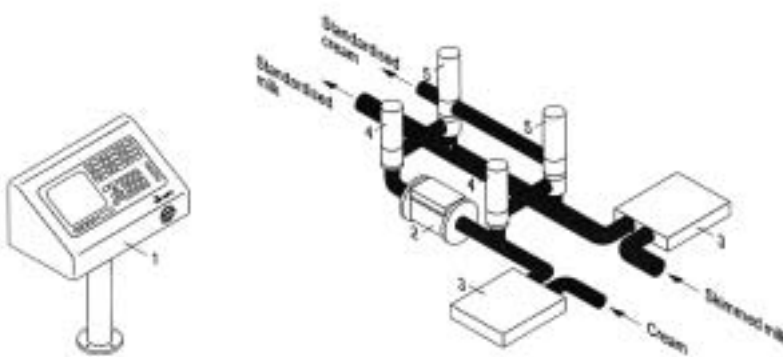
Fig. 3.15   An illustration of fully automatic in-line standardising system
1, Control panel; 2, flow meter; 3, density transmitters; 4, regulating valves; 5, on/off valves.
Reproduced by courtesy of APV Nordic, Denmark.
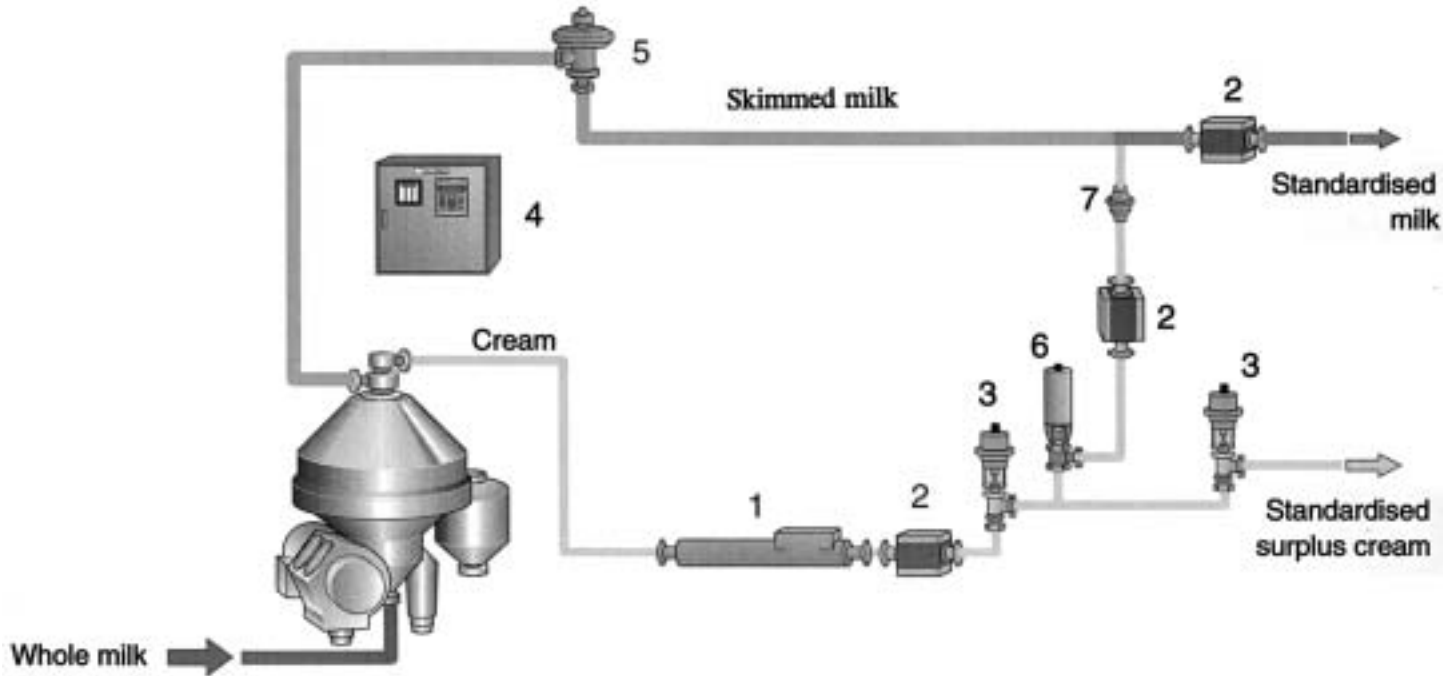
Fig. 3.16 Illustration of an automatic direct standardisation (ADS) system for milk and cream

1. Density transmitter; 2, flow transmitter; 3, control valve; 4, control panel; 5, constant pressure valve; 6, shut-off valve; 7, check valve.

Reproduced by courtesy of Tetra Pak (Processing Systems Division) A/B, Lund, Sweden.

The Compomaster has capacities ranging between 7000 and 45000 l hour^{-1}. It is delivered as a compact unit ready for installation and connections need to be made to the product inlet, air-line and the mains electricity supply. According to Hansen (1996), the Compomaster type KCC standardising system needs to be calibrated only once every second year reflecting the high precision of the unit. This system also contains in-line mixers for special applications (i.e. mixing cream and skimmed milk) without the use of a separator; furthermore, this system is suitable for CIP application.

Automatic Direct Standardisation (ADS) Systems – These methods of standardisation of the milk and cream are very accurate and depend on a careful choice of components and the design and engineering of the system. A typical system is shown in Fig. 3.16, where the components within the system are clearly identified. In brief, according to Bird (1993) and Bylund (1995), the ADS system can be described as follows.

The set points for standardised cream (or surplus cream) and milk fat content are fed into the process control unit. The pressure control system at the skimmed milk outlet (Fig. 3.16 (5), constant pressure valve) maintains a constant pressure, regardless of fluctuations in the pressure drop over downstream equipment. The cream regulating system maintains a constant fat content in the cream discharged from the separator by adjusting the flow of cream discharged. The ratio controller mixes cream of constant fat content with skimmed milk in the correct proportion to give standardised milk with a specified fat content. The accuracy of the system, based on standard deviation of repeatability, should be <0.03% for milk and about 0.25% for cream (see also Hellström, 1986; Anon., 1992).

The application of these systems to the manufacture of yoghurt could be considered under the following conditions: (a) if the solids content of the milk is fortified using an evaporator (Fig. 3.13 and 3.17), then it is necessary to standardise the fat content in the milk before the concentration process commences, (b) skimmed milk could be concentrated by evaporation and then before further treatments (i.e. homogenisation and heat treatment) the concentrated skim could be standardised with cream, (c) concentrated skimmed milk may be standardised with cream, and (d) membrane filtration (UF (ultrafiltration) or reverse osmosis (RO)) is sometimes used to concentrate the milk base. Normally, the fat is separated from whole milk and the skimmed milk is concentrated to the desired level of solids; the concentrated skim fraction is then standardised with the cream.

In general, therefore, the milk base is standardised for fat content before evaporation commences but, if the skimmed milk is concentrated in a UF plant, the addition of cream takes place later. The reason for adding the fat to the concentrated skimmed milk in the latter method is that the high pressure used during the concentration process could damage some of the physical properties of the fat, which in turn may affect the quality of yoghurt (e.g. an oiling-off or a churning effect).

3.3.3 Fortification of milk solids

The level of milk solids in the milk base can be raised by one or more of the following methods.

3.3.3.1 Traditional process

Boiling the milk can be carried out in a tank similar to a batch pasteuriser. The aim of this approach is the evaporation of one-third of the milk volume under

Fig. 3.17 View of an internal evaporation and de-aeration plant used on a yoghurt
processing line

Reproduced by courtesy of APV Nordic, Denmark.

atmospheric pressure. However, this method of concentration of the milk solids is
not used under industrial conditions, mainly due to the high cost involved, but also
because the generation of too much steam in the processing area can be unaccept-
able to personnel.

3.3.3.2   *Addition of milk powder*

Different types of milk powder can be used to fortify the yoghurt milk (see Chapter
2), although skimmed milk powder is used most widely. The dried ingredients are
incorporated into the aqueous phase which could be whole milk, skimmed milk or
water, and the available equipment is designed to provide: (a) complete dispersion
of the dried ingredients into the aqueous phase, (b) complete hydration of the dried
particles with no residual lumps, (c) minimal incorporation of air in order to reduce
the problems of foaming, and (d) the capability to clean and sanitise the unit easily.

The powder-handling equipment found in a dairy is dependent on the daily
throughput and the method of bulk delivery. Basically, milk powder is packed into
either small capacity units (25–50 kg multilayer paper sacks with polythene liners),
or medium capacity units (up to a tonne in metal or plastic containers), or road
tankers for bulk storage in metal silos. The machinery available for emptying the
powder also varies, so that while the sacks (small quantities) may be emptied
directly into reconstitution units, larger volumes are emptied into a sifter for deliv-
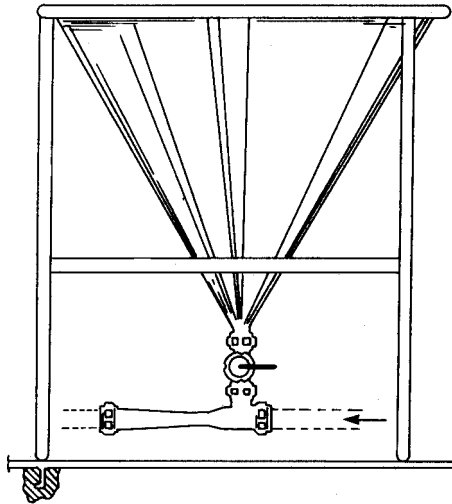
Fig. 3.18 Illustration of a mixing funnel/hopper used for the reconstitution of milk powders

The sack of milk powder is placed on the table and then emptied into the funnel. The force of the circulating liquid causes the powder to be aspirated downward and mixed with the water. Circulation is continued until the powder is dissolved. Notice that the funnel has a valve connection, which has a slight constriction/restriction in the pipe to provide a venturi effect.

ery into the mixing unit. The powder stored in metal/plastic bins or silos is transferred using either a screw-feed (of variable speed) or a blower; dust filters must be used to recover any fine particles, especially in plants handling large capacities. Some examples of milk powder mixing units are given below.

Mixing funnel/hopper – Reconstitution of the powder is carried out in batches and a "closed circuit" consisting of a tank, pipe connection, centrifugal pump and the funnel/hopper assembly is required. The tank is normally filled with the aqueous phase at around 40–50°C and the circulation started. The positioning of the hopper in relation to the centrifugal pump is important and two options are available (see Fig. 3.18).

First, if the hopper is assembled on the suction side of the centrifugal pump, it offers the advantage of rapid dispersal and adequate dissolution of the powder due to the action of the pump; the disadvantage is that frequent blockages may occur in the hopper.

Second, by placing the hopper on the outlet side of the centrifugal pump directly after a specially designed venturi unit, the problem of blockage is overcome, since the venturi unit creates a vacuum within the pipe causing the powder to be sucked into the recirculating solution; full dispersal of powder may be a little slower (Newstead *et al.*, 1979; Sanderson, 1982).

The former circuit is illustrated in Fig. 3.19. It is noticeable that, in the latter approach, any suction of air is returned to the tank rather than the suction side of the pump, because if air is introduced into the system, the action of the pump's impeller can increase the amount of air incorporated into the product. Furthermore, a reduction in aeration and/or frothing can be achieved by installing a special valve
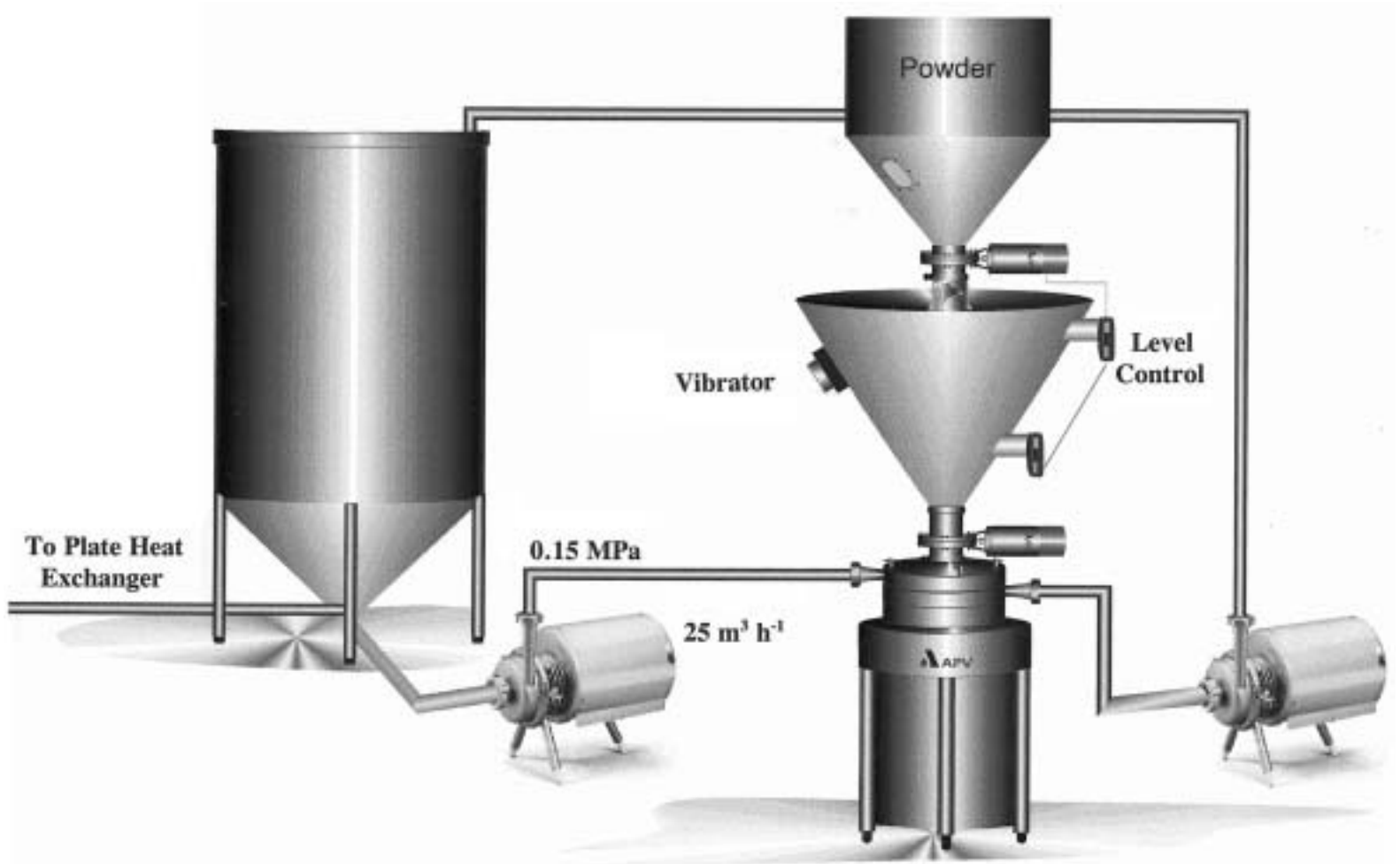
Fig. 3.19 Schematic illustration of TPM-1 powder mixer
Reproduced by courtesy of APV U.K. Co. Ltd., Crawley, U.K.

Fig. 3.20   Tri-Clovers dual-stage Tri-Blender®

Reproduced by courtesy of Tri-Clover Inc., Kenosha, U.S.A.

on the mixing hopper and ensuring that the return line in the mixing tank is below the level of the liquid. If additional mixing of the added powder is required, one of the following units could be employed: (a) in-line static mixer, (b) high speed agitator in the mixing tank, and (c) high velocity liquid jet.

An alternative method to the funnel/hopper installation is the in-line mixer, and some examples of such units are as follows.

Tri-Blender® – This mixing unit is supplied by the Tri-Clover Inc. of Wisconsin, U.S.A. The principle of this mixing unit is that the venturi jet mixer is replaced with a dual stage blending process (see Fig. 3.20). The system is designed for continuous in-line or batch blending of dry ingredients at a rate of up to 45 kg min^{-1}. The product passes through the initial liquid/dry ingredient blending chamber to a second blending chamber which effectively serves as a discharge pump. This double blend feature improves end-product consistency and provides a smoother and more uniform blend. With the discharge pump function handled within the blender itself, it is possible to achieve significantly higher vacuum rates over a wider range of process conditions. The increased vacuum rates contribute to fast and consistent flow rates throughout an entire production run, and with such a blending system, the additional strainers and a discharge pump are not required. Incidentally, this unit is rather compact and occupies only 50 × 75 cm of floor space (see Fig. 3.20).

Silverson mixers – These types of mixer operate at very high speed and exert an homogenising effect during the recombining of dried ingredients. The models, which

Fig. 3.21 The "Flashmix" that can be used for in-line mixing of powders
Reproduced by courtesy of Silverson Machines, Chesham, U.K.

could be used for the reconstitution of milk powder, are known as the "In-Line" and the "Flashmix". The latter unit is shown in Fig. 3.21. These machines are designed for continuous operation at high speeds and each has incorporated a high shear rotor/stator processing workhead; the In-Line mixer has one such head and the Flashmix has two. The upper head is normally a general purpose disintegrating unit, whilst the lower head is a square hole type with high shear screen. The operating characteristics of these workheads are briefly described by the manufacturer:

- The liquid is gravity fed or pumped into the hopper and is rapidly drawn down by the two rotor/stator workheads; a vortex is created by the flow of liquid through the Flashmix, and it is into this vortex that the powder is added.
- The liquid/solid mixture is drawn down the vortex into the mixing chamber and has no way of bypassing the workhead(s) assembly ensuring that all the solids are totally dispersed before leaving the mixing chamber.

Two advantages claimed for the unit are that the workheads can be changed to suit each individual product and that by using the appropriate feeding/metering equipment, the liquid/solid ratio of flow can be precisely controlled. However, a similar

unit known as Flashblend can also be used to wet and disperse powders into liquids rapidly but the mode of operation is different.

The use of an In-Line mixer alone has its limitations, because the delivery of milk powder through a funnel into a recirculating circuit inevitably leads to "arching". However, the use of a Flashmix mixer overcomes this difficulty due to the fact that the liquid and solid ingredients are fed simultaneously into a specially designed hopper before being sucked immediately into the upper rotor/stator. This workhead converts the milk powder/liquid phase into a slurry which is then dispersed as the result of the high speed shearing effect of the bottom or second workhead. It is obvious that each mixer is designed for a particular purpose and a combination of these two types of mixer in the recombining process brings the advantages of both units, that is, the mixing process involves three workheads rather than one or two, so ensuring complete dissolution of the powder with the minimum incorporation of air. Some degree of homogenisation of the mix can be obtained by using different types of stator head or screen on the high speed mixer, so that, for example, a disintegrating effect is achieved using large circular holes or slots, a fine screen produces an emulsification/homogenisation effect and a screen with square holes imparts a high shearing effect.

Vacucam^{TM} – This type of an in-line powder mixer was developed by the Semi-Bulk Systems Inc. in the U.S.A. An overall illustration is shown in Fig. 3.22. The system has the following features: (a) an air-pallet/ejector mixer section conveys, wets and dispenses the powder into the liquid; since the design generates its own vacuum to draw in the dairy powders, the mixer allows total separation of dry handling from wet processing, and also, by introducing the powder within the liquid stream, powder plugging is avoided, (b) the in-line ejector/mixer conveys and mixes the powder on a "skidded system" without using mechanical equipment (e.g. conveyors, rotary valves, receivers and in-tank mixers); this system can be fully automated including CIP, and can operate on batch recycle, single pass or continuous modes and (c) the air cone hopper is designed for easy discharge of powders that can cause delivery problems; details of the construction and principles of operation have been given (Anon., 1996b) and the use of low pressure air or other gases eliminates the bridging effect of the powder in the hopper and facilitates discharge. Incidentally, this system of mixing can be easily used to dissolve sugar into the milk base.

In-tank mixing unit – Efficient mixing of powder in a tank relies entirely on the agitation system provided. The familiar flow pattern which occurs during liquid mixing is illustrated in Fig. 3.23. These patterns are largely influenced by:

- Shape and size of the agitator system (e.g. paddle, turbine, propeller, scraped surface, anchor, etc.).
- Position of the agitator, i.e. top or bottom entering, perpendicular or sloped, and/or centrally mounted or not.
- Speed of rotation of the agitator.
- Shape of the processing vessel, while more specifically the efficiency of mixing is related to speed of rotation of the agitator, velocity difference between the bulk fluid and the agitator; the creation of a vortex, incorporation of air into the bulk fluid and any shearing effects.

All these factors are relevant to the dispersal of powder into the bulk fluid and hence an equipment manufacturer has various options in terms of design.
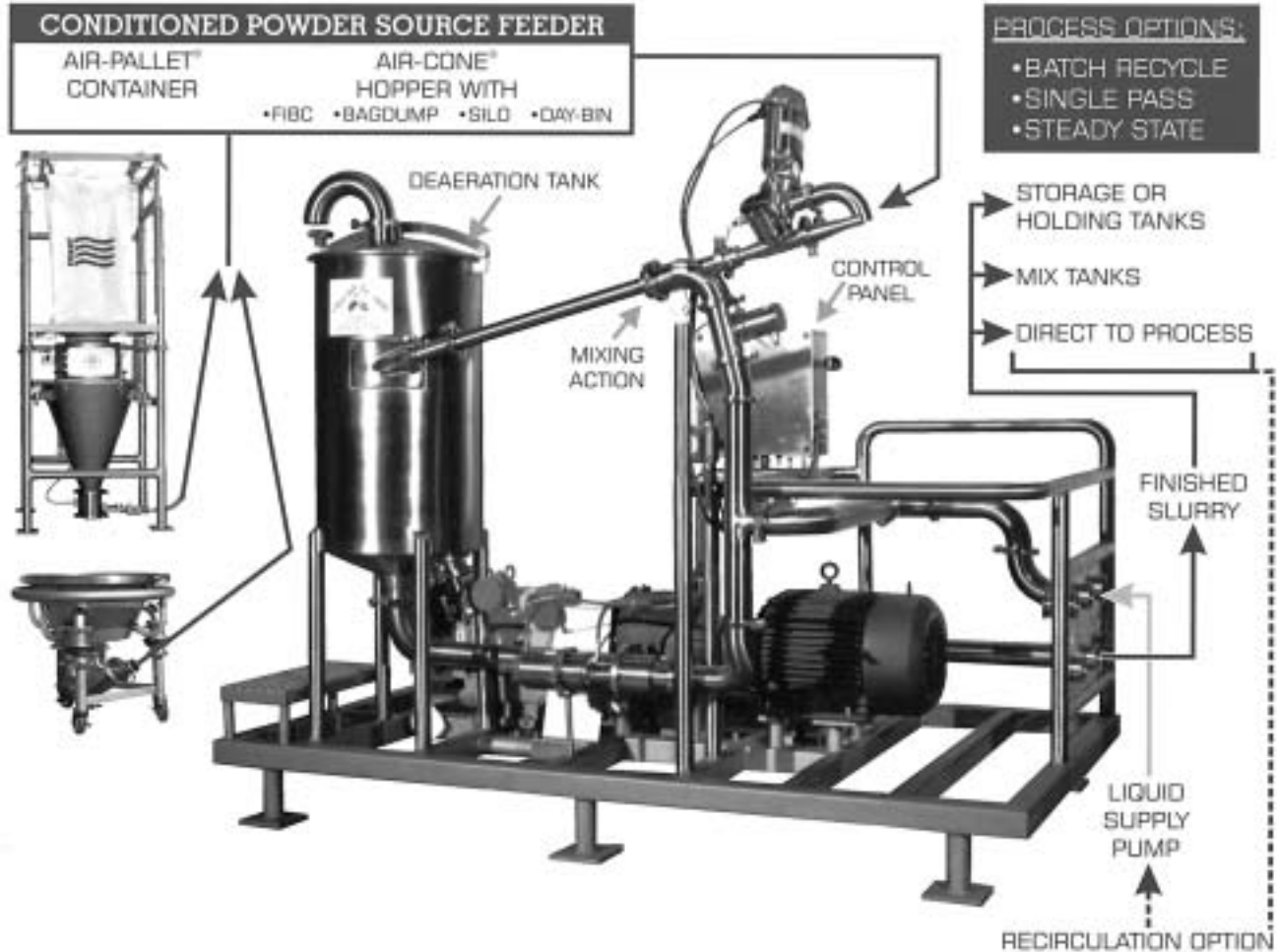
Fig. 3.22 Vacucam™ continuous in-line powder mixing system
Reproduced by courtesy of Semi-Bulk Systems Inc., Missouri, U.S.A.
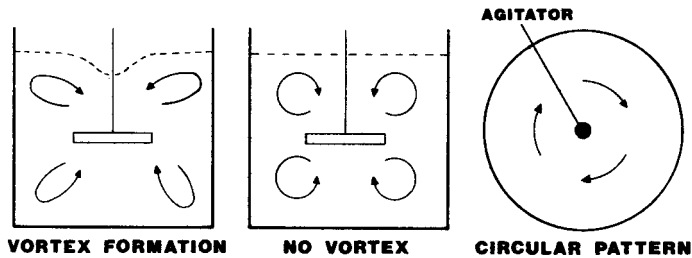
Fig. 3.23   Liquid mixing flow patterns
Note: the paddles are perpendicular, top entering and centrally mounted.
After Tamime and Greig (1979). Reprinted with permission of *Dairy Industries International*.

Multipurpose processing tank – This type of tank (i.e. the batch pasteuriser) can be utilised during all stages of yoghurt making (see Fig. 3.6), since the agitation system consists of a high speed motor which is operated during the preparation and processing of the milk, a slow speed motor for mixing in the starter and later for cooling the coagulum, and the drive shaft of the slow speed motor can be fitted with a one- or two-propeller agitators and is usually top entering and sloped (see also Bylund, 1995).

Simple mixing tank – Different types of high speed mixer (Silverson and Greaves) could be used in simple tanks that resemble a batch pasteuriser, but do not have a properly mounted agitation system. Thus in yoghurt production, two of these tanks will be installed in parallel for preparation of the milk base, so that while one tank is being emptied, the other tank is normally being filled up; a continuous flow of yoghurt milk to the incubation tanks can be achieved in this way. In practice, a tank is filled with water or milk warmed to around 40–50°C and the milk powder is emptied from the sacks. Recombination is achieved using a high shear mixer/homogeniser and the mixers can be mounted permanently in each tank, or alternatively can be removed from one tank to the other with the aid of an hydraulic lift (see Fig. 3.24).

An alternative older type of high speed in-tank mixer is the Ystral mixer described by Dalhuisen (1972). The powder mixing procedure is: (a) powder is emptied into the special chute, (b) the high speed action of the mixing head creates a vacuum at the tip of the powder delivery pipe, thus transferring the powder down the pipe from the chute, and (c) powder/liquid mixing takes place in the absence of air; there is little risk of the powder forming clumps.

Crepaco "Multiverter" – This is a specially designed tank that provides rapid and complete dispersion of the dried ingredients into the liquid slurry. The tank has a 15° or 35° cone bottom which facilitates easy and rapid unloading and it is fitted with a high-speed motor which drives a special centrifugal agitator. This unique agitator incorporates a "squirrel cage" design which results in a dual blending action combining an overall swirl with a deep-draw vortex that quickly and effectively disperses the milk powder into the aqueous phase with a minimum of foam. Although the tank is specifically designed to emulsify two or more immiscible products, the blending action is especially effective in dispersing any fatty constituents in the yoghurt milk. Furthermore, the tank can also be fitted with a CIP system.
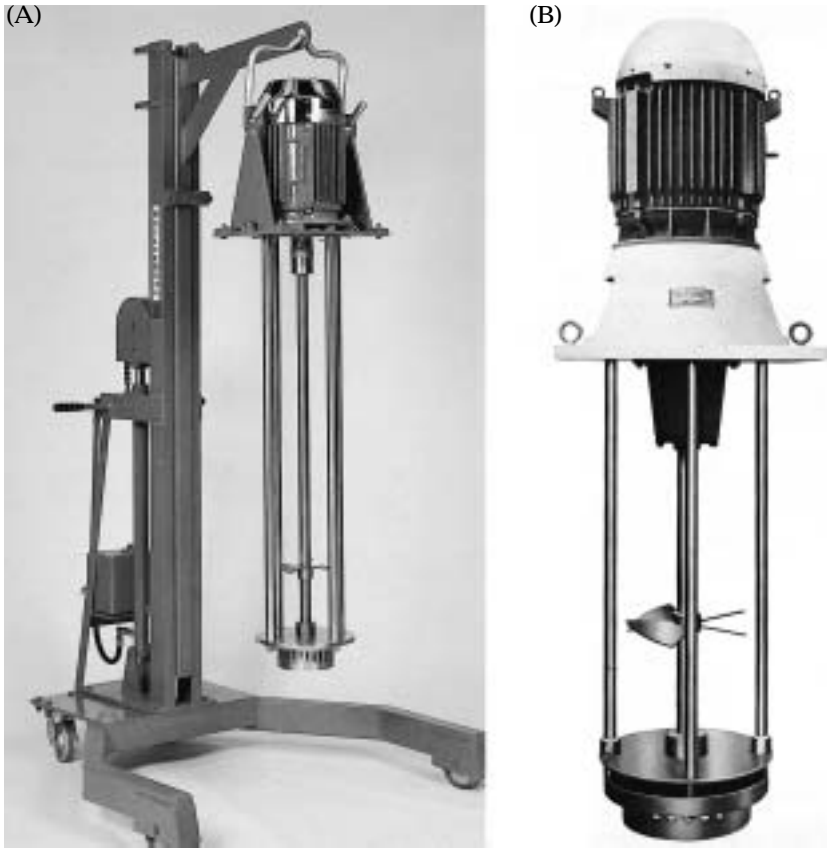
Fig. 3.24 Examples of high speed mobile mixers (A) Silverson and (B) Greaves
Reproduced by courtesy of Silverson Machines, Chesham, U.K. and Joshua Greaves and Sons, Bury, U.K.

Crepaco "Liquiverter" – This high speed mixer/blender is capable of both dispersing the dry ingredients and incorporating fat into the liquid phase. The impeller/agitator is centrally mounted from the bottom of the square tank and the action of the Liquiverter pulls the added milk powder through the liquid vortex at the centre and forces the mixture up the walls in continuous circulation.

Large-scale recombination plant – Two systems could be used during the large-scale production of a milk base (Bylund, 1995; see also Aneja, 1990). In the first system, the fat is dosed into the mixing tank (see Fig. 3.25).

Potable grade water is heated in a PHE to facilitate easy rehydration of the SMP and is metered into one of the storage tanks (see Fig. 3.25 (7)). The circulation pump (5) is started when the tank is half full and water flows through a bypass line from the mixing tank to a high speed powder blending unit (4). The feed rate of skimmed milk powder (SMP) through the blending system is up to 45 kg min^{-1}. A vacuum is created by an interplay between the circulation pump (5) and the booster pump (6) which causes the blender to draw the ingredients into the eye of the centrifugal impeller. The agitator in the mixing tank is started at the same time as the
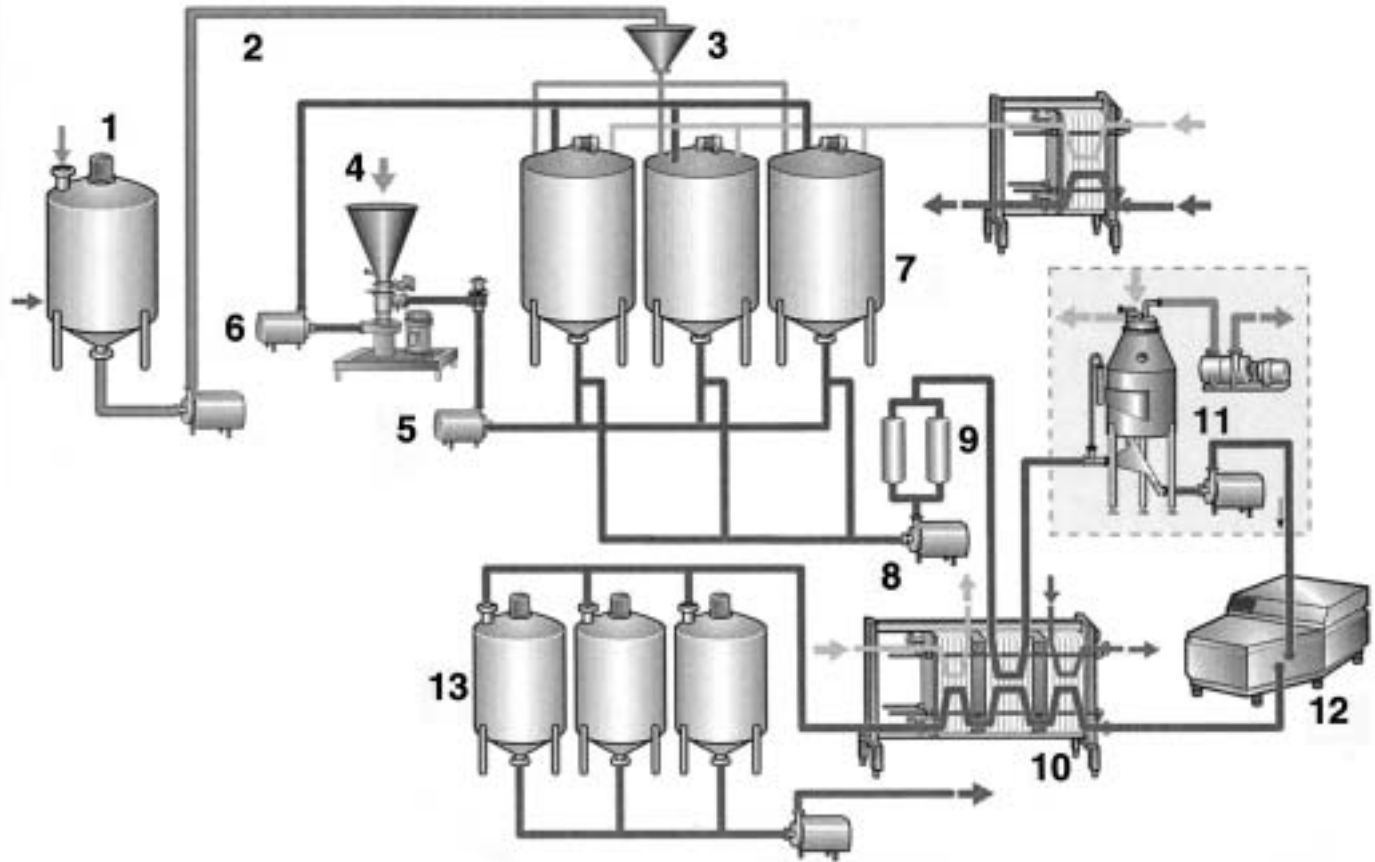
Fig. 3.25   Recombination in a large-scale plant where the fat is added in the mixing tanks

1, Tank containing melted fat (*e.g.* cream or anhydrous milk fat (AMF); 2, insulated pipe for delivery of fat; 3, weighing funnel for fat; 4, funnel with high speed blender (see Fig. 3.20); 5, circulating pump; 6, booster pump; 7, mixing tank; 8, discharge pump; 9, filters; 10, PHE; 11, vacuum de-aerator (optional); 12, homogeniser; 13, storage tanks.

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circulation pump. Water continues to flow into the tank while mixing is in progress until the specified quantity has been supplied.

When all the SMP has been added, the agitator and the circulation loop are stopped and the contents of the tank are left until the SMP has dissolved completely. At a water temperature of 35–45°C this will take about 20 min. At the end of this period the agitator is restarted. In the meantime, the blender is reconnected for the next batch to be recombined. AMF is now added from the fat storage tank (1). The quantity is measured in the weighing funnel (3). The agitator, specially designed for optimum fat dispersion, runs for several minutes and finely disperses the fat in the skimmed milk. The piping for the warm fat fraction is normally insulated to prevent the temperature of the fat from falling below the melting point.

When all the ingredients have been mixed and added to one tank, the process is repeated in the next tank. The skimmed milk/fat mixture is drawn from the full mixing tank by pump (8) which forwards the mixture through duplex filters (9). After being preheated in the PHE (10), the product is pumped to the homogeniser (12) where the dispersion of fat globules is completed. During recombination, air might be incorporated into the milk base, and a vacuum de-aerator vessel (11) can be installed in the line before the homogeniser to eliminate this; such a unit can reduce the air content from 1.3–1.8% to 0.1–0.2% which can improve the texture and consistency of the yoghurt (Rage *et al.*, 1987). The product is preheated to 7–8°C above homogenisation temperature before being flashed in the de-aerator, where the vacuum is adjusted so that the outgoing product has the correct homogenisation temperature, typically 65°C. The homogenised milk is pasteurised and chilled in the PHE (10) and is then pumped to the storage tanks (13) or direct to packaging. However, for yoghurt production the milk is heated to higher temperature as described in Fig. 3.13.

Alternatively, in-line fat mixing (Fig. 3.26) can be used in which the recombination of the powder is similar to that described in Fig. 3.25 (Bylund, 1995). In this system, the process could be described as follows. When a mixing tank has been filled and the contents have been given time for complete hydration of the SMP, the reconstituted skimmed milk is pumped through duplex filters (6) to a balance tank (7) (see Fig. 3.26). This ensures a constant flow rate to the process. A centrifugal pump (8) feeds the skimmed milk through a preheating section of the PHE (9). Although the addition of fat can suppress foaming in the skimmed milk, in this instance, a de-aerator vessel (10) is required. The milk base is preheated and homogenised in the manner described in Fig. 3.25, but then the milk flows through an in-line injector (13) where liquid fat from the fat-melting tank (11) is continuously metered into the flow by a positive displacement proportioning pump (12). Blending is completed in an in-line mixer (14) downstream of the injector. Immediately after mixing, the recombined milk continues to a high capacity homogeniser (15) and then returns to the PHE (9) for further processing as described in Fig. 3.13.

When dealing with the recombination of milk powder, two conditions in the reconstituted milk have to be monitored. First, not all the particles of milk may dissolve during the recombining process, perhaps through the use of poor quality powders, inefficient mixing equipment and/or the presence of scorched particles. Any undissolved particles must be removed using in-line stainless steel mesh, or a stainless steel mesh and nylon filter called the duplex, or centrifugal clarifiers. Clarifiers are excellent for the removal of any fine or undissolved particles and any
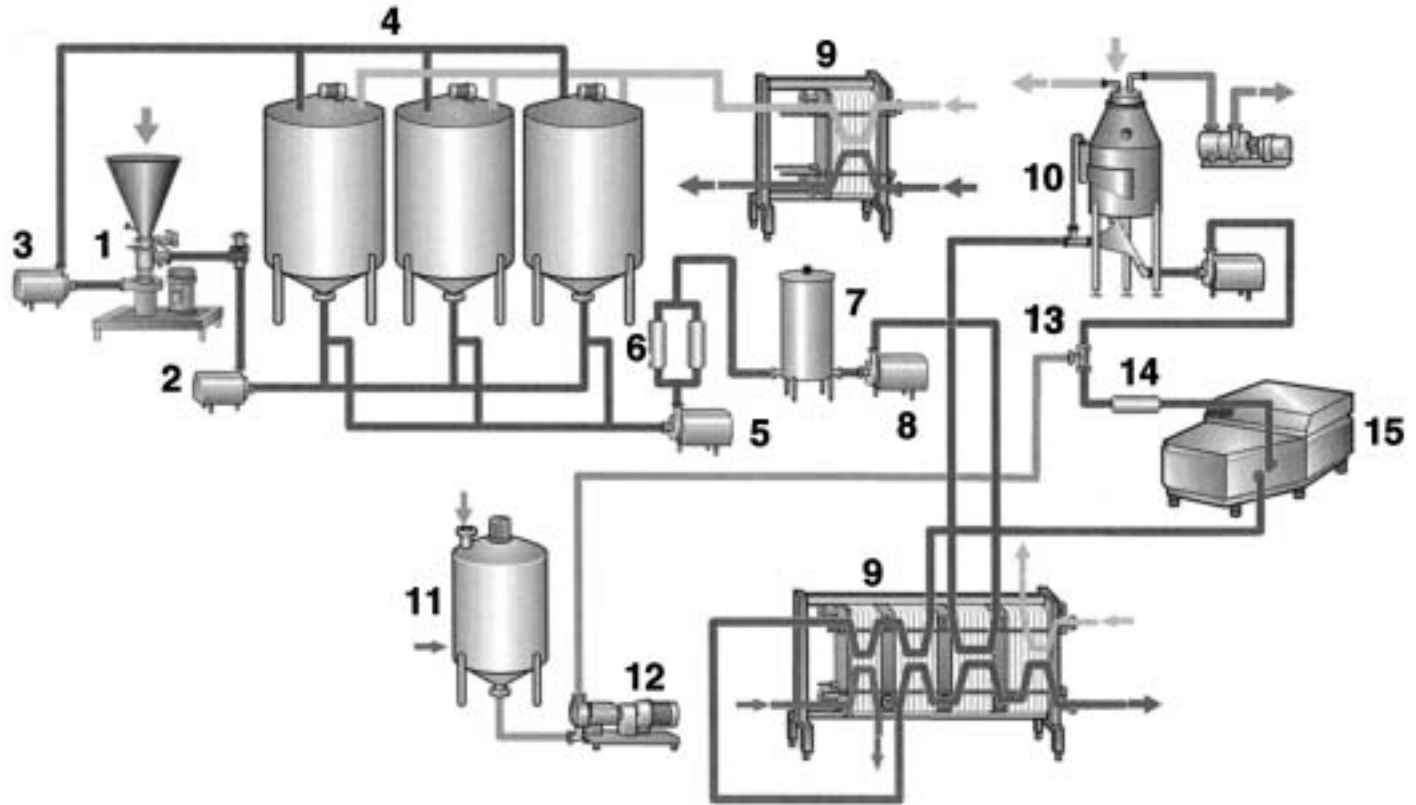
Fig. 3.26 Large-scale recombination plant with in-line fat mixing

1, Funnel with high-speed mixer (see Fig. 3.20); 2, pump for circulation; 3, booster pump; 4, mixing tanks; 5, discharge pump; 6, filters; 7, balance tank; 8, feed pump; 9, PHE; 10, vacuum de-aerator; 11, tank containing melted fat (e.g. cream or AMF); 12, positive displacement pump; 13, fat injector; 14, in-line mixer; 15, homogeniser.

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extraneous matter but, for convenience, filters are more commonly used. Normally two interchangeable filters are installed in a milk reconstitution line, especially in large dairies, so that in the case of clogging, the flow of milk can be easily diverted while one of the filters is being cleaned. The removal of such particles is essential, since their presence in the milk can damage the orifices in an homogeniser and/or increase soiling in heat exchangers. Second, reconstituted powders require up to 15 min to achieve complete hydration, otherwise sedimentation becomes evident. The hydration effect may not be important during the manufacture of yoghurt, since the time elapsing between recombination and the end of heat treatment of the milk can be as long as 15 min anyway.

3.3.3.3 Evaporation of milk

Concentration of standardised milk base can be achieved by use of an evaporator, in which the average amount of water removed is $10–25 \, g \, 100 \, g^{-1}$ and the total solids is increased by $1.5–3 \, g \, 100 \, g^{-1}$, corresponding to the recommended fortification with milk powder (Bylund, 1995). In order to remove the desired amount of water and avoid damage to the milk constituents at high temperatures, the process of evaporation is normally carried out under vacuum.

Single-effect evaporators can be used directly in a yoghurt processing line. The milk base is pumped from the balance tank to the condenser where it is preheated and then enters the plate section of the evaporator for further heating. After reaching the preset temperature, the milk flows to the separator section and water vapour is removed from the milk; the cycle is repeated until the desired concentration of total solids in the milk base has been reached. Heat recovery during the evaporation process is very efficient and is achieved using a thermocompressor, that is, factory steam is mixed with the vapour produced from the evaporator.

Another type of single-effect evaporator which could be used to concentrate the milk base is supplied by Tetra Pak A/B. The sequence of operations is as follows. The standardised milk base is preheated to 70°C in the regeneration section of the PHE using the condensate from the evaporator (see Fig. 3.13). Subsequently the milk is heated to 85–90°C in the heating section of the PHE and the preheated milk enters the vacuum chamber where the inlet is shaped as an expansion tube to prevent burning of the milk. The milk is recirculated four to five times until the desired degree of concentration is achieved. The recirculation cycle is controlled by the capacity of the vacuum chamber, evacuation pump and the float controller; during each recycle, about $3–4 \, g \, 100 \, g^{-1}$ of water is removed. The capacity of such evaporators is up to 8000 l hour^{-1}, but for larger plants, different types of evaporators are used with capacities up to 30 000 l hour^{-1}.

In general these evaporators offer the advantages of minimum requirement for space, efficient heat recovery and immediacy of use. Furthermore, yoghurt made from milk concentrated in this way exhibits an excellent organoleptic quality.

3.3.3.4 Membrane concentration of milk

An alternative method of fortification of the milk base is by concentration of the milk (whole and/or skim) by membrane filtration (i.e. UF and RO). The basic differences between the UF and the RO systems are first that the operational pressures are much higher in the case of RO, and second that the RO membrane is less permeable than the UF membrane; the pore size for RO is $<4 \, \text{Å}$ and for UF is $>20 \, \text{Å}$ ($1 \, mm = 10^7 \, \text{Å}$; see Fig. 3.27).
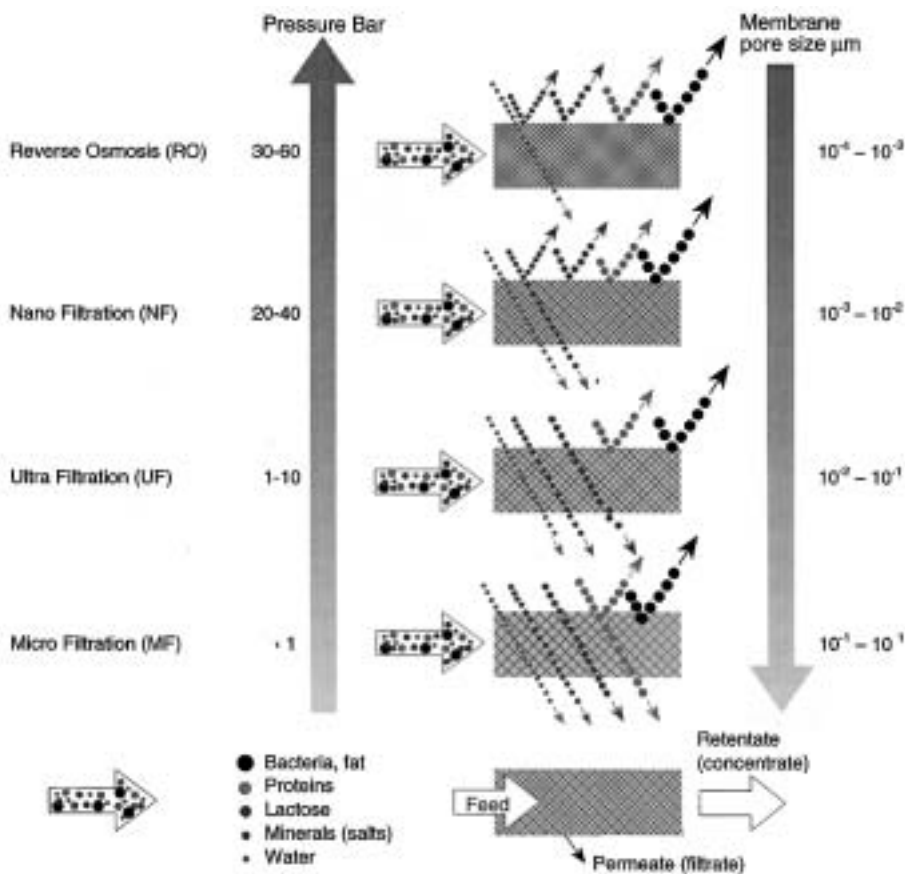
Fig. 3.27   Principles of membrane filtration systems of milk

Note: 10 Bar = 1 MPa; refer to Chapter 2 for milk constituent losses in the permeate.

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The milk constituents that pass through a membrane are referred to as the permeate, and the material that does not pass through the membrane (i.e. concentrated fraction) is known as the retentate. The different components present in milk can be divided into three main groups based on the molecular weight, that is, large molecules (proteins and fats), medium (lactose and salts) and small (water). The RO membrane allows only the small molecules (water) to pass through the membrane and the retentate consists of a concentrate of all the milk constituents, while the UF membrane permits small and medium size molecular weight solutes (e.g. water, lactose and salts) to pass through; the retentate is a concentration of the macromolecules of proteins and fats. The differences in the composition of the permeate and the retentate of the RO and UF processes are illustrated in Fig. 3.27.

The application of membrane filtration in the yoghurt industry is most likely to involve the use of UF (see also Ottosen, 1988, 1990; Cheryan, 1998), since it has the advantage of giving an increased concentration of proteins, but a reduced level of lactose in the milk base. Recently, Lankes *et al.* (1998) reported that set and stirred yoghurts made from UF skimmed milk (16 g total solids (TS) 100 g^{-1}) had better gel

Fig. 3.28 View of an ultrafiltration plant which can be employed for yoghurt production
Reproduced by courtesy of APV Nordic, Denmark.

strength compared with yoghurt that had been fortified with SMP or concentrated by vacuum evaporator (VE). Figure 3.28 shows a UF plant in a dairy in Denmark being employed during the production of yoghurt.

3.3.4 Homogenisation

Homogenisers are used mainly for the purpose of providing stable fat-in-water emulsions so that the fat in the milk base does not separate, but homogenisation also brings about some desirable physical changes in the milk base which contribute towards:

- whiter and more attractive colour of the milk
- improved mouthfeel of the product
- increased viscosity.

The homogenisation process was invented by Gaulin in 1899 who described it as "fixer a composition des liquides" (Bylund, 1995). However, the primary action of the homogeniser is to cause disruption of the fat globules to give ones of smaller diameter. As a consequence, the homogenisation process diminishes the creaming effect of the milk fat and reduces the tendency of the fat globules to coalesce or clump. Such an effect is achieved by forcing full fat milk through a small passage at high velocity.

The theoretical concept of homogenisation has been reported by Bylund (1995), Anon. (1996c, d) and Pandolfe and Baekgaard (1997). At present, it is accepted that homogenisation reduces the fat globule size in the milk due to turbulence or cavitation. The former theory suggests that the energy dissipating in the liquid generates turbulent eddies. However, the intense energy of the turbulence and localised pressure differences then tear apart the droplets, reducing their average size.
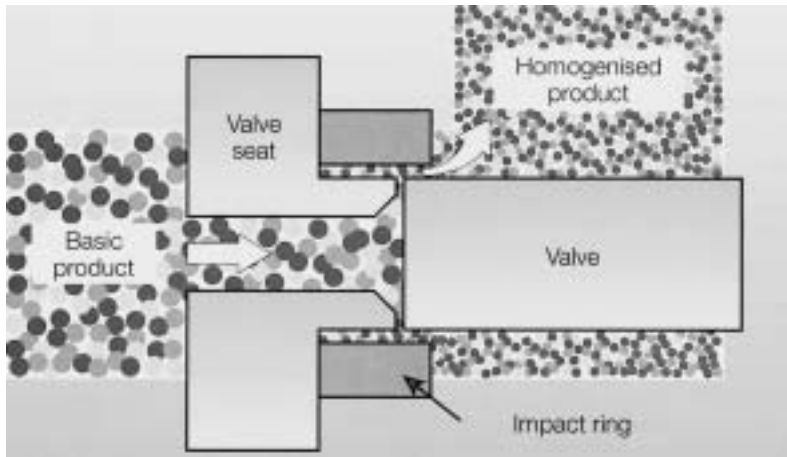
Fig. 3.29 Schematic diagram illustrating how the homogenising plug-type valve works
Reproduced by courtesy of APV Nordic, Denmark.

In the cavitation theory, the liquid encounters intense cavitation because of the large pressure drop through the valve. When the pressure drop is large enough, the vapour pressure of the liquid exceeds the ambient pressure causing the formation of vapour bubbles (cavities in the liquid). When the cavitation bubbles implode (collapse of the cavities), shock waves are generated in the liquid and these shock waves break apart the dispersed fat droplets. However, it has been suggested that some of the effects associated with turbulence and cavitation are similar, therefore making it difficult to distinguish clearly between the two (Anon., 1996c).

By tracing the path of flow of the full fat milk through the homogenising valve, it will be easier to understand the concept of homogenisation. However, many types of valve are available (see Harper *et al.*, 1976). Figure 3.29 shows a plug-type homogenising valve and a standard valve seat. The non-homogenised product enters the valve seat from the pump cylinder at a relatively low velocity (for example 3.1–6.1 m s^{-1}), but at high pressure (20.7 MPa). The pressure is generated by the positive displacement pump and the restriction to flow is caused by the valve being forced against the seat. Also, the positive displacement pump provides a relatively constant flow and, therefore, will generate the required pressures as the area between the valve and seat is increased or decreased. As the velocity of product flow between the valve and seat increases, the pressure decreases producing an instantaneous pressure drop. Then the liquid impinges on the impact or wear ring (see Fig. 3.29) and is finally discharged as an homogenised product.

High pressure homogenisers (see Fig. 3.30) are generally needed when high efficiency homogenisation is required. The product enters the pump block and is pressurised by the piston pump. The pressure that is achieved is determined by the back pressure given by the distance between the forcer and seat in the homogenisation device. This pressure, P1, is always designated the homogenisation pressure. P2 is the back pressure to the first stage or the inlet pressure to the second stage in two-stage homogenisers. The piston pump is driven by a powerful electric motor (Fig. 3.30 (1)) through a crankshaft and connecting-rod transmission which converts
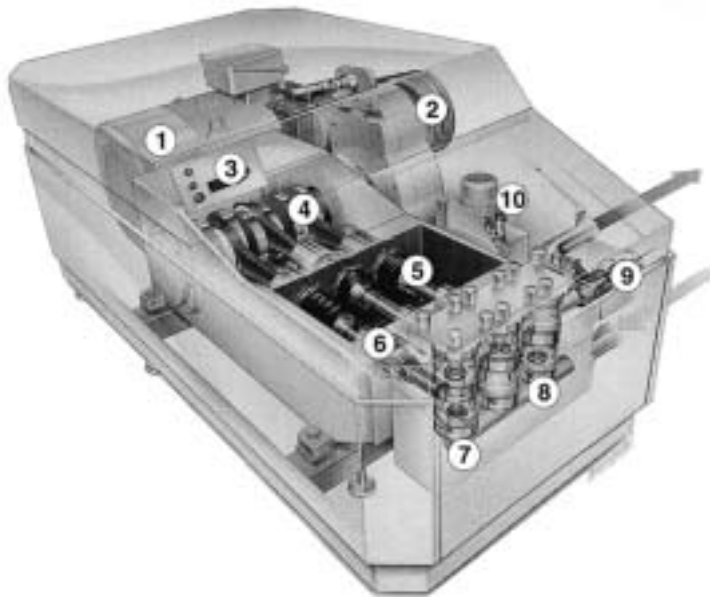
Fig. 3.30 View of a homogeniser which is a large high pressure pump with a back pressure device

1, Main drive motor; 2, V-belt transmission; 3, pressure indication; 4, crankcase; 5, piston; 6, piston seal cartridge; 7, solid stainless steel pump block; 8, valves; 9, homogenising device; 10, hydraulic pressure setting system.

Reproduced by courtesy of Tetra Pak (Processing Systems Division) A/B, Lund, Sweden.

the rotary motion of the motor to the reciprocating motion of the pump pistons. The pistons (Fig. 3.30 (5)) run in cylinders in a high pressure block; they are made of highly wear-resistant materials. The machine is fitted with double piston seals and water is supplied to the space between the seals to cool the pistons. Hot condensate can also be supplied to prevent reinfection when the homogeniser is placed downstream in aseptic processes.

Milk is supplied at high pressure to the space between the seat and forcer. The width of the gap is about 0.1 mm or 100 times the size of the fat globules in homogenised milk. The velocity of the liquid is normally $100-400 \, \text{m s}^{-1}$ in the narrow annular gap and homogenisation takes place in $10-15 \, \mu\text{s}$. During this time, all the pressure energy delivered by the piston pump is converted to kinetic energy. Part of this energy is converted back to pressure again later and the other part is released as heat. Every 4 MPa drop in pressure across the gap gives a temperature rise of 1°C. In fact, less than 1% of the energy is utilised for actual homogenisation but, nevertheless, high pressure remains the most efficient method available to handle emulsions.

With regard to the impact of homogenisation of the milk base on the quality of yoghurt, a number of aspects have to be considered (see also Hong, 1995), for example, the use of single- or two-stage homogenisation and the positioning of the homogeniser (i.e. before or after heat treatment). Since most of the yoghurts produced in different countries of the world contain fat $\leq 3.0 \, \text{g} \, 100 \, \text{g}^{-1}$, it is arguable

whether two-stage homogenisation is necessary. Kessler (1998) has examined a number of factors that can influence the firmness of yoghurt made from a milk base containing $10\,g\,fat\,100\,g^{-1}$ and his findings can be briefly summarised as follows:

- Use a mixture of 50:50 WPC and SMP instead of SMP alone.
- Denature β-Lg to ≥90% (for more information refer to Chapter 2) or heat treat the milk base at high temperatures, e.g. 95°C for 80 s.
- Increase the single-stage homogenisation pressure to 30 MPa; however, circulating the milk with up to four passes through the single-stage homogeniser at 20 MPa increased the firmness of the gel, or reduction of the fat globule diameter from 1.8 μm to 1.1 μm resulted in a doubling of the firmness of the product.
- Homogenisation of the milk base after the heat treatment stage produces a firmer product because the homogeniser causes the casein micelles to be torn apart by surface-active forces while new fat globules are being formed; during acidification, hydrophobic interactions result in a more stable protein network.

3.3.5 Heat treatment

The purpose of the heat treatment of the milk base has been discussed in detail in Chapter 2, and hence only the important technical aspects will be reviewed in this section (see also Klupsch, 1984, 1985; Lucey *et al.*, 1998; Kessler, 1998).

The heating of the milk base and the cooling of the coagulum both involve one fundamental aspect of thermodynamics, heat transfer (Hall, 1976; Loncin and Merson, 1979; Kessler, 1981, 1998; Hall *et al.*, 1986; Fikiin *et al.*, 1987; Brennan *et al.*, 1990; Bylund, 1995; Fryer *et al.*, 1997). In general, the flow of heat takes place from a warmer medium to a cooler and the greater the temperature differential between the two media, the greater and/or more rapid the heat flow. This transfer of heat can be either by conduction, convection or radiation (see Table 3.1) but, in the dairy industry, the former two processes are more important. The actual application of heat may be carried out in a direct or indirect manner but, for practical reasons, the latter is most widely used. Thus, instead of steam (food grade) being injected into the milk during the heating stage, the heating medium and the milk never come into contact with each other; the chemical composition of the milk base remains unaltered during the heat treatment. Similarly, the indirect method of heat transfer is used for cooling the coagulum.

The types of equipment that can be employed for heat treatment of milk include:

- The batch process (batch pasteurisers or multipurpose tanks) in which the milk can be heated by direct steam injection into the milk, or indirectly by one of the following methods: (a) steam injection into the jacket (this system allows excellent heat transfer, but may lead to severe denaturation of the milk due to localised heating), and (b) steam injection into the water jacket (this system of heating is widely used); alternatively, the water can be heated by gas or electricity and such processing tanks are very popular with the small-scale producers.
- The continuous process (plate, tubular or scraped surface heat exchangers) in which the milk is heated by the indirect method using either direct steam (under reduced pressure) in the heating section of the heat exchanger or alternatively hot water.

The types of equipment used for heating the milk base are as follows.

Table 3.1 Brief definition of types of heat transfer and factors affecting thermal conductivity

Types of heat transfer	Factors affecting thermal conductivity
Conduction is the transfer of thermal energy from one molecule to another and this may take place through solid bodies or through layers of liquid at rest in which no physical flow or mixing takes place in the direction of heat transfer.	• Area • Thickness or length of heat transfer path • Temperature difference
Convection is the transfer of thermal energy due to the movement of mass and this occurs when particles at high temperatures are mixed with particles at a lower temperature.	• As above • Movement of fluid • Fluid characteristics (thickness, viscosity, turbulence, velocity and temperature of fluid)
Radiation is the emission of thermal energy by radiation (hot or cold) across an absolute vacuum in which the electromagnetic radiation of a body causes molecules to vibrate and emit radiant energy.	• Surface property of the body ⎤ e.g. a black body • Temperature of the body ⎦ shows good absorbance and emission of heat

3.3.5.1 Batch or multipurpose tanks

These tanks resemble batch pasteurisers and they are normally water jacketed. Steam is injected into the water during the heating stage of the yoghurt milk and chilled water is circulated during the cooling of the coagulum. The capacity of these tanks is several thousand litres, and according to Kessler (1981) the time required to heat the milk base with vigorous stirring can be calculated from the following equation:

$$
\text{Time of heating}
$$

$$
= \frac{\text{Volume } (\text{m}^3) \times \text{Density } (\text{kg m}^{-3}) \times \text{Specific heat } (\text{J kg}^{-1}\text{K}^{-1})}{\text{Effective heat exchange area } (\text{m}^2) \times \text{Heat transfer coeffient } (\text{W m}^{-2}\text{K}^{-1})}
$$

$$
\times \ln \frac{\text{Temperature of heated medium} - \text{Starting temperature of the milk base}}{\text{Temperature of heated medium} - \text{Desired temperature required to heat the milk base}}
$$

$$
\ln = \text{natural logarithm}
$$

In a large processing plant, a series of these tanks could be used at regular intervals for the production of yoghurt on a semi-continuous basis. A typical processing cycle using multipurpose tanks could involve the following stages:

- filling the tank with fortified and homogenised milk at ≥60°C;
- heating the milk base to 85–90°C for 15–30 min;
- cooling the milk to the incubation temperature, i.e. 40–45°C (short set) or to 30°C (long set);
- incubating the milk to the desired acidity;
- cooling the coagulum to 20°C or <10°C.

Examples of multipurpose yoghurt processing tanks have been reviewed by Tamime and Greig (1979) and Robinson and Tamime (1990, 1993) and the design of such tanks should cover the following aspects:

- Provision of a heat exchange medium (e.g. direct steam or hot water) for circulation in the jacket and high speed agitation for use during heating of the milk.
- For in-tank cooling (optional, refer to subsequent text), glycol or chilled water is circulated in the jacket and slow speed agitation must be provided during the cooling of the coagulum.
- These tanks usually have a conical base to facilitate easy emptying of the cooled yoghurt.

3.3.5.2 Continuous process

The types of heat exchangers most commonly used in the dairy industry are:

- plate heat exchanger (PHE)
- tubular heat exchanger, including the multitube or multichannel designs
- scraped/swept surface heat exchanger.

The former two types are widely installed in yoghurt plants for the heat treatment of the milk base, but the swept surface heat exchanger is used for the heat treatment of fruit preparations. These heat exchangers can be visualised as two-channel units in which the heating medium (hot water) flows in one channel and is separated by a partition from the yoghurt milk flowing in the other (Bylund, 1995; Anon., 1996e). The milk is processed, therefore, on a continuous basis and, when compared with the batch process systems, offers the following advantages: (a) a small floor area is required, (b) less energy is required due to the improved efficiency of heat transfer; (c) productivity can be increased by utilising the fermentation tanks more than once per day, and (d) the system is more versatile, for example the processed milk could be removed from the plant at a certain temperature to be homogenised.

A PHE is a unit which consists of a series of corrugated stainless steel plates held together in a frame and a rubber gasket is fitted to prevent leakage between the milk and water passages along the boundaries between the plates. The corrugation of the plate helps to increase the turbulence of the liquid flow and/or the surface area of the plate and hence improve the efficiency of heat transfer. Also, the shape of the partition in a PHE may differ depending on the product to be treated and thermal efficiency requirements. The thickness of the gasket does, of course, alter the space between the plates, and while a narrow gap is desirable for the heat treatment of milk (e.g. 2.5 mm), a larger gap (e.g. up to 6 mm) is recommended for cooling of the coagulum. In the former instance, the milk flows in a thin film across the width of the plate, so that heat transfer is rapid, but the large gap is necessary during the cooling of the yoghurt in order to avoid too great a drop in viscosity.

According to Bylund (1995), the necessary size and configuration of any type of heat exchanger are governed by multitude of factors such as:

- product flow rate
- physical properties of the liquids to be processed
- temperature programme
- permitted pressure drops
- heat exchanger design
- cleaning requirements
- required running or operation time.

Thus, the general formula, which is used to calculate the required heat transfer area of a heat exchanger, is

$$A = \frac{V \times \rho \times c_p \times \Delta t}{\Delta t_m \times k}$$

where A is the required heat transfer area, V is the product flow rate, ρ is the density of the product, c_p is the specific heat of the product, Δt is the temperature change of the product, Δt_m is the logarithmic mean temperature difference (LMTD) and k is the overall heat transfer coefficient.

In practice, a PHE consists of several sections in which different treatments of the milk may take place, for example, preheating/regeneration, final heating, holding and/or cooling sections. The heating medium is normally hot water, but if the milk is to be heated to temperatures above 100°C, steam (under reduced pressure) may be used. The cooling medium can be cold water, chilled water or brine, and the type of coolant circulated in a PHE is dependent on the desired outlet temperature of the product.

The flow of both milk and heating/cooling medium in a PHE can run alternately (i.e. single-channel operation), but the efficiency of heat transfer is difficult to maintain. To overcome this disadvantage, the flow of fluids in a PHE may be arranged into special patterns, and one example has a combination of a $4 \times 2 / 2 \times 4$ (Bylund, 1995). Such a combination means that the heating medium is in four parallel channels and changes its direction twice, and the flow of milk is in two parallel channels and changes direction four times.

The tubular heat exchanger is, as the name indicates, constructed from tubes or pipes and may be in the form of a single-tube heat exchanger, or may consist of a bundle of tubes or multichannel tubes. In a single-tube type, the heat exchanger consists of one tube inside another (heating/cooling medium) tube (coaxial double tube), but if a larger surface area is required, the product/medium tubes can be arranged spirally within an upright cylindrical tank. This latter type of a heat exchanger is manufactured by Stork-Amsterdam. The flow of liquids in this unit can be either parallel or counter current, and the latter is usually recommended for the heat treatment of the milk base. A more recent development is a multichannel tubular heat exchanger in which a number of coaxial tubes are fitted inside each other; the heating medium flows in the spaces of these tubes and the milk flows through the middle of each tube. In the other type of tubular heat exchanger, bundles of tubes are enclosed within an outer shell and while the milk flows through the pipes, the heating/cooling medium circulates inside the shell.

For the heat treatment of viscous products the scraped/swept surface heat type is used and the unit consists of a jacketed cylinder fitted with a scraper blade. The blades, which rotate at a high speed, remove the continuously processed product from the heated surface and, as a result, the effective surface area is large; heat trans-
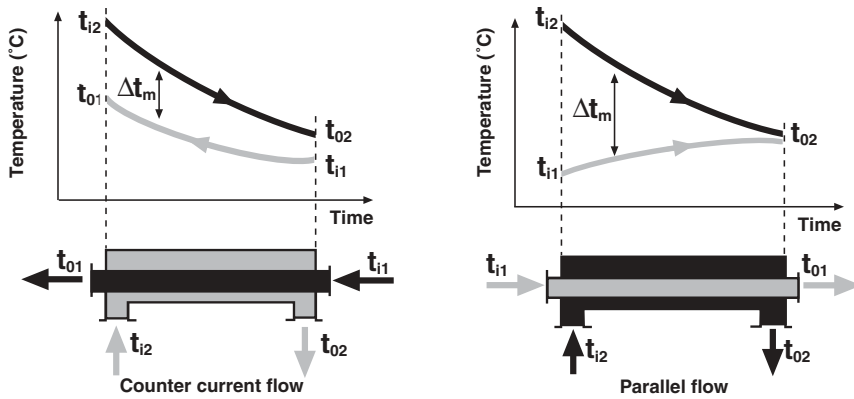
Fig. 3.31 Differences in the temperature profiles for heat transfer in a PHE either with parallel or counter-current flow

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fer is normally rapid, depending on the speed of rotation of the blades. These heat exchangers can be mounted vertically or horizontally.

In principle, irrespective of what type of heat exchanger is used, it is safe to assume that heat transfer through a partition wall resembles the profile illustrated by Bylund (1995). The flow of fluids (i.e. hot water and milk) in a heat exchanger can be either in the same direction (parallel flow) or in the opposite direction (counter-current flow) and, in each situation, the profile of temperature changes during the heat treatment of milk is different (see Fig. 3.31). In counter current flow, the milk and the heating medium enter the heat exchanger from opposite ends (i.e. the cold milk meets the cooled heating medium) and the temperature is progressively raised as it passes through the heat exchanger. The overall temperature of the heated milk is always a few degrees below the temperature of the heating medium at the corresponding point (see Fig. 3.31). However, in parallel flow, both the milk and the heating medium enter the heat exchanger from the same end and, as a result, the increase in temperature of the product is never higher than if the milk and the hot water were mixed together (see Fig. 3.31). Different efficiencies of heat transfer are, therefore, obtained from the contrasted types of flow and Kessler (1981) reported a 50% maximum efficiency for parallel flow; the efficiency was much higher with the counter-current system.

As mentioned earlier, the equipment for continuous heat processing is made up from different sections. In a plant designed for the heat treatment of the milk base, these sections are:

• regeneration section
• heating/cooling section
• holding unit.

It is also important that the plant is installed with a balance tank in order to maintain a continuous flow of milk. Balance tanks are normally situated in the area where the milk is being fortified and/or standardised. Different types of balance tank are available on the market, fitted either with a special float or with level sensors that ensure that milk is always available.

Regeneration section – In this section the incoming cold yoghurt milk is pre-warmed by the heated milk and vice versa, with the aim of utilising energy more efficiently and economically. For example, if the temperature of the milk base is raised from 5°C to 90°C (hot water) and then cooled to 40–45°C (cold water), the energy demand is high; energy is required to heat the hot water and also to cool the cold water. However, if the heat energy can be utilised in the regeneration sections of the plant, the result is energy conservation and the efficiency of regeneration is sometimes expressed as a percentage. Fearn (personal communication) has provided the following energy data relating to two different types of Tetra Pak yoghurt processing plants.

In the first example the capacity of the plant was 4000 l hour^{-1} and the milk base was fortified by the addition of SMP. The temperature progression using a plate heat exchanger fitted with a regeneration section was as follows:

- The temperature of the milk base was raised from 5°C to 45°C by regeneration, i.e. utilising the heat from the already heated milk, and the temperature change was 40°C. The prewarmed milk was heated from 45°C to 90°C by hot water (incidentally, at around 60–70°C the milk left the heat exchanger to be homogenised before returning to the plant for final heating).
- The heated milk was cooled from 90°C to 50°C by regeneration, i.e. transferring the energy to the incoming cold milk, and the temperature change was 40°C.
- The partially cooled milk at 50°C was further cooled to 40–45°C (incubation temperature) by water.

It can be observed that the milk base was heated from 5°C to 90°C (a temperature increase of 85°C) and that the increase in the regeneration section was 40°C. Therefore, according to Bylund (1995), the percentage of regeneration calculated from the following formula was equal to:

$$R = \frac{(t_r - t_i)}{(t_p - t_i)} \times 100$$

where R is the regeneration efficiency %, t_r is the milk temperature after regeneration (45°C), t_i is the temperature of raw incoming milk (5°C), t_p is the temperature after heat treatment (90°C),

$$R = \frac{(45 - 5)}{(90 - 5)} \times 100 = \frac{40}{85} \times 100 = 47\%$$

Although this figure is relatively low compared with a normal pasteuriser HTST (high temperature short time) or ultra high temperature (UHT) plants which may be about 94% efficient, the contrast is due to the fact that the product outlet temperature in the case of HTST and UHT milks is around 5°C and 20°C, respectively, compared with the milk base at 40–45°C. Thus, the energy requirements of the 4000 l hour^{-1} plant are 325 kg hour^{-1} of steam and 4000 l hour^{-1} of water.

In the second example the same capacity plant (4000 l hour^{-1}) was used for heat treatment of the milk base, but the plant was installed with a single-effect evaporator to concentrate the milk to the desired level of solids (see Fig. 3.13). The temperature progression was as follows:

- The incoming cold milk was prewarmed from 5°C to 60°C by regeneration, i.e. utilising the energy available in the condensate from the evaporator.

- The partially heated milk at 60°C was then heated by hot water to 90°C before entering the evaporator (in order to achieve the correct concentration of solids in the milk base, the milk was circulated within the evaporator and the heating section of heat exchanger at a flow rate of 19000 l hour^{-1}).
- The concentrated milk left the evaporator at 70°C and was homogenised; later it was heated to 82°C by regeneration, i.e. utilising the energy available from already heated milk.
- The concentrated milk at 82°C was heated to 90°C with hot water.
- The heated milk base was cooled from 90°C to 78°C by regeneration, i.e. a transfer of energy to the concentrated milk at 70°C.
- The milk base was then cooled from 78°C to 40–45°C (e.g. the incubation temperature) by cold water.

To calculate the percentage regeneration of this system is more complicated than with the first example, and the simplest approach is to divide the overall thermal load by the amount of heat obtained from regeneration which when multiplied by 100 is equal to the percentage regeneration. If the specific heat and the density of the milk base are assumed to be the same as water, that is 1, the calculations are as follows:

- 5–60°C (regeneration) – heat obtained is $55 \times 4700 = 258500$ kcal hour^{-1}
- 60–90°C (hot water) – thermal load is $30 \times 19700 = 591000$ kcal hour^{-1}
- 70–82°C (regeneration) – heat obtained is $12 \times 4000 = 48000$ kcal hour^{-1}
- 82–90°C (hot water) – thermal load is $8 \times 4000 = 32000$ kcal hour^{-1}

Therefore the overall thermal load is:

- $258500 + 591000 + 48000 + 32000 = 929500$ kcal hour^{-1}

The heat obtained by regeneration is:

- $258500 + 48000 = 306500$ kcal hour^{-1}

The percentage of regeneration is:

- $\dfrac{306\,500}{929\,500} \times 100 = \sim 33\%$

Thus, the energy requirements of such a plant are 840 kg hour^{-1} of steam and 9200 l hour^{-1} of water.

Although the percentage regeneration in the second example is slightly lower than in the former case, two factors must not be overlooked, the cost of the SMP and the quality of yoghurt produced from concentrated milk. The latter aspect has already been illustrated in Fig. 2.13 (see Chapter 2).

Heating section – In this part of the heat exchanger, the milk base is heated to the desired temperature and under commercial practice the final temperature may range from 85 to 115°C.

Holding section – The holding section of a heat exchanger is that part of the plant in which the heated milk can be maintained at temperature for a specified period of time. The objective is to provide for those time–temperature relationships that comply with existing legislation, for example pasteurised milk (HTST) must be heated to 72°C and held at that temperature for 15 s. There are, of course, no regulations regarding the heat treatment of the milk base for yoghurt, so in practice the time–temperature combination is chosen both to ensure the destruction of

pathogens and to bring about the physicochemical changes desired in the milk (refer to Chapter 2).

In the holding section no heating or cooling of the milk takes place and depending on the holding time desired, the unit can be built either as part of the heat exchanger or as a separate unit on its own.

Different time–temperature relationships have been employed for the heat treatment of the milk base and some examples of these combinations are:

- 30 min at 85°C (long holding time)
- 5 min at 90–95°C (medium holding time)
- 3 s at 115°C (short holding time).

It is evident, however, that the holding section of a yoghurt processing plant will, in most cases, have to be built as an external unit linked to the heat exchanger. The equipment available for holding milk for the specified times includes:

- Holding for "long time" – in order to provide a 30 min holding time in a continuous processing plant, a well-insulated or water-jacketed tank can be used instead of the usual holding unit. This method of holding requires a large floor area, but was widely used in the yoghurt industry in 1980s. At present, the long time holding system is rarely used in large yoghurt plants.
- Holding for "medium time" – spiral or zig-zag arrangements of pipework are often used as holding units for up to 5–6 min, and two typical examples are: the Tetra Pak (Fig. 3.32) or APV holding tube which is constructed from two spirals of stainless steel pipe enclosed in an insulated, upright cylindrical tank; a modified version, in which a 6 min holding time can be achieved, has been designed with large diameter zig-zag piping (see Robinson and Tamime, 1993).
- Holding for "short time" – in this case the holding section can be incorporated into the heat exchanger but, if a larger capacity of holding unit is required, the pipe can be installed outside the plant.
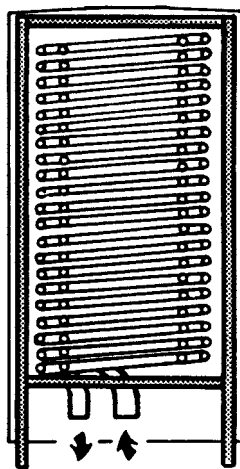
Fig. 3.32   Schematic illustration of holding tube/cell for medium time treatments up to 5 min

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The appropriate tube length for the required holding time can be calculated when the hourly capacity and the inner diameter of the holding tube are known (Bylund, 1995). As the velocity profile in the holding tube is not uniform, some milk molecules will move faster than average. To ensure that even the fastest molecule is sufficiently pasteurised, an efficiency factor must be used. This factor depends on the design of the holding tube, but is often in the range of 0.8–0.9. The formulae required for the calculations are:

$$V = \frac{Q \times HT}{3600 \times \eta} \, \text{dm}^3$$

$$L = \frac{V \times 4}{\pi \times D^2} \, \text{dm}$$

where Q is the flow rate at pasteurisation (l hour^{-1}), HT is the holding time in s, L is the length of holding tube in dm, corresponding to Q and HT, D is the inner diameter of holding tube in dm, to be known or adapted to the other pipework, V is the volume of milk in litres or dm^3 corresponding to Q and HT, η is the efficiency factor and π is 3.14.

For example, a holding time (HT) of 15 s is required in a pasteurisation plant with a capacity (Q) of 10 000 l hour^{-1}. The inner diameter (D) of the pipe to be used is 48.5 mm – 0.485 dm. Calculate the length (L) of the holding tube, with an efficiency factor of 0.85.

$$V = \frac{10\,000 \times 15}{3600 \times 0.85} = 49.0 \, \text{dm}^3$$

$$L = \frac{49.0 \times 4}{\pi \times 0.485^2} = 265.5 \, \text{dm or } 26.5 \, \text{m}$$

Thus, the length of the holding tube should be about 26.5 m.

Heat processing plants (e.g. for HTST and UHT milks) are fitted with a temperature-sensor safety device known as flow diversion valve (FDV). At the start of the processing operation the milk is normally diverted back to the balance tank until the right temperature is achieved and maintained, and only then does the milk flow through the rest of the plant to complete the processing cycle. However, the milk is always diverted back to the balance tank at any time that the temperature drops, so making sure that all the processed milk is heat treated to the specified temperature. The FDV unit is not, however, normally installed in a yoghurt plant, for if the temperature of the heated milk starts to drop, manual diversion of the milk back to the balance tank via a special arrangement of pipes is quite acceptable.

Normally, at the start of the heat treatment process, water is circulated through the plant both to sanitise the pipework and to warm the plant to the desired processing temperatures. Warming the plant avoids prolonged circulation of the initial milk intake.

3.3.6 Fermentation/incubation of the milk

At this stage of yoghurt manufacture, the processed milk (i.e. standardised/fortified, homogenised and heated milk) is cooled to the incubation temperature, which would be in the range of 40–45°C (short fermentation: $2\frac{1}{2}$–3 hours) or 30°C (long fermentation: overnight) and there are many different types of fermentation vessel

6

11

10

9

8

7

(A)
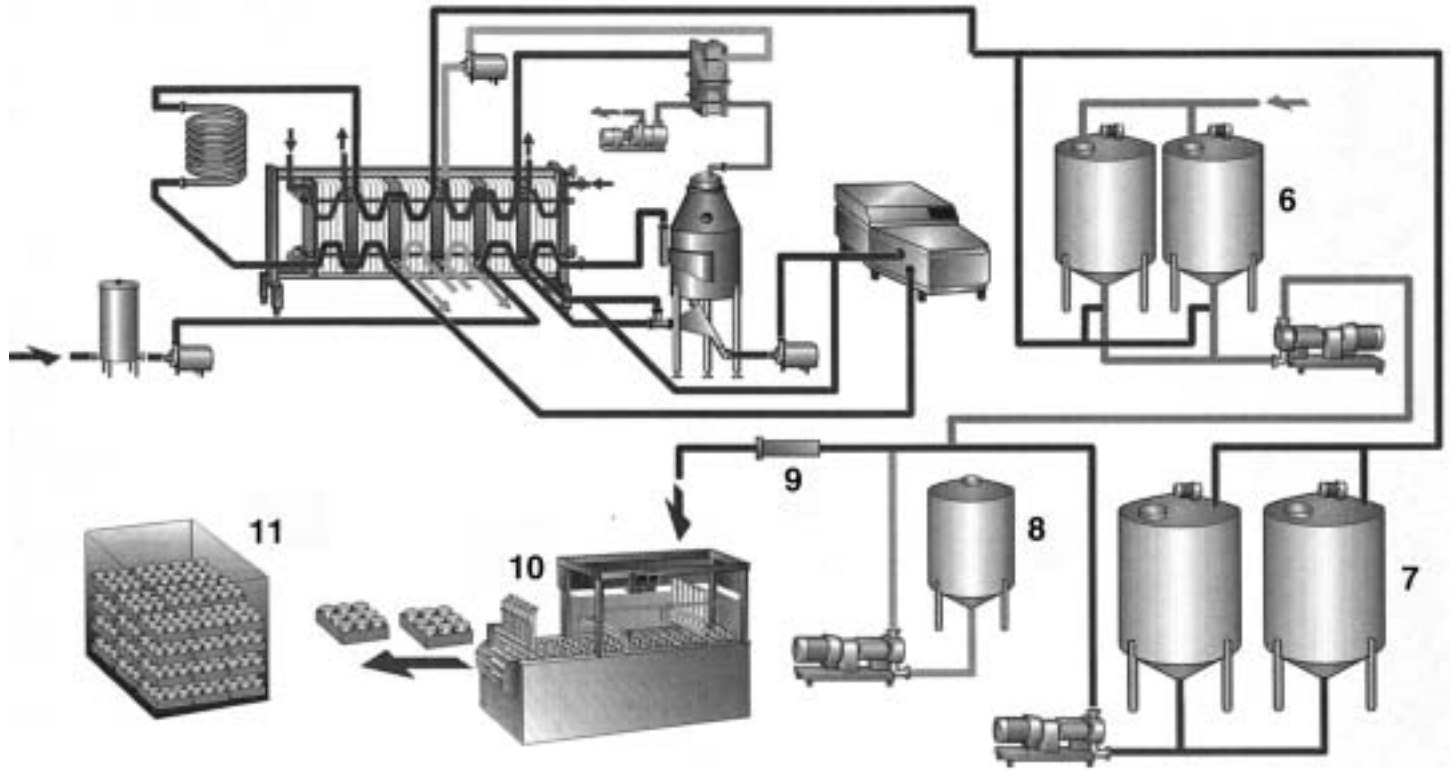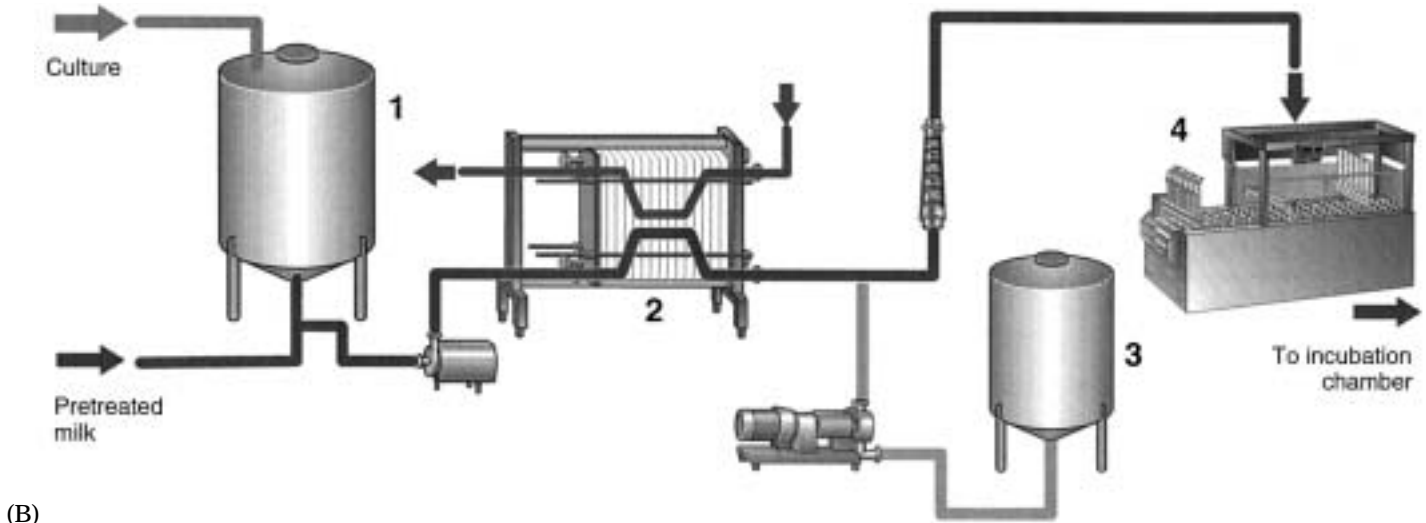
Fig. 3.33 Production lines for set yoghurt

A: Details of pretreatment of the milk base are given in Fig. 3.13; 6, bulk starter tanks; 7, buffer tanks; 8, flavouring tank; 9, in-line mixer; 10, filling machine; 11, incubation (see text). B: 1, Buffer tank; 2, PHE; 3, flavouring tank; 4, filling machine.

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which can be used. Basically the equipment is designed to provide and maintain the necessary processing condition(s), especially temperature and the form of the equipment depends on the type of yoghurt produced, that is, set or stirred.

3.3.6.1 Equipment for the production of set yoghurt

The fermentation/coagulation of the milk base takes place in the retail container. In brief, the process may involve the following stages:

- Cool the processed milk base to 40–45°C or 30°C.
- Add the starter culture and, if desired, flavouring materials and/or colouring matter to the milk. Incidentally, for the production of fruit set yoghurt (sundae style), the fruit is delivered into the retail container followed by the inoculated milk.
- Seal the retail containers, incubate, cool and dispatch.

It is evident that the same plant that processes the milk base (see Fig. 3.13) can be used for the production of both set and stirred yoghurt and, as a consequence, the installation costs can be reduced. An overall illustration of the plant is shown in Fig. 3.33A (Bylund, 1995). The starter culture is metered into the processed milk base (at the correct temperature) as it is pumped from an intermediate/buffer storage tank to the packaging machine. Also, the flavouring(s) can be continuously metered into the milk stream prior to packaging. As mentioned earlier, fruit pieces and other additives should be dosed into the yoghurt cups before they are filled with the inoculated milk.

Since the daily production of set yoghurt may be small, an alternative production system may be used (see Fig. 3.33B) which offers flexibility in production planning because the size of plant does not necessarily have to match the pretreatment capacity of the milk base or the capacity of the filling machines. The processed milk is cooled to <10°C and thoroughly mixed with the starter culture (e.g. DVI type). Matching the capacity of the selected machine, the milk/starter mixture is warmed in a PHE to the incubation temperature, mixed with flavours and finally packaged. Alternatively, bulk starter culture can be metered in-line to the warmed milk at the same time as the flavours (Bylund, 1995).

The correct conditions for fermentation are provided by employing one of the following approaches.

Water baths or tanks – In this system, the yoghurt containers, which are often glass bottles, are placed in metal trays immersed in shallow tanks of warm water; details of this old method were reported by Crawford (1962). The water level is maintained just below the tops of the bottles to avoid contamination of the product and, after the coagulation period, the warm water is replaced by circulating cold water that cools the coagulum very quickly. When the yoghurt is partially cooled, the trays are removed and transferred to the refrigerated store for final chilling. Since this method necessitates the use of glass bottles, the use of water baths/tanks is of limited popularity.

Cabinets – In the cabinet system, incubation takes place in small insulated chambers with average capacities ranging from 250 to 750l. Forced hot air is circulated during the fermentation stage and later it is replaced by chilled air. In order to improve the heat transfer characteristics of these units, the cabinet has the facility to moisten the hot air; if the retail container is moisture sensitive, then hot dry air is recommended.
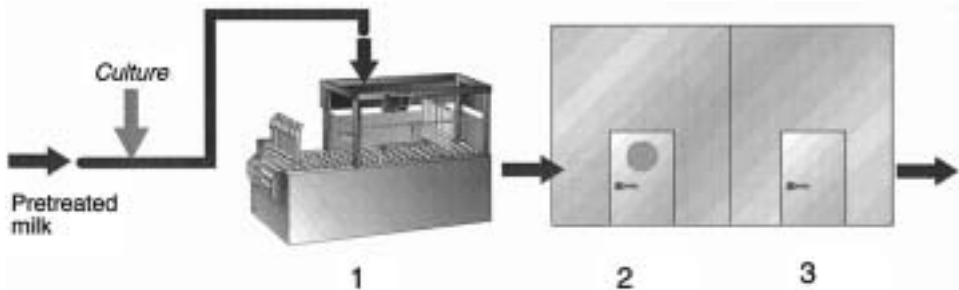
Fig. 3.34 Illustration of set yoghurt production showing incubation and rapid cooling rooms

1, Filling machine; 2, incubation cabinet; 3, rapid cooling room.

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Rapid cooling at the end of the fermentation stage is achieved by circulating chilled air. The yoghurt is then left until dispatched or, when the product temperature is low enough, moved to the main cold store. All units of this type are electrically operated and some incubator/cooler cabinets are fitted with a pH controller so that the fermentation/cooling cycle can be automated; in many cases the processing cycle is worked on a time basis. Nevertheless, the production of a uniform yoghurt does necessitate attention to the following points:

- The cabinets must be relatively small in size, so that the pallets can be stacked very quickly; the time lag between the first and the last yoghurt containers being placed in the cabinet should be very short.
- The air must circulate uniformly to all parts of the cabinet.
- There must be provision for accurate and reliable temperature control in the cabinet.

In some instances the cabinets are used only as incubators and the yoghurt is cooled in another cabinet (Fig. 3.34) or a refrigerated cold store. The disadvantage of this approach is that the coagulum is in motion while it is still warm and hence may suffer some structural damage and/or whey separation. However, the system may be less expensive to install in the first instance.

Tunnel – Large quantities of set yoghurt could be produced in batteries of individual cabinets, but the process can be mechanised for continuous production by adopting a tunnel system; however, it should be emphasised at this stage that the concept of continuous yoghurt production is different. For further details refer to Section 3.5. The pallets containing the yoghurt pots are placed on smooth rollers/conveyor belt and travel through a tunnel consisting of two sections. Warm air is circulated in the incubation part of the tunnel and the speed of the pallet is governed by the speed of the conveyor belt, which in turn is regulated by the rate of lactic acid production in the milk. At the end of the fermentation period, which is equivalent to pH 4.5, the pallets pass through the cooling section and the hot air is replaced by a blast of chilled air. The yoghurt is partially cooled in this section and final cooling takes place in the cold store. Since the yoghurt is in motion during the incubation/cooling periods, extreme care must be exercised to avoid damage to the coagulum.

Table 3.2 Different systems of incubators/coolers used for the manufacture of set yoghurt

	Waterbath tanks	Cabinet	Tunnel
Incubation/cooling in the same compartment	Yes	Yes/alternatively cabinets may be used only as incubators and then pallets removed and cooled in cold store	The first part of the tunnel is the incubator and the final section is used as a blast cooler
Heating and cooling agent	Water	Air	Air
System of production	Batch[a]	Batch[a]	Continuous
Packaging material	Glass	Glass, cartons or plastic	Glass, cartons or plastic
Variation in the quality of yoghurt	Yes[b]	Yes[b]	Slightly
Energy consumption	High	High	Low
Processing floor area	Large	Medium	Small

[a] Semi-continuous production line can be achieved if water tanks or cabinets are in series. [b] If filling time of pallets exceeds 15 min.

After Tamime and Grieg (1979). Reprinted with permission of *Dairy Industries International*.

A combined system for the production of set yoghurt consisting of incubation rooms and a cooling tunnel is used at the S.V. Inza Co-operative in Belgium (Cottenie, 1978). The advantage of this approach may be that, while the yoghurt cups are not in motion during the incubation period, the cooling rate of yoghurt containers in a tunnel is much faster than can be achieved with other methods. Thus, in practice the milk is acidified to pH 4.5 in the incubation room and then transported to the cooling tunnel where the temperature of the yoghurt reaches 10°C in around $1\frac{1}{2}$ hour (see also Kessler and Bäurle, 1980; Kessler, 1981, Anon., 1983b). Incidentally, the cooling tunnel is connected directly to the cold store so that the palletised cups of yoghurt can be transferred easily using a forklift truck.

The different systems used for the manufacture of set yoghurt have been evaluated by Cottenie (1978), and a summary of their main features is shown in Table 3.2. The conclusion emerges that, while the water bath system was at one time popular in Europe for the production of set yoghurt, the present trend is to use cabinets for medium-size production runs and the tunnel system for more extensive batches.

An update of the tunnel system has been reported by Bylund (1995) (Fig. 3.35). The filled packages/containers of inoculated milk are placed in crates of open design and at a certain distance from each other so that the circulating warm/cold air for the incubation and cooling stages can reach every individual container and provide accurate temperature control. When the empirically determined optimum pH (typically 4.5) is reached, it is time to start cooling. The normal target temperature is 18–20°C; it is important to stop further growth quickly, which means that a temperature of about 35°C should be reached within 30 min, and 18–20°C after a further 30–40 min.

Final cooling, normally down to 5°C, takes place in the chill store, where the products are held to await distribution. Cooling efficiency depends on the size of the
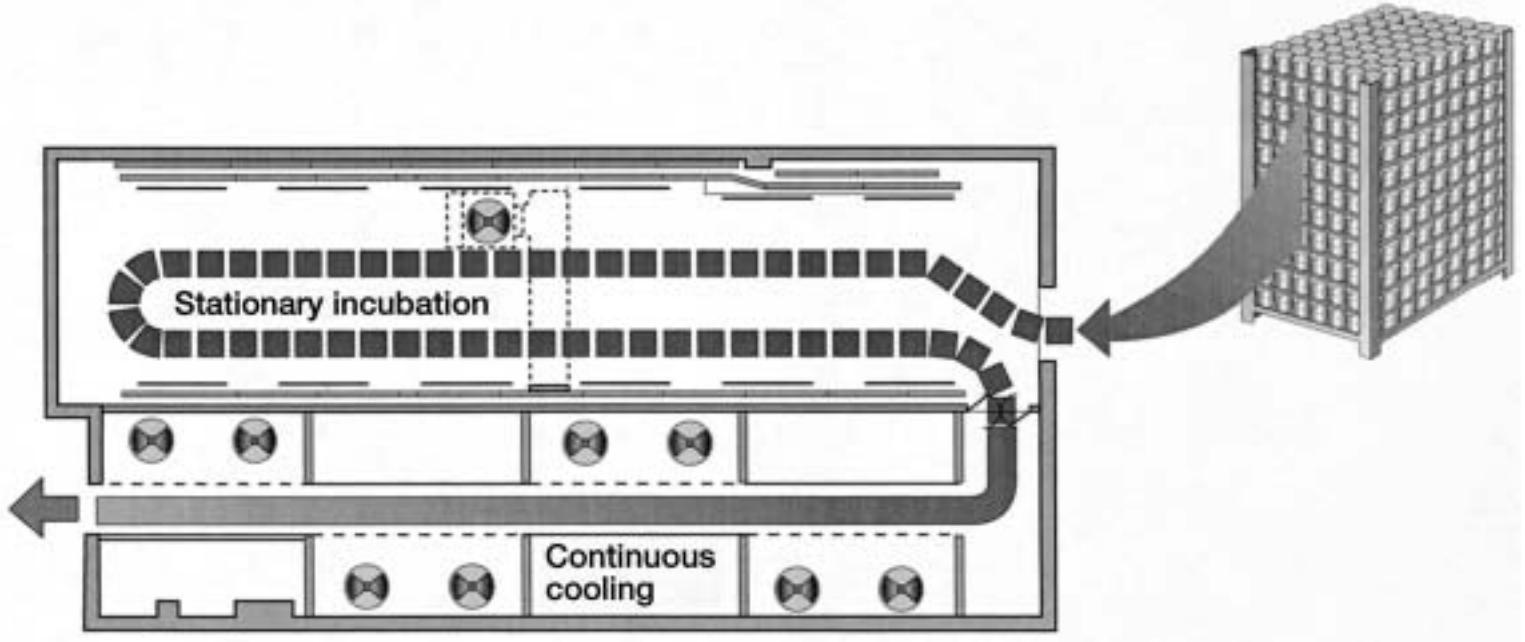
Fig. 3.35   Combined system (incubation room and cooling tunnel) for the production of set yoghurt
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individual package, the design and material of the packages, the depth of the crate stack, the spacing between individual packages in each crate and the design of the crates. For a depth of one metre, for example, the cross section of the stack allowing free air-flow must be not less than 25% of the total area. A smaller free cross section will require higher airflows, which also means higher energy consumption.

The pallets (crates) are stationary during incubation. They are placed in the incubation section of the tunnel in such a way as to facilitate first in/first out handling. In a typical incubation period of $3–3\frac{1}{2}$ hours, it is very important that the product is not exposed to any mechanical disturbance during the last $2–2\frac{1}{2}$ hours, when it is most sensitive to the risk of whey separation.

The cooling capacity should be adequate to achieve the above mentioned temperature programme. As a guide, the total cooling time is about 65–70 min for small packages (0.175–0.2 kg sizes) and about 80–90 min for large packages (0.5 kg size). Eventually, regardless of the type of incubation/cooling chamber, the set yoghurt is cooled to about 5°C in the chill store.

3.3.6.2 *Equipment for the production of stirred yoghurt*

By contrast, the coagulum of stirred yoghurt is produced in bulk and the gel structure is broken before or during the cooling and packaging stages. However, processing the milk base for the manufacture of stirred yoghurt is similar to that described earlier (see Fig. 3.13). An illustration of a typical plant is shown in Fig. 3.36 where the processed milk base is cooled to 40–45°C or 30°C before delivery to the fermentation tanks. The types of fermentation tank used in the industry for the production of stirred yoghurt could be classified as follows.

Multipurpose tank – This type of tank has been discussed elsewhere and is designated as a multiple duty unit, that is, (a) milk processing (heating) and fermentation, (b) same as (a) but also used for cooling the coagulum, and (c) fermentation and cooling only.

The tanks are water jacketed so that steam can be used during the heating stage and circulating cold water is used to cool the milk to 40–45°C. The temperature is maintained at 42°C during the fermentation period. Finally, chilled water is circulated to cool the coagulum.

Fermentation only tank – These tanks are only insulated in order to maintain an even temperature during the incubation period. The agitation system in such tanks is optional, since the cone-shaped base facilitates easy removal of the coagulum (see Fig. 3.37). However, agitators in a yoghurt fermentation tank may be required, especially if DVI starter cultures are used and there is need to ensure rehydration and/or proper mixing into the milk; if a bulk starter culture is used, it is metered into the processed milk and hence no agitation is required (see Fig. 3.36).

Fermentation/cooling tank – This type of tank is water jacketed and warm water at 40–45°C is circulated during the incubation period, followed by cold or chilled water for partial cooling of the coagulum (see Section 3.3.7); illustrations of these tanks and others mentioned elsewhere have been reported by Tamime and Greig (1979) and Robinson and Tamime (1993). One such example is the Goavec tank which has an increased surface area to improve the efficiency of cooling the yoghurt (Goavec, 1983).

Aseptic fermentation tank – This type of tank is a modified version of the standard fermentation unit. The tank is used for the production of yoghurt under aseptic conditions. The overall specifications are:
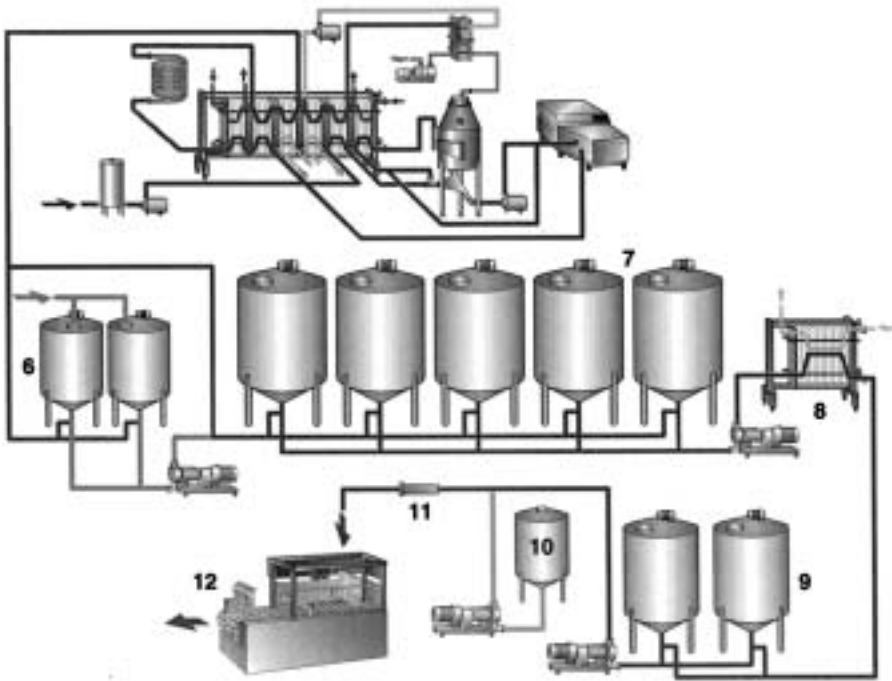
Fig. 3.36 Production line for stirred yoghurt

Details of pretreatment of the milk base are given in Fig. 3.13; 6, bulk starter tanks; 7, fermentation tanks; 8, plate cooler; 9, buffer tanks; 10, fruit flavour tank; 11, in-line yoghurt/fruit mixer; 12, filling machine.

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Fig. 3.37 Conical-shaped fermentation tank designed for easy discharge of yoghurt from the base

Reproduced by courtesy of Delta Dairy S.A., Athens, Greece.

- The tank is insulated.
- It is fitted with two pH electrodes and a resistance thermometer.
- The air entering or leaving the tank is filtered.
- The agitator has a double-shaft seal with steam barrier to minimise contamination.

The primary objective of using an aseptic fermentation tank is to minimise contamination of the yoghurt with yeasts and moulds. As mentioned earlier the aseptic tank is permanently pressurised under sterile air; a similar concept is used for the production of bulk starter using an aseptic tank (see Fig. 8.8 in Chapter 8). According to Bylund (1995), the air filtration system required for four fermentation tanks would consist of:

- one air fan delivering $400 \, m^3 \, hour^{-1}$ of filtered air (about $100 \, m^3 \, hour^{-1}$ per tank)
- one filter capable of trapping particles $>0.3 \, \mu m$
- one casing for the filter and one basic duct
- four connecting pipes, valves and manometers.

As a safeguard, each tank is equipped with an extra pipe for the air and a safety system to prevent the tank from imploding as a result of the vacuum created by the drop in temperature after cleaning. The air velocity is about $0.5 \, m \, s^{-1}$ and the tank is positively pressurised to about 5–10 m water gauge which is equivalent to 0.005–0.01 MPa (see also Müller, 1995).

It is important to note that all the tanks mentioned above have a foam-reducing inlet fitting that decreases the problem of froth formation in the tank. In addition, most modern yoghurt fermentation tanks are fitted with pH sensors to monitor lactic acid production by the starter organisms. Reviews of such developments have been published by Watanabe *et al.* (1994), Corrieu *et al.* (1994) and Mulchandani *et al.* (1995).

3.3.7   Cooling

At the desired level of acidification, cooling of the coagulum commences, so that the temperature is reduced from 40–45°C to 20°C or in some cases <10°C (Anon., 1977). The basic objective is, of course, to slow down the metabolic activity of *S. thermophilus, L. delbrueckii* subsp. *bulgaricus* and the bio starter cultures and, if the cooling process is delayed, the yoghurt or related product may become unpalatable due to the presence of too high a level of acidity. As mentioned elsewhere (see Section 2.11 in Chapter 2) the cooling of yoghurt may be carried out in stages. Therefore, depending on the type of equipment used for cooling the yoghurt and the duration of the cooling period, it is recommended that cooling should start at around $0.8–1.0 \, g \, 100 \, g^{-1}$ lactic acid, so that the acidity of the cool yoghurt will be between $1.2–1.4 \, g \, 100 \, g^{-1}$ lactic acid. The systems available for cooling the yoghurt are as follows.

3.3.7.1   *Chilled air*

This method of cooling is widely employed in two areas in the yoghurt industry. Chilled air is circulated in cabinets and tunnels to cool set yoghurt at the end of the fermentation period. It is also circulated in the cold store, transport vehicles and retail stores. The recommended temperature for yoghurt during storage, distribu-

tion and retailing is <10°C, otherwise the keeping quality of the product will be severely impaired.

3.3.7.2 In-tank cooling

The system by which yoghurt is cooled in the fermentation or multipurpose tank is known as in-tank cooling and chilled water is usually circulated in the jacket during the cooling period. The rate of cooling the coagulum from 40–45°C to 20°C or <10°C is governed by:

- area of the contact surface
- speed of agitation
- temperature differential between the cooling medium and the product
- mass flow rate of the cooling agent
- contact time between the product and the cooling surface.

Therefore, a fast rate of cooling can be achieved by providing: (a) as large a cooling surface as possible, (b) a rapid flow rate of the cooling agent by forced circulation, (c) a steep temperature gradient between the yoghurt (40–45°C) and the cooling agent (i.e. chilled brine at −3.8 to −4.0°C), and (d) adjustment of the contact time between product and cooling surface, that is, by continually replacing the cooled yoghurt with warm yoghurt.

These factors are, of course, interrelated, but for convenience their effect on the efficiency of in-tank yoghurt coolers can be assessed separately.

Surface area – The surface area available for cooling yoghurt may vary considerably from one tank to another. Figure 3.38 shows how this area could be maximised. The in-tank cooling rates of yoghurt can differ widely, and while, for example, the 5000 l tank (Fig. 3.38, type (2)) may require 4 hours to cool the yoghurt from the incubation temperature to about 5°C, yoghurt in tank type (4) (see Fig. 3.38) requires $\frac{1}{2}$ hour to cool from 45°C to 20°C (Jay, personal communication; Hale, personal communication).

Agitation system – The different flow patterns which occur during liquid mixing are discussed elsewhere (see Fig. 3.23), and the factors that affect the performance of an in-tank yoghurt cooler are basically:

- shape of the tank
- shape of the agitator system (paddle, propeller, scrape surface or anchor)
- size and position of the agitator
- speed of rotation
- velocity difference between the bulk fluid and the agitator.
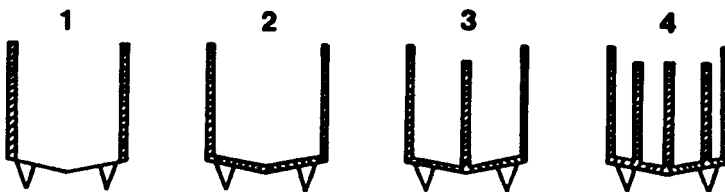
Fig. 3.38 Diagram of the surface areas of some in-tank yoghurt coolers
Shaded area is the cooled region; 1, side of tank; 2, side and bottom of tank; 3, side, bottom and inner cylinder; 4, same as in (3) plus in-tank cooling coils.

The creation of a vortex and/or incorporation of air into the bulk of the yoghurt are not desirable, and similarly, stirring of the warm coagulum may cause shearing; these effects can be minimised by controlling the speed of rotation and adjusting the shape of the agitator. The shearing effect is also influenced by the difference in velocity between the bulk yoghurt and the agitator tip, and a reduction in velocity differential will minimise the rate of shear. It is for this reason that more than one type of agitation system may be provided in a yoghurt tank. The design of the agitation system seeks, therefore, to minimise structural damage to the coagulum. Some examples of suitable systems are first, the scraped surface agitator plus, for example, a centrally mounted helical paddle, second, the contra rotating paddle, third, the scraped surface agitator only; however the 35° cone-base tank assists in turning the yoghurt gel with minimum structural damage and the scraping action of the agitator continually replaces the cool yoghurt with warm yoghurt, thus improving the rate of cooling, and fourth, a paddle agitator plus fixed baffles along the side of the tank.

Speed of rotation – The agitator speed is reduced as much as possible to give effective mixing of the coagulum but minimum shearing. Some commercially available tanks reflect this aim with the speed of rotation ranging between 8 and <50 rpm. In some instances, two-agitator systems rotating in opposite directions may be installed in a tank or one agitator paddle may be needed which can rotate clockwise or anti-clockwise alternately. Nevertheless, the in-tank cooling of yoghurt requires a long time and according to Kessler (1981), the formula used to measure the heat transfer in a tank during the heating of milk can be used to calculate the time required to cool the yoghurt. He illustrated this point with the following example:

Time of cooling (s)

$$= \frac{\text{Volume } (\text{m}^3) \times \text{Density of yoghurt } (\text{kg m}^{-3}) \times \text{Specific heat } (\text{J kg}^{-1}\text{K}^{-1})}{\text{Effective heat}/\text{cool exchange area } (\text{m}^3) \times \text{Heat transfer coefficient } (\text{W m}^{-2}\text{K}^{-1})} \ln \frac{\text{Temperature of warm yoghurt} - \text{Temperature of cooling medium}}{\text{Temperature of cool yoghurt} - \text{Temperature of cooling medium}}$$

$$= \frac{3 \times 1040 \times 3800}{9.55 \times 150} \qquad \ln = \frac{40 - 15}{20 - 15} = 1.61$$

$$= \frac{3 \times 1040 \times 3800}{9.55 \times 150} \times 1.61$$

$$= 13\,235 \text{ s} = 222 \text{ min} = 3.7 \text{ hours}$$

where ln is the natural logarithm. High speed agitation was used during cooling.

It is clear that chilled water rather than mains water should be used in order to maximise the temperature differential between the warm yoghurt and the cooling agent and if the surface area can be increased, the cooling time will also be reduced.

An alternative technique for the in-tank cooling of yoghurt would be the insertion of a heat exchanger (plate or coil) into the coagulum at the end of the fermentation stage (Ehrmann, 1972). However, this type of apparatus restricts the use
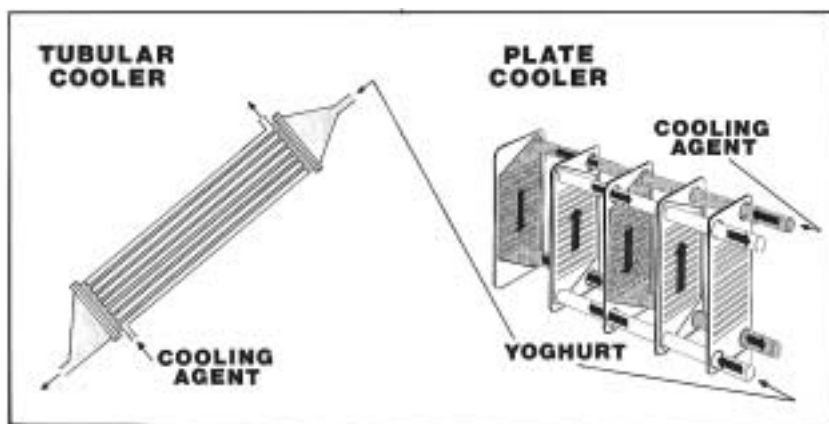
Fig. 3.39 Flow of yoghurt through different types of coolers
Notice that the streams of yoghurt and cooling agent run in counter current mode.
After Tamime and Greig (1979). Reproduced with permission of *Dairy Industries International.*

of agitators in the tank and since these coolers are inserted into the coagulum after the incubation period, problems of contamination may arise.

3.3.7.3 Continuous coolers

In contrast to the slow heat transfer of in-tank or batch coolers, more rapid cooling of yoghurt can be achieved using either plate or tubular heat exchangers. The flow pattern of yoghurt through a heat exchanger is illustrated in Fig. 3.39. It is normally accepted that the throughput/unit time of plate or tubular cooler should be roughly double the capacity of the processing plant, so that if the plant capacity ranges from 3500 to 4000 l hour^{-1}, then the capacity of the cooler should be in the region of 8000 l hour^{-1}.

The plate cooler is similar in design to the conventional plate heat exchanger described earlier, except that the gap between the plates is much larger (e.g. up to 6 mm compared with 2.5 mm), so minimising the risk of structural damage to the coagulum. In addition, because of the tendency of back pressure to build up in a plate cooler, either the passage of yoghurt has to be restricted, or alternatively, the gap between the plates is increased progressively across the unit. It is further recommended that the throughput of a plant should be increased by installing a number of small units in parallel rather than by increasing the number of plates on a large unit. The cooling agent in a plate cooler is usually chilled water and an approximate water consumption of 40 000 l hour^{-1} can be anticipated for a plate heat exchanger cooling 8000 l of yoghurt hour^{-1}.

The tubular cooler is constructed of a bundle of tubes enclosed in a shell and, as the product passes through the tubes, a counter current flow of cooling agent passes around them. Some technical specifications of this type of cooler, which is produced by Terlet/Zutphen, are: (a) sizes range from 1000 to 10 000 l hour^{-1}, and it is recommended that capacity should be the same as the filling machine, (b) chilled water flows counter current to the yoghurt and the consumption of water is roughly five

times the product volume, (c) the time required to cool yoghurt from the incubation temperature (40–45°C) to 8°C is 1 hour and the velocity of the yoghurt through the tubes is 0.65 cm s⁻¹, (d) any reduction in viscosity is minimised by transferring the yoghurt from the tank to the cooler by providing the right plant installations (see Sections 3.3.8 to 3.3.12), and (e) plant design is simplified by the fact that these coolers can be placed in various positions (i.e. vertically or horizontally).

It is inevitable that some structural damage to the coagulum will occur during the passage of yoghurt through either plate or tubular coolers, but Steenbergen (1971a) and Piersma and Steenbergen (1973) concluded that least loss of viscosity occurred in a tubular cooler. However, purging of the product at the end of the production may be necessary to minimise yoghurt loses before the CIP stage.

3.3.8 Pumps

A variety of different pumps are used in the dairy industry, depending on their intended function. For simplicity the production line can be divided into the following sections:

- liquid milk handling and processing
- coagulum production and handling
- fruit/yoghurt blending and packaging.

The physical characteristics/consistency of the materials differs in each section and it is vital that the type of pump is suitable for its duty. This is especially true after the formation of the coagulum, since any harsh mechanical treatment can ultimately affect the viscosity of the product. Nevertheless, in large yoghurt plants the milk base is pumped through long pipelines with many valves, and through heat exchangers, filters and other equipment which may result in high pressure drops. Therefore, pumps are used in different parts of the processing plants and it is important that the right type of pump is installed at the right place in order to avoid problems. According to Castaigne *et al.* (1985) and Bylund (1995), aspects to be considered are:

- pump installation
- suction and delivery lines
- type and size of pump required should be selected with regard to flow rate, product to be pumped, viscosity, density, temperature, pressure in the system and/or material in the pump.

3.3.8.1 Centrifugal pump

Basically, this pump consists of an electric motor (to supply the energy), a rotating impeller enclosed in a casing and a delivery chamber. The fluid enters the impeller chamber and is accelerated centrifugally until it is forced outward along the tip of the impeller. As a result, the fluid is discharged into the delivery chamber and out through a port in the casing of the pump. The pressure generated is always equivalent to the flow resistance of the process line and the efficiency of the pump, that is the transmission of energy from the motor to the liquid via the impeller, is equal to:

$$\text{Efficiency of centrifugal pump} = \frac{\text{Kinetic energy} + \text{Pressure energy imparted to the liquid at discharge}}{\text{Energy delivered by the motor}}$$

Note that the energy loss in the form of heat is ignored.

All centrifugal pumps are the same in principle, but the design of the impeller can vary and certain other factors have to be considered, namely:

- discharge pressure at the pump,
- flow rate or velocity of the liquid,
- degree of cavitation (this is the result of liquid being transferred from one side of the pump to the other, thus creating a vacuum; the new liquid enters the pump by suction),
- viscosity of the product can affect pressure loss in the pump and losses are higher when viscous products are being moved due to an increase in friction,
- if pressure losses occur in the processing line, the velocity of the fluid is controlled either by installing regulating valves, or by using a speed control, or by changing the diameter of the impeller.

The centrifugal action of these pumps is capable of producing high shear forces in the liquid being pumped and hence their application in yoghurt processing is restricted to liquid milk handling and the pumping of water (hot or cold) through the heat exchangers.

3.3.8.2   Piston pump
A piston pump could be described as a piston that reciprocates in a cylinder; inlet and outlet valves control the flow of liquid so that it flows in the right direction. In general, piston pumps are used in dairies as metering pumps. However, a homogeniser could be also considered as a type of piston pump. Thus, this type of pump can be used to achieve high pressures during the processing of the milk base (see Chapter 2 and Section 3.3.4).

3.3.8.3   Positive displacement pumps
The positive displacement pumps are classified into three different groups, rotary, reciprocating and miscellaneous. The principle of a positive displacement pump is that for each revolution (i.e. rotary type pump) or each reciprocating movement, a net amount of liquid or product is pumped regardless of manometric head (H) (Bylund, 1995). However, when pumping non-viscous products (i.e. milk) some slip or internal leakage may occur as the pressure builds up, and this will reduce the flow per revolution or stroke (i.e. in the reciprocating type pump). The incidence of slip is reduced with an increase in viscosity, as is the case with yoghurt.

Throttling the outlet of a positive displacement pump will increase the pressure dramatically. Hence it is important that no valves after the pump should be closed, and that the pump should be fitted with a pressure relief valve built into the pump or as a by-pass valve. When using these types of pump, the flow is normally controlled by regulating the speed of the pump or adjusting the stroke of a reciprocating pump. When pumping high viscosity products (e.g. yoghurt), the following precautionary measures most also be considered. First, the pump should be located very close to the product feed tank and second, the pipe diameter must be large (see later). These precautions ensure that only low pressure drops occur, otherwise if the pressure drop is high, the pump will cavitate. The same conditions also apply to the outlet side of the pump where high pressure can occur if long and narrow diameter pipelines are installed.

A pump classified as a reciprocating displacer is, in effect, a low pressure piston pump and although not used for the direct movement of the yoghurt coagulum, the majority of filling machines incorporate the basic design. Thus, although this type of

pump may exert a slight shearing effect, damage to the coagulum is minimised due to the short contact time between the pump and the yoghurt, the low temperature of filling, i.e. $\leq 20°C$ and the absence of back pressure.

Lobe-type rotary pumps – These rotating displacement pumps are the most popular type for yoghurt, for the product moves through a rotating cavity between two rotors each constructed with bi-, tri- or multilobes. The design of the rotor lobes makes them suitable for pumping yoghurt containing delicate solids (e.g. large fruit pieces). The flow pattern of yoghurt through these different pumps is illustrated in Fig. 3.40. When the rotors rotate, a vacuum is created at the inlet side of the pump which draws the yoghurt into the pump. The product then flows along the periphery of the pump casing towards the outlet side of the pump; there the volume of yoghurt is reduced and the product is forced through the outlet (see Fig. 3.40). In general, each rotor is independently driven by a timing gear located at the back of the pump; however, the rotors do not touch each other or the pump casing even though the clearances between all parts in the pump are very small (Bylund, 1995).

According to Tamime and Greig (1979), the advantages of these positive displacement pumps, compared with reciprocating pumps, are:

- cheaper drive train,
- can operate at a higher speed (these pumps are cheaper and smaller than piston pumps with comparable delivery rates; however, since the speed of pumping affects the viscosity of yoghurt, the application of high speed is not recommended,
- negligible surges of flow,
- pumps are self priming,
- suitable for applications where large heads are involved,
- high delivery rates,
- suitable for pumping viscous products (e.g. yoghurt), or mixtures of solids and liquids (the suspended solids should not be sharp or abrasive),
- volumetric efficiency hardly diminishes with increasing counter pressure.

Some illustrations of bi- (SK range) and tri-lobe (SR range) positive displacement pumps are shown in Fig. 3.41. However, it could be argued that the bi-lobe pump provides smoother displacement and low shear. The top inlet of the product and
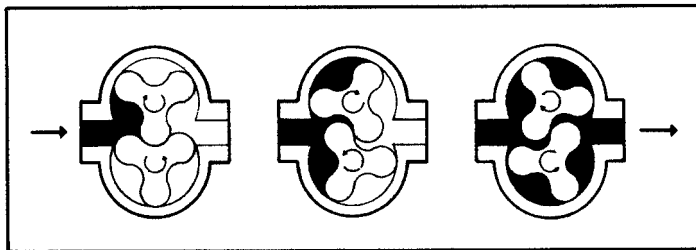
Fig. 3.40   Flow of yoghurt through a lobe rotor of a positive displacement pump
After Tamime and Greig (1979). Reprinted with permission of *Dairy Industries International*.

(A)

(B)

Fig. 3.41 Illustration of bi- (A) and tri-lobe (B) SSP rotary positive displacement pumps
Reproduced by courtesy of Alfa Laval Pumps, Eastbourne, U.K.

bottom outlet design provides full drain down between batches with virtually no product residue to cause contamination. In the past, one criticism of the rotary displacement pump was that the seals were prone to leakage and Harper *et al.* (1976) have pointed out that the seals between the pressure and suction sides are not as efficient as in reciprocating pumps. The seals between the rotary gears and the face plate are also prone to leakage. Regular inspection of the seals can reduce these problems to a minimum. However, over the years the design of these pumps has been improved to meet specific applications in the industry. For further details refer to Anon. (1984, 1985, 1989a), Verheij and Langeveld (1985) and Maynard (1991). More recently, the use of titanium (i.e. parts of machinery made from or lined with) and in particular pump components for the dairy industry has been discussed by Repenning (1995).

Wing-type rotary pumps – An alternative lobe design for the rotors of a positive displacement pump uses a single or twin-wing rotor. An example of such a pump is the Waukesha Universal Series, shown in Fig. 3.42. The single wing rotor ensures minimum breakage and better filling of fluids with discrete particles such as fruit

Fig. 3.42   Illustration of twin-wing rotor used in a rotary positive displacement pump
Reproduced by courtesy of Alpha Technical Services, London, U.K.

Fig. 3.43   The Mono S range screw-type rotary pump
Reproduced by courtesy of Mono Pumps Dresser, Manchester, U.K.

flavoured yoghurt, fruit preserves, pie fillings and large curd cottage cheese (see also Anon., 1985, 1989b, 1991).

Screw pump – Another type of positive displacement pump is known as the eccentric screw, helical or screw pump, widely used for pumping fruit yoghurt. It consists of a single helical rotor turning within a resilient stator. The fruit/yoghurt travels along a continuous spiral path without changing volume; in this way the yoghurt coagulum is treated gently and the fruit particles remain intact (Fig. 3.43). This type of pump must be filled with yoghurt before starting. However, the primary

Product inlet   **Product outlet**

Direction of flow
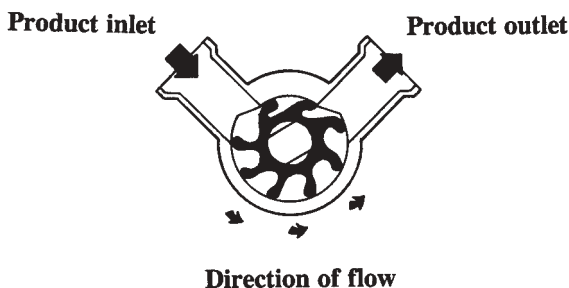
Fig. 3.44   Diagram to show the operation of a flexible impeller pump

objective of the initial filling is not for priming purposes, but to provide the necessary lubrication of the stator until the pump primes itself. Therefore, the pump should never be run in a dry condition because the stator will be damaged.

Some screw-type pumps (i.e. Mono Dresser) are provided with a "Flexishaft" that links the drive shaft to the helical rotor. In other pumps the shaft drive is reversible so that it can be driven in either direction (e.g. PCM Moineau pump).

3.3.8.4   *Flexible impeller pump*

This pump works on the principle that as the impeller blade leaves an offset plate it creates a vacuum, so that on start-up, air in the inlet pipe is displaced and yoghurt is drawn into the pump and then carried through to be discharged from the outlet at a steady flow rate (see Fig. 3.44). As the flexible vanes of the impeller come into contact with the offset plate again they bend and the squeezing action forces the product to be discharged continuously. The impeller can be manufactured from various types of inert material (e.g. Neoprene) which is widely employed in the dairy industry for continuous operation at temperatures up to 65°C, and up to 90°C for CIP. A typical pump of this type is made by ITT Jabsco; incidentally, the same company manufactures a wide range of pumps that can be used in the yoghurt industry (see Fig. 3.45). A rotor with a scimitar design is supplied by the same pump manufacturer.

3.3.8.5   *Diaphragm pump*

Air-operated diaphragm pumps are used in the yoghurt industry to transport a product including fruit pieces without any damage. A typical example is shown in Fig. 3.46 (L-series), made from highly polished stainless steel for hygienic processes. However, mechanically powered diaphragm pumps are better suited as metering pumps because, in the air-operated type, there are pulsations in the outlet pressure and the capacity will change with changing product pressures since the air pressure is kept constant (Bylund, 1995).

In principle, the air-operated diaphragm pump is a double-acting positive displacement pump with two alternating pump chambers (see Fig. 3.46). Compressed air, which is required to operate the unit, is admitted through a control valve at the rear of each diaphram in turn; this action displaces the yoghurt from the alternate pump chamber; also the diaphragm ensures that the pumped yoghurt is separated from the air. As the diaphragm retracts, a vacuum is created within the unit and the
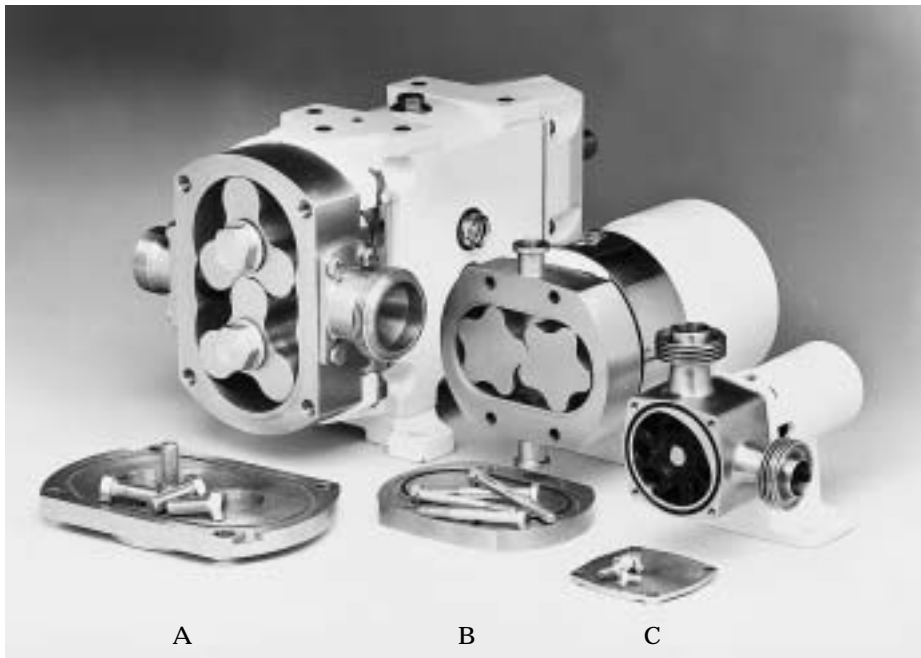
Fig. 3.45 Illustration of different types of Jabsco rotary pump

A, Tri-lobe 24 series; B, 5 lobe rotor 55 series including the scimitar lobe (design not shown); C, flexible impeller 28 series.

Reproduced by courtesy of ITT Jabsco, Hoddeson, U.K.

product flows into the chamber. At the same instance, the volume of the opposite chamber is reduced and the yoghurt is discharged through the upper ball valve (see Fig. 3.46). A common piston rod (see large arrows in Fig. 3.46) connects the two diaphragms together. Since the pressure is the same (i.e. compressed air section and pumping chambers) during each stroke, the actual diaphrams are not subjected to a large pressure differential and hence last for long operational periods.

3.3.8.6   *Peristaltic pump*

This type of pump consists of three parts, a flexible plastic pipe, a curved track which houses the plastic pipe and a motor that drives a series of rollers which, in turn, occlude the tube and thus push the fluid along. The action of the roller also creates a powerful suction or vacuum in the tube and, as a result, fluid is drawn in to replace that being driven forward; the flow rate is governed by the speed of the roller and the internal diameter of the flexible plastic pipe. According to Bylund (1995) the volume between the rollers is equal to half the volume conveyed per rotation. Therefore, the product is pumped to the outlet connection during rotation and, at the same time, the same amount is drawn in on the suction side of the pump.

Incidentally, this type of pump is also referred to as a hose pump, and although it can be used for transportation of the product, in the yoghurt industry it is used for accurate in-line metering of colouring matter and/or liquid (flavour) essences into the processed milk during the production of set-type flavoured yoghurt and/or
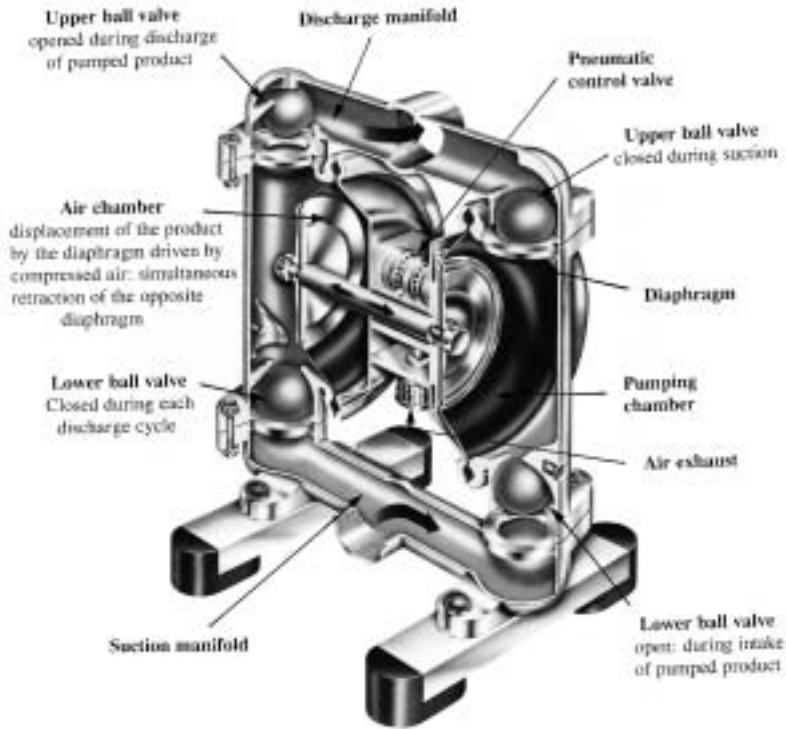
Fig. 3.46 DEPP air operated diaphram pump – series L
Reproduced by courtesy of Alfa Laval Pumps, Eastbourne, U.K.

drinking yoghurt. As this type of pump is self priming, it is suitable for emptying containers as well.

Different types of pump are used in the yoghurt processing line and in choosing the right pump for the right job, a number of interrelated factors must be taken into account. Some practical considerations may include:

- Length and diameter of the piping used on the suction and the discharge sides of the pump.
- Number and types of fitting installed, i.e. elbows, T-pieces and types of valve
- Types of metering/mixing device used.
- Manufacturer's specifications provided on the plate/tubular coolers intended for cooling the coagulum.
- Restrictions in the processing line, e.g. static in-line mixers, strainers or structurisers (see later).
- Product variables, which may include: (a) level of solids in the milk/yoghurt, (b) effect of shear on the product, (c) final viscosity of the yoghurt, (d) ability of the product to withstand high pressure pumping, (e) product type, e.g. level of pH, presence of particulate solids (fruit pieces), and (f) type of fluid flow in the system, e.g. laminar flow for yoghurt, $R < 2000$.
- Total pressure losses in the system.

Few data are available regarding the damage that may result from pumping the yoghurt coagulum from one point to another, but the classical study by
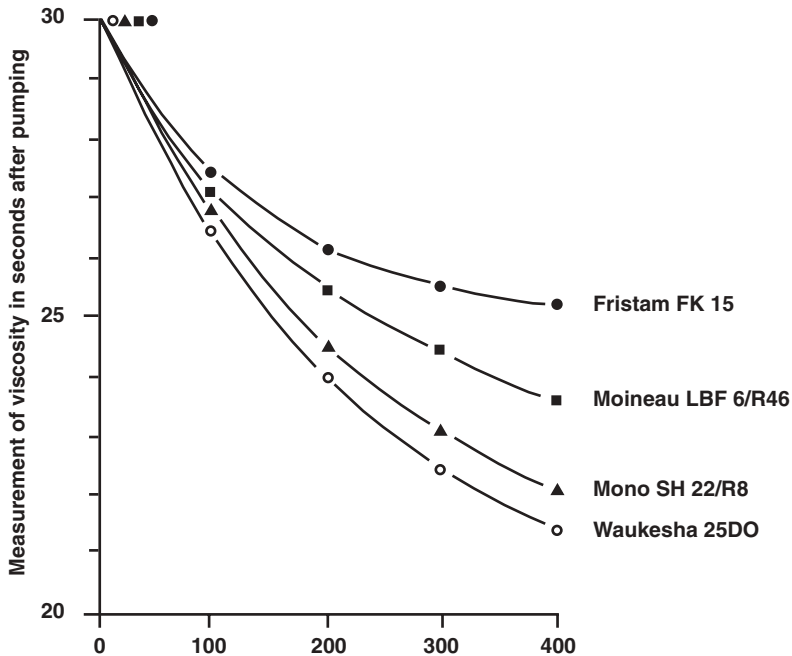
Fig. 3.47 Effect of pumping on the viscosity of yoghurt

Note that viscosity measurement was carried out using the Posthumus funnel (see Galesloot, 1958)
and counter or back pressure was 0 MPa.

Adapted from Steenbergen (1971b).

Steenbergen (1971b) has shown the effect of pumping on the viscosity of yoghurt (see Fig. 3.47) and it was concluded that the important aspects were:

- speed of the pump
- shape and type of the impeller
- counter pressure in the processing line.

Figure 3.47 shows that minimal reductions in viscosity occurred when the speed of the pump was maintained at 100 rpm; the loss in viscosity varied from 8.3 to 11.7% depending on the type of pump used. However, as the speed of the pump was gradually increased from 100 to 400 rpm, structural damage to the coagulum did occur so that if an increase in throughput is required, it is advisable to choose a pump with a larger stroke volume, rather than to increase the speed of the pump.

The development of counter pressure in any type of yoghurt plant is the result of a multitude of factors, for example, the type and number of fittings, arrangement of pipework and/or heat exchangers, and the greater the counter pressure in the system, the lower the viscosity of the yoghurt after pumping. Table 3.3 illustrates this effect. However, a high fluid flow is desirable in the plant during the CIP stage and hence the pumps used between the fermentation tanks and the filling machines must be of variable speed.

According to Nilsson and Hallström (1990), the correct selection and design of the plate or tubular coolers is essential in order to maintain optimal product quality.

Table 3.3 Reduction in the viscosity of yoghurt as affected by counter/back pressure

| Pressure (MPa) | Pump speed (rpm) | Viscosity | | |
		Initial (s)	Observed (s)	Reduction (%)
0.0	100	60	51.0	15.0
0.1	100	60	47.5	20.8
0.2	100	60	45.0	25.0

Type of pump – Waukesha 25 DO. Viscosity measurement by Posthumus funnel (see Galesloot, 1958).
Adapted from Steenbergen (1971b).

These cooling units should be designed for low product velocity, as this results in a low shear force and low pressure drop; these effects could also minimise the mechanical damage caused by the pump as the total pressure in the system is then lower. For example, the use of ice-water as a cooling agent is not recommended because too low a product temperature may be reached locally and, thus, increase viscosity and ultimately lower or even block the flow. For this reason the yoghurt is partially cooled, mixed with the fruit and finally packaged. Therefore, the cooling temperature will influence the final viscosity of the product as follows:
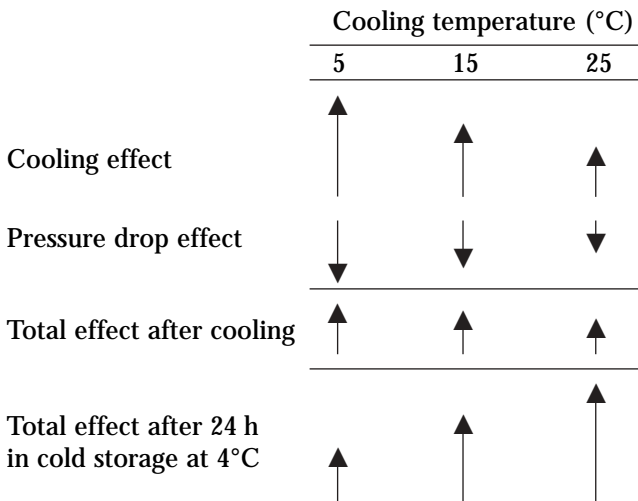
Whatever precautions are taken, however, mechanical handling of the coagulum does ultimately reduce its viscosity and some recommended precautionary measures include:

• Fortification of the yoghurt milk to a higher total solids content
• Addition of stabilisers (this may be prohibited in some countries)
• Use of an exopolysaccharide (EPS) starter culture
• Agitation of the coagulum should be avoided in the fermentation tank
• Partially cool yoghurt before fruit mixing and packaging

Table 3.4 Effect of transport through pipes on yoghurt viscosity

Length of pipe (m)	Yoghurt I (initial viscosity) 30 s			Yoghurt II (initial viscosity) 40 s		
	10	20	30	10	20	30
			Reduction in viscosity (s)			
Diameter of pipe (cm)						
3.81	10	14	17	17	22	24
5.08	6	9	12	11	16	20
6.35	3	5	8	6	10	14
7.62	1	4	6	2	8	11

The flow rate is 3600 l hour^{-1}. Viscosity measurement by Posthumus funnel (see Galesloot, 1958).

After Steenbergen (1971c).

3.3.9 Miscellaneous fittings

Different items of equipment in a yoghurt processing line are linked together by a series of pipes, fittings (elbows, T-pieces, pipe couplings, etc.), valves, and sometimes strainers, and the passage of the yoghurt through these miscellaneous parts of the plant can cause some structural damage to the coagulum. The ways in which this damage may arise are given in the following.

3.3.9.1   Pipes

As the yoghurt is pumped at a low velocity, it is safe to assume that the flow pattern through the pipes is laminar. However, other factors can affect this flow pattern, namely:

- length and diameter of the pipe
- internal roughness of the pipe surface
- fluctuations in fluid velocity

Steenbergen (1971b, c) studied the effect of pipe length and diameter on the viscosity of yoghurt and some of his results are shown in Table 3.4. From these data it can be concluded that: (a) if the velocity and diameter of pipe are kept constant, reduction in the viscosity of yoghurt is proportional to the length of the pipe, and (b) if the velocity and length of pipe are kept constant, the larger the diameter of the pipe, the least structural damage occurs to the coagulum.

It is recommended, therefore, that large diameter pipes should be installed between the fermentation tanks and the filling machines, and that at the same time, the connections should be as short as possible.

3.3.9.2   Fittings

Fittings, valves and other restrictions in a processing line can interfere with the flow pattern of the yoghurt and, as a result, affect the viscosity of the product. Steenbergen (1971c, 1973) evaluated the effect of these different fittings and observed that the viscosity of yoghurt was reduced by between 0.2 s and 20 s (the initial viscosity of the product was 30 s as measured by the Posthumus funnel), which is equivalent to lowering the consistency of the yoghurt by 0.7% and 67%, respec-

tively. The most severe structural damage to the coagulum took place where fittings reduced the diameter of the piping, and if such fittings were avoided, the reduction in viscosity was minimised.

3.3.9.3 Screens, strainers or structurisers

One fault which sometimes occurs during the manufacture of stirred yoghurt is the appearance of non-dispersible particles referred to as nodules, lumpiness, granules or graininess. The nature and/or origin(s) of nodule formation is not well established (see Robinson, 1981). Although the fault can be avoided by fermenting the milk at precisely 42°C (short set) and not disturbing the gel during the coagulation period, an alternative approach is to disperse the nodules by pumping the coagulum through a stainless steel mesh. This restriction in the pipe line does affect the viscosity of yoghurt, but the advantage is that it produces a smooth textured coagulum free from nodules, a feature confirmed by Nielsen (1972); unfortunately no figure was given in relation to loss in viscosity.

One such unit, sometimes known as a structuriser, is shown in Fig. 3.48. In commercial practice, the warm coagulum is pumped through the strainer in order to

(A)

(B)
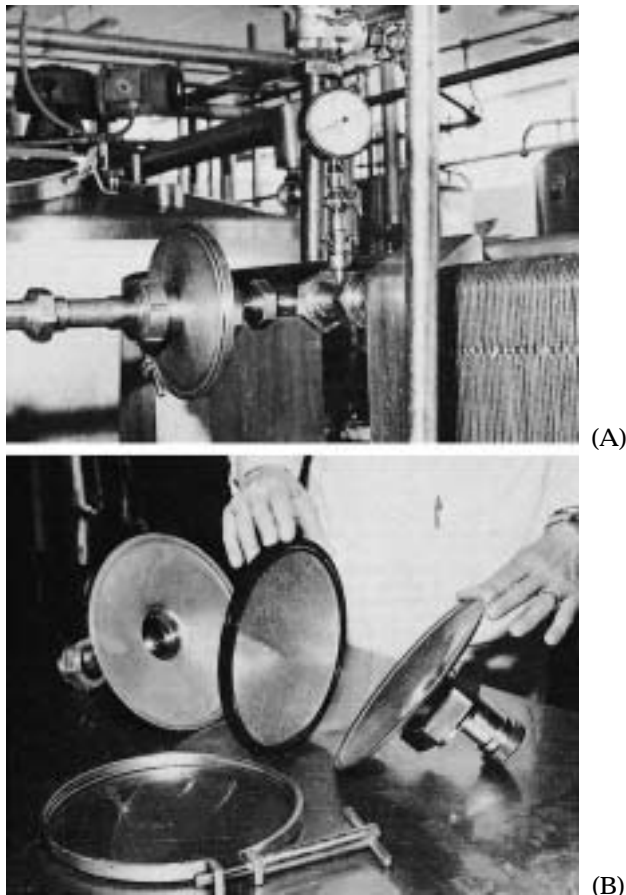
Fig. 3.48 On-site illustration (A) of a Tetra Pak 'structuriser' on a yoghurt processing plant and an exploded view of a dismantled unit (B)

Fig. 3.49   General view of Ytron®-Z "Yoghurt – Stretching" unit on a yoghurt processing plant

Note: The insert (bottom right hand) shows the labyrinth design of the rotor-stator.

Reproduced by courtesy of YTRON Process Technology, Bernau am Chiemsee, Germany.

break up the nodules. According to Fergusson (1985) the yoghurt coagulum is pumped through a filter to retain particles >1 mm in diameter and then passes through a perforated plate (strainer) followed by cooling to 5–10°C in a PHE ready for packaging. A similar type of perforated structure or sieve has been reported by Driessen *et al*. (1989); however, pumping cold yoghurt through such a strainer would severely damage the viscosity of the product, since high pressures would be required to achieve the necessary flow.

3.3.9.4   *Ytron®-Z machine*

This machine (see Fig. 3.49) has been developed in Germany. In it the yoghurt co-agulum is subjected to an extremely short and intensive burst of shearing to smooth the product. The viscosity of the yoghurt is improved due to the mechanical action of the Ytron®-Z which causes stretching of the protein molecules. Thus, the expression "Yoghurt–Stretching"™ is associated with Ytron®, and such an effect has the following advantages:

- Gel stability is improved and the consistency of the product resembles cottage cheese or quark.
- Complete elimination of syneresis and grit or nodule formation and the product is smooth.
- The protein content in the milk base can be reduced by $0.2\,g\,100\,g^{-1}$.

The "Yoghurt-Stretching"^{TM} effect is achieved in the rotor-stator-reactor which is constructed from toothed cages increasing progressively in slot width (see the insert in Fig. 3.49). Hence, the speed of the rotor, the slot widths in the rotor-stator set and the number of Ytron®-Z units installed ensure optimum results. The path of the yoghurt through the rotor-stator labyrinth ensures a consistent effect on the rheological properties of the product (see also Anon., 1995, 1996f).

3.3.9.5   *On-line viscometer*
Continuous viscosity measurements during the manufacture of fermented milks could be used to monitor the rheological properties of the product. A vibrating rod sensor has been developed by Picque and Corrieu (1988) to determine the changes in viscosity in a bioreactor during xanthan gum and fermented milk production. The sensor signals decreased non-linearly as the viscosity of the product(s) increased; if such units were to be installed on-line at different points in a yoghurt plant it would record the rheological changes in the product during pumping and/or other operations possibly contributing towards reducing the viscosity of the yoghurt. No published data are available.

3.3.10   Fruit handling and mixing units
The cool (e.g. at 20°C) or cold (e.g. at 10°C) yoghurt is delivered to an intermediate storage tank prior to further processing, that is, fruit mixing followed by packaging. The yoghurt will be retained in this tank for a short period of time or, alternatively, stored overnight, and the primary purposes of these tanks are as follows:

- The tanks are insulated and hence the temperature of yoghurt can be maintained at any desired level.
- In the event of breakdown in another section of the yoghurt factory, the tanks can act as buffer vessels.
- Overnight storage of yoghurt in the intermediate tanks can provide sufficient reserves for packaging to start first thing in the morning, rather than the machines remaining idle until the freshly produced yoghurt is available.

In this section of the processing line, equipment is required for handling the fruit, and mixing the fruit with the yoghurt. Some appropriate units are as follows.

3.3.10.1   *Equipment for fruit handling*
As mentioned elsewhere (see Chapter 2), the processed fruit used in the yoghurt industry is usually packaged either in metal cans, polypropylene containers (drums or buckets), flexible pouches or stainless steel tanks.

The packaging of fruit in metal cans is very popular and these cans are widely used by small- and medium-scale yoghurt manufacturers. However, large-scale producers only obtain fruit in metal cans if the demand is low and the popular flavours are either processed in the dairy or obtained in bulk in stainless steel tanks. If metal

Fig. 3.50 View of opener for metal cans

Reproduced by courtesy of D.C. Norris & Company (Engineering) Ltd., Sandy, U.K.

cans are used, a number of different types of can opener can be used, that is, hand-operated (see Fig. 3.50), semi-automatic or fully automated. The hand-operated openers employ either an electric motor or compressed air to cut the metal and remove the lid. However, the can opener shown in Fig. 3.50 is model 150 which is pneumatically operated and features stainless steel construction for all parts, has a dual safety circuit for two-handed operation, an opener that removes the metal lid in 2 s, a fully enclosed knife for safety operation, and removal of the whole lid without metal chips, together with ease of cleaning.

A semi-automated can opening line can be built around, for example, model 150 (see Fig. 3.50) and the equipment might include:

- loading tables
- gravity roller in-feed section
- can washer and air blow drier
- can opener stand
- discharge section with or without product drain tray
- can product rinse
- product discharge pump
- mobile dolleys for product, empty cans and lids
- magnetic traps.

Fig. 3.51 View of an automatic can opening system
Reproduced by courtesy of D.C. Norris & Company (Engineering) Ltd., Sandy, U.K.

A semi-automatic type opener can give a throughput of 1000 cans hour^{-1}, whilst the fully automated design has a throughput up to 2500 cans hour^{-1} (see Fig. 3.51). The fully automated model has all the features listed above including automatic inversion and emptying of cans, metered water jet to clean inside the can after emptying the fruit, automatic crushing of cans, a unit constructed from heavy duty stainless steel which can be fully hoseproof for easy cleaning, and facility for CIP.

Fruits in plastic containers have to be handled manually, but if the ingredients are received in stainless steel tanks, the normal approach is to meter them directly into the yoghurt immediately prior to packaging. However, in some yoghurt processing lines the fruit is emptied onto an inclined stainless steel table and the fruit is inspected for any residual plant matter (i.e. stems and/or leaves) before mixing it with the yoghurt. As a further precautionary measure, the fruit may also be subjected to screening by metal detectors. If such a system is used, care should be exercised to minimise contamination of fruit prior to mixing it with the yoghurt.

3.3.10.2 *Equipment for fruit/yoghurt blending*
In large yoghurt plants, the fruit is blended with the product using either batch or continuous blending methods. However, manual blending may be used when producing fruit flavoured yoghurts of limited consumer demand (Robinson and Tamime, 1993). Examples of the equipment for fruit/yoghurt blending are:

Manual blending – This method of fruit/yoghurt mixing is illustrated in Fig. 3.7. Two tanks are used in parallel. In each tank, the required amount of fruit is added to a given volume of yoghurt, mixed gently with a plunger, and the finished blend is pumped to the packaging machines. While the first tank is being emptied, the second one is being prepared, so that the process can, in practice, become continuous.

Batch blending – In principle, the approach is similar to that described for manual blending, except that the volume of the mix is larger and hence the fruit and yoghurt are metered into a tank, mixed and then pumped to the packaging machine. Again

the process becomes, in effect, continuous through the installation of two tanks in parallel.

Continuous blending – A continuous fruit/yoghurt mixer consists of three different units: first, a metering device for dosing the correct amount of fruit into the yoghurt line, second, a metering device for measuring the required volume of yoghurt and third, a mixing chamber that ensures uniform distribution of the fruit into the yoghurt. Different types of continuous mixer are available on the market (see also Unterholzner and Maurer, 1987; Pröepper, 1988). The primary requirements are:

- Proper mixing of the fruit and yoghurt
- Minimum structural damage to the coagulum
- The fruit metering unit must be accurate to allow different fruits to be mixed with the yoghurt in the desired proportions
- Eary to dismantle for cleaning, or suitable for CIP
- All contact surfaces to be of good quality stainless steel.

Some continuous fruit/yoghurt blenders that meet these requirements are as follows.

Static-in-line mixer – Many dairy fabrication companies supply the industry with different designs for this type of mixer. A typical example is shown in Fig. 3.52, consisting of a stainless steel pipe into which a number of helical blades are welded. In practice, the static mixer is, if possible, built into the product pipeline (see Fig. 3.36 (11)) where the fruit is metered from the tank into the yoghurt stream. The flow of yoghurt/fruit through the twisted blades in the mixer ensures uniform distribution of the fruit throughout the coagulum. The specifications of such mixers are: (a) flow rates up to 10 000 l hour^{-1}, (b) pipe diameter up to 6.35 cm, (c) lengths of the mixer ranges from 75 to 115 cm, and (d) number of blades is up to 10. Although such units can be cleaned using a CIP system, it is usually recommended that the mixers should be dismantled and rinsed before starting the CIP programme.

A portable fruit feed unit (Clarendon) fitted with a static-in-line mixer is shown in Fig. 3.53. Such a unit can operate up to 250 l hour^{-1} and a flexible impeller-type pump accurately meters the fruit into the yoghurt. In order to obtain a fine adjustment of the volume of metered fruit, a trimming device can be installed within the control cabinet and the output flow is then easily controlled by means of a knurled knob located on the front of the control cabinet. An optional attachment is a six nozzle ripple head (as is used in the ice-cream industry) which can provide an effective method of incorporating both fruits and flavour into the yoghurt.
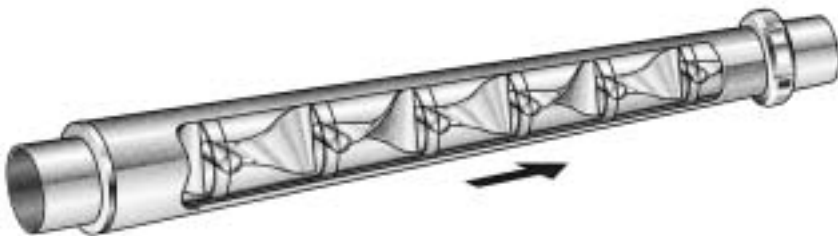
Fig. 3.52   Example of a static-in-line fruit/yoghurt mixer built into the pipeline
Reproduced by courtesy of Tetra Pak (Processing Systems Division) A/B, Lund, Sweden.

Fig. 3.53   View of the Clarendon fruit feeder/ripple pump with a static-in-line mixer
Reproduced by courtesy of Clarendon Food & Dairy Equipment, Leaming Spa, U.K.

The dimensions of this compact unit are $75 \times 35 \times 100$ cm high. However, for larger installations, the AutoBlend®, which is supplied by Bran Luebbe, accurately meters the fruit and yoghurt continuously and the two are blended uniformly via a static-in-line mixer.

As mentioned elsewhere (see Section 3.3.8), screw-type pumps, for example the Allweiler, are widely used for metering purposes. Fig. 3.54 illustrates the pumping of a fruit preparation from a tank into a yoghurt production line (see also Bedwell, 1984).

Gasti DOGAmix 60 – This unit (Benz & Hilgers GmbH, Germany) consists of two feeding pumps that draw yoghurt base and fruit into a mixing chamber (see Fig. 3.55). The maximum discharge rate of the yoghurt pump is 60l min^{-1}, whilst the discharge rate of the fruit pump can be adjusted to provide the desired mixing ratio in a range between 1:5 and 1:20; however, the accuracy of metering is $\pm 0.5\%$. Both yoghurt base and fruit are discharged through a common pipe to the mixing chamber which is fitted with a dynamic agitator. The product mix is homogeneous and uniform and is fed straight to the hopper of the filling machine. The feed rate of the yoghurt/fruit mix, up to 0.3 MPa back pressure, is 75l min^{-1}. Bacterial contamination of either the yoghurt base or the fruit during the mixing stages is avoided by isolating the moving parts of the DOGAmix (i.e. the rods of the plunger pumps and the mixer drive of the dynamic agitator) from the surrounding atmosphere by sterile air chambers. The dimensions of the Gasti DOGAmix 60 are $100 \times 115 \times 110$ cm in height.

Fig. 3.54 Illustration showing the use of screw type pump to meter fruit from a tank into the yoghurt line

Reproduced by courtesy of Allweiller Pumps, Poole, U.K.
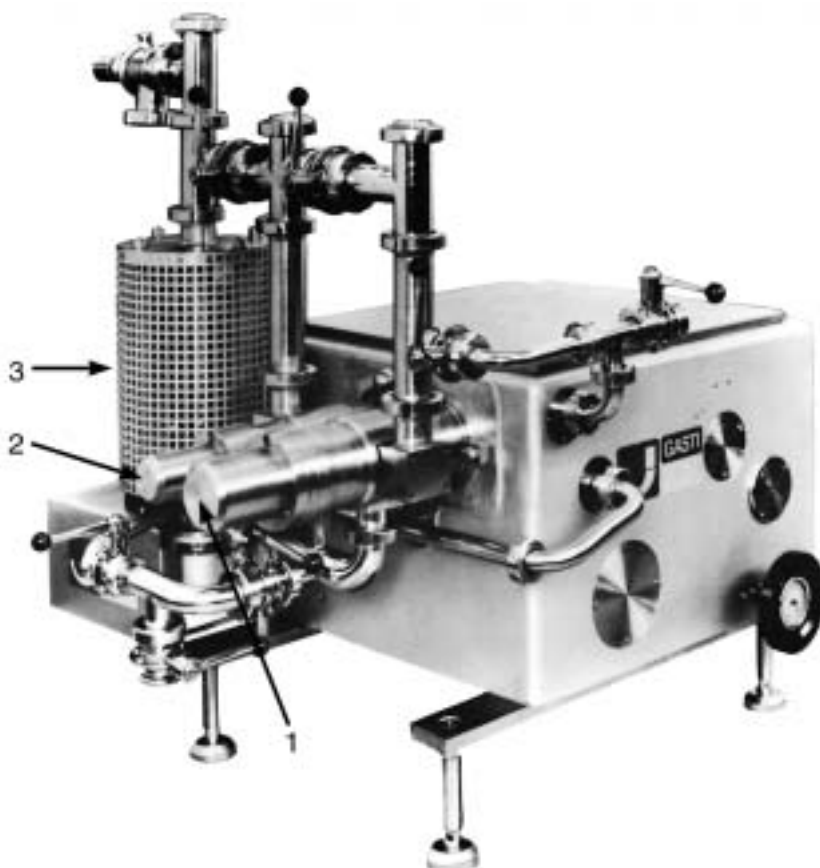
Fig. 3.55 Front view of the Gasti DOGAmix 60

1, Pump for yoghurt; 2, pump for fruit; 3, mixing tank with dynamic agitator.

Reproduced by courtesy of Jagenberg (London) Ltd., Purley, U.K.

The unit is capable of being cleaned by CIP (e.g. 1–2 g 100 ml^{-1} caustic at 80°C or 1–2 ml 100 ml^{-1} nitric acid at 80°C) and sterilised using steam at 140°C; this latter facility can be advantageous to ensure that yeasts do not build up at any point. However, the sterilisation of the air is achieved as follows:

- Compressed air passes through a filter with a water trap and automatic condensate draining, and then through a pressure regulating valve.
- Air then passes through a second high performance filter consisting of: (a) a layer of boron silicate micro-glass fibre weave, and (b) an activated carbon filter for the exclusion of oil vapour and odours.
- Finally, the air passes through sterile filter as in (a) above of 0.1–1 μm thickness; this filter is sterilised by steam (0.3 MPa pressure) at up to 140°C.

Burtech dynamic loop mixer – This in-line mixer is manufactured by Burtech Burgent Technology GmbH in Germany, also known as Burdosa Technology. Basically, the Burtech dynamic loop mixer (e.g. Supramix SLR or Unimix SLM) has a wide application in the dairy and food industries. The Supramix SLR is designed for mixing applications where high shear forces are used, whilst the Unimix SLM is designed where effective but low shear forces are required to protect the product against damage during the mixing stage.

The cross section of the Burtech dynamic loop mixer is shown in Fig. 3.56. The working principles are:

- Continuous product flow, which is made up of different ingredients that are metered into the inlet side of the mixer, is directed through the mixing chamber where, for example, yoghurt and fruit particles are constantly circulated.
- Recirculation is achieved by a central mixing tube (see Fig. 3.56) in which a rotating helical displacer supplies the energy required for particle mixing.

Other features of this type of mixer are: (a) the unit is totally closed and of hygienic design, (b) high throughput, but with small volume mixing chamber, (c) the mixer can be cleaned using CIP without dismantling, (d) trouble-free during start/stop operation, (e) low energy input and space saving design, and (f) the mixer has a flushed mechanical seal.

3.3.11 Filling machines

The fundamentals and principles of packaging, including the different types of packaging materials used in the yoghurt industry, have been given in detail in Chapter 2. However, some other relevant aspects of yoghurt packaging are: (a) the use of controlled and/or modified atmosphere packaging processes to improve the shelf life of yoghurt, cheese and other dairy products (Honer, 1988), (b) the advantages of tamper evident packaging include increased consumer acceptability, reduced product leakage and spoilage during storage, distribution and retailing (Herner, 1987; Hotchkiss, 1987), (c) the use of a sterile air chamber where the yoghurt cups can be filled (Anon., 1990a) or the sterilisation of the packaging containers using steam or hot air (Reinecke, 1985; Turtschan, 1986; Savaria, 1986; Doty, 1986; Maurel, 1996), (d) the use of a Serac R20T2OE/72A rotary-type filler equipped with 24 nozzles for aseptically packaging UHT drinking yoghurt in high density polyethylene bottles; the filling capacity is 8000 × 1 l or 11 000 × 0.5 l bottles hour^{-1} (Anon., 1989b) and (e) the development of sensors for inspection of the outer containers of
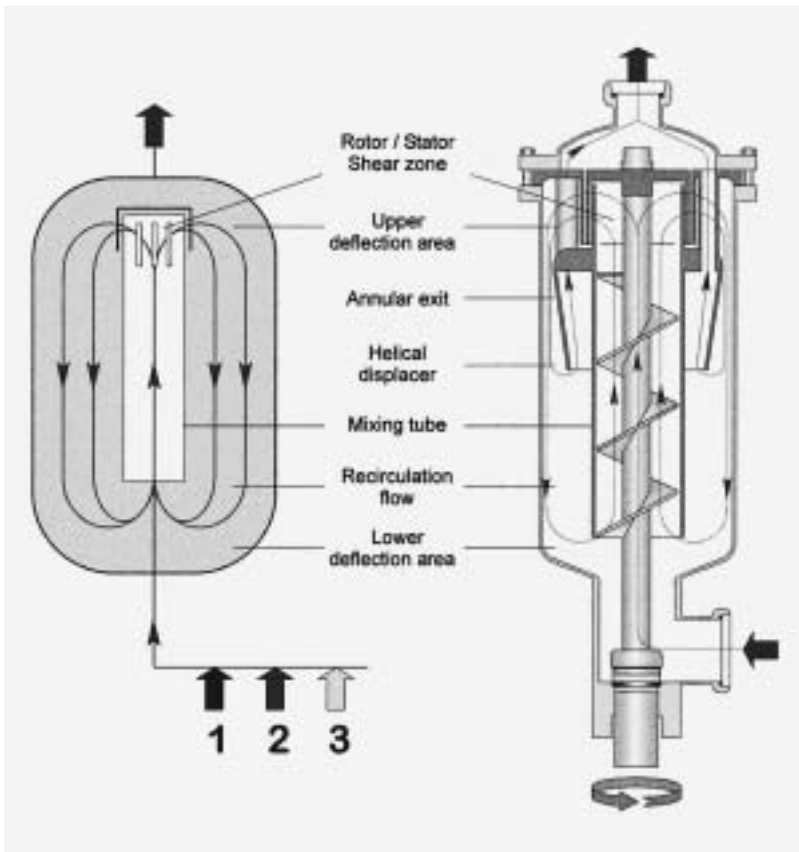
Fig. 3.56   Cross section of the Burtech dynamic in-line loop mixer
Reproduced by courtesy of Burdosa Technology, Wembley, U.K.

yoghurt for detecting, for example, defects in the quality of printing by the ink jet printer (Tomita and Shibata, 1994). Currently, there is interest in the yoghurt industry in the use of biodegradable "Eco cups" that are fully compostable in two months (Stratton, 1998); the thermoplastic container is made from polyactic acid which is derived from maize or beetroot. There is also a tendency within the industry to replace the aluminium foil laminates used to seal the plastic cups with plastic material so that metal detection in the filled cups becomes easy.

A multitude of high speed yoghurt filling machines are available on the market and although capital cost could be one of the major factors in choosing a certain piece of equipment, from a technical point of view, certain important specifications must not be overlooked. For example:

• Proposed method of filling and sealing,
• Type of unit container being used,
• Desirability of filling under a controlled atmosphere,
• Degree of automation being sought,
• Need for a high standard of hygiene (e.g. all contact surfaces must be stainless steel and accessible for sterilisation/sanitisation),

- Time required to change from one flavour to another or from one volume of carton to another,
- Versatility and reliability of the machine,
- Accuracy of filling and the elimination of drip between individual fills,
- Power and labour requirements of the machine,
- Other specifications such as availability of date marking, method of dispensing the cups, and safety measures (e.g. no cup no fill).

It would be impractical to discuss all the different types of yoghurt filling machines in detail, but it is safe to assume that the use of the positive displacement or piston pump is almost universal and that the measures are volumetric. In addition, most filling machines are equipped with marking attachments (e.g. best before date) and/or label application units (e.g. for large containers with snap-on lids). Some examples of these yoghurt filling machines follow.

3.3.11.1 Machines for filling yoghurt into preformed plastic containers

DOGAtherm 81 CIP – There are two versions of this machine which is manufactured by Benz & Hilgers GmbH in Germany. The common specifications of these models are: (a) automatic cup loader, (b) two lane filling conveyor with eight filling heads, (c) closure of the cups by heat sealing, (d) maximum output from 10 000 to 15 000 cups hour^{-1}, and (e) machine cleaning using a CIP system.

The DOGAtherm 81 CIP is fitted with a clean air cabinet over the machine so that filling takes place in a controlled atmosphere and the shelf life of the product is extended. Also, UV irradiation is used to sterilise the plastic cups and lids and the main dosing unit (i.e. two filling heads) has an option for two product filling. However, before placing the aluminium foil lid on the filled cup for heat sealing, the lid is stamped; provision can be made for two stamping zones (see Anon., 1990b).

For smaller operations the FLEXOtherm model can be used and both machines could be fitted with a prefiller for double layer cup filling or twin-chamber filling. A reusable plastic snap-on lid can also be applied.

Remy 54 volumeter – This machine is capable of packaging yoghurt into 500 g plastic containers with heat-sealed foil covers. The plastic cups are dispensed from an enclosed magazine holder and the filling and sealing stations can be in a sterile, laminar air flow cabinet that reduces contamination of the yoghurt. The capacity of this machine, depending on the number of lanes, ranges from 8400 to 16 800 containers hour^{-1}; the packaging machine is also fitted with an automatic tray packer.

COMBIseptic CS 41, 61 & 81 – These conveyor filling machines (Benz & Hilgers GmbH, Germany) are enclosed in a chamber with sterile air overpressure and have sealed, insulating doors to protect against H_2O_2 vapour emissions or noise (see Fig. 3.57). The complete line consists of a cup feeder, tray erector, integrated or separate tray packer, palletiser and separate foil lid press. Sterilisation of the packaging material is with H_2O_2 and/or UV irradiation lamps. For the latter systems, quartz screens are provided for maximum product/operator protection; this section is also air cooled and is fitted with safety guards.

The outputs of the COMBIseptic models are 9000, 12 000 and 15 000 cup hour^{-1}, respectively. The CONTItherm models are supplied by the same manufacturer and have ultraclean facilities as in the COMBIseptic, but the production output is 12 000–19 200 cups hour^{-1} (model 82) and 15 000–25 000 cups hour^{-1} (model 123).
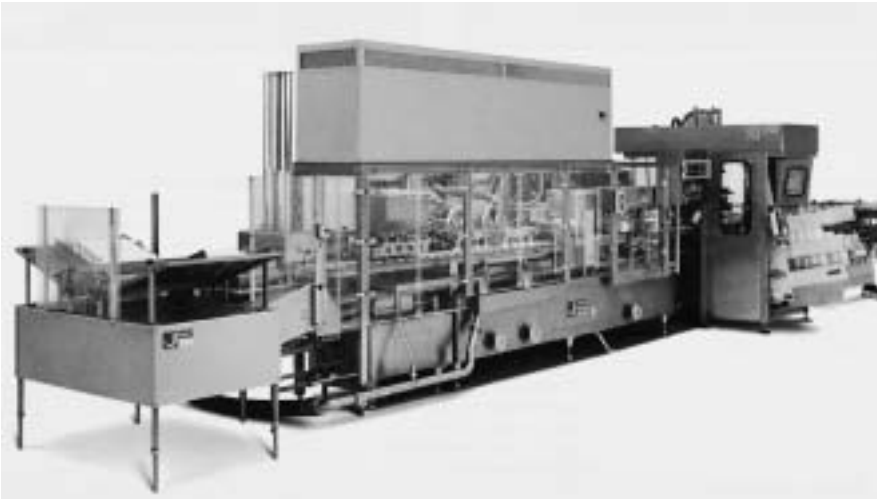
Fig. 3.57 COMBIseptic cup filling machine

Reproduced by courtesy of Jagenberg (London) Ltd., Purley, U.K.

Waldner Dosomat 20 – This is a fully automatic cup filler with a filling capacity up to 60 000 cups hour^{-1}. The Dosomat 20 (Hermann Waldner GmbH, Germany) has an automatic cup feeder and filled cups are nested in trays or cardboard boxes. The filler is long enough for the cup loading section, production area and the final packaging section to be separated from each other, thus providing an ideal hygienic layout.

H_2O_2 is used to sterilise the plastic cups but, in the Dosomat 20, rather than spraying H_2O_2 into an air stream, it is vaporised using a special evaporator. Subsequently, the vaporised H_2O_2 is mixed into a hot air stream and blown into the cups through special pump nozzles. This system ensures good wetting of the cup surfaces without forming drops and has another advantage in that, as the optimum reactive temperature is used, there is no need of an unnecessary supply of hot air. Then the cups are dried with hot air on three other stations and the air charged with H_2O_2 is discharged over a catalyser into the open.

All the subsequent stations for filling and sealing the cups are located in a hermetically sealed environment which is flushed continuously with sterile air to minimise recontamination of the cups or the product. The dosing unit is piston driven with outlet tappet valves. A sterile valve junction before the dosing unit, which is hermetically sealed, also ensures that the unit is free from recontamination even after running the filler for a long time. UV irradiation is used to sterilise the aluminium foil lids; alternatively, infrared or H_2O_2 sterilisation systems could be used.

Remy 900 volumeter – This type of yoghurt filling machine can be ordered in three versions, clean with laminar flow, ultraclean and aseptic. In general, the machine offers high standards of hygiene, and in particular:

- An automatic cup loader and dispensing unit
- In the ultraclean version, the cups are sprayed with H_2O_2, dried with sterile hot air and then filled with yoghurt in a sterile, laminar air flow cabinet; in the aseptic
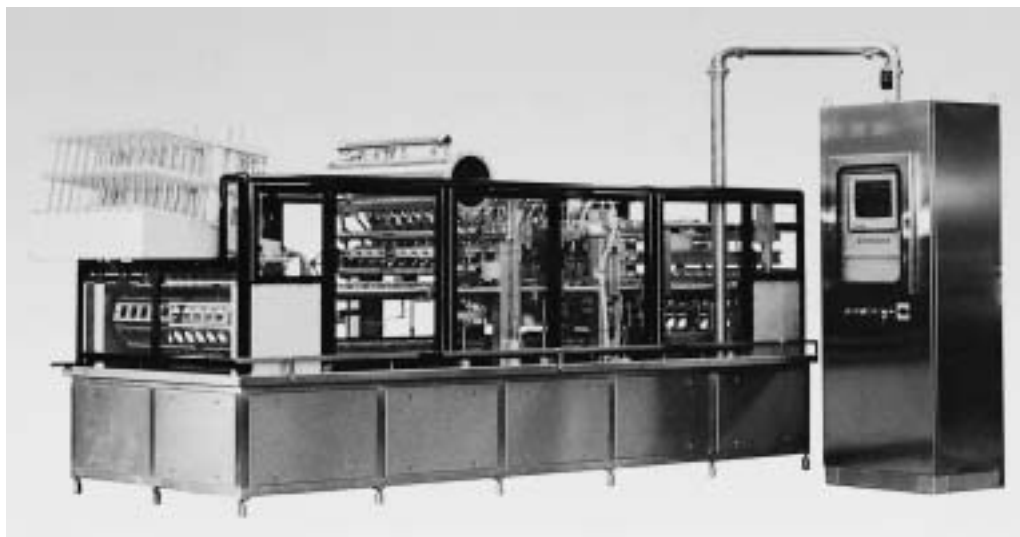
Fig. 3.58 A general view of ILPRA® fill seal 20 000 – the basic model
Reproduced by courtesy of ILPRA SpA, Corso Pavia, Italy.

version, the filling station is equipped with a sterile water barrier, and both the cups and foil lids are sterilised and conditioned inside a sterile watertight tunnel.
• The lids are heat sealed and the filled cups are fed to an automatic tray packer.

The capacity of the machines ranges between 8000 and 54 000 cups hour^{-1} depending on the number of filling lanes. The maximum diameter of the flanges that can be installed are 81, 85, 120 and 150 mm. Incidentally, these machines can be used to fill yoghurt into glass jars too.

ILPRA® Fill Seal System – A wide range of cup filling machines are manufactured by ILPRA SpA in Corso Pavia in Italy and the smaller machines have an output ranging from 1500 to 6500 cups hour^{-1}. However, the larger filling machines (i.e. models 10 000 and 20 000) have a throughput of 11 000 and 24 000 cups hour^{-1}, respectively (see Fig. 3.58). The filling quantity ranges between 50 ml and 500 ml, and both machines may be obtained in different versions: (a) basic, where the cups and the aluminium foil are not sterilised prior to filling, (b) steam cleaned, including a sanitisation system for the packaging materials and where the filler is enclosed within a laminar flow compartment, and (c) aseptic type which is similar to (b) but the cups and foil are sterilised with hydrogen peroxide (H_2O_2).

These filling machines (e.g. models 10 000 and 20 000) have four or ten filling lanes, respectively, including cup holder, destacker, volumetric product dosing head that is suitable for CIP and a heat sealer. The filling machines are equipped with a detector device (e.g. no cup/no fill), a coder station and ejection station. Some other optional facilities include snap-on lid applicator, foil dispensing from a reel for multicups (e.g. 12, 16, 20 or 24) nesting in cartons, plastic trays or boxes.

Hittpac AKH-051 series – These are versatile rotary yoghurt cup fillers for packaging singles including twin-chamber and multipacks of 2, 4 or 6 (see Platt, 1990). The Hittpac AKH-051 series (Lapp-Textima AG, Switzerland) can handle 3000 up

Fig. 3.59   View showing the rotary Hittpac AKH-051 series cup filler
Note the two × 6 cup pocket for multipack yoghurts.
Reproduced by courtesy of Corporation Packaging, Farnham, U.K.

to 15 400 and/or 25 000 cups hour^{-1} using up to 12 individual pockets per cycle, and for "Petite Suisse" using the SU 8/8 model. The volumetric dosing station has an air brush or membrane nozzle for positive cut-off of the yoghurt to minimise drips between the pots. The fillers are available as aseptic or ultraclean version using hygienic technologies such as UV irradiation or H_2O_2 treatment with hot sterile air (see Fig. 3.59).

Trepko cup filler – High speed 4-, 6- or 8-lane filling machines are made in Denmark and the filling capacity ranges between 10 000 and 20 000 cup hour^{-1} (see Fig. 3.60). The most basic versions are for filling the plastic containers to be heat sealed with aluminium foil, but most models could be supplied with the facility for UV irradiation of the packaging materials (e.g. cups and foil) and the product filling zone is provided with sterile air in a laminal flow cabinet (see Fig. 3.60). Some cup filling models could be provided with facilities for handling:

Fig. 3.60   An eight-lane Trepko cup filler

Reproduced by courtesy of Trepko Fyldemaskiner A/S, Taastrup, Denmark.

- snap-on plastic lids
- heat sealing with aluminium foil only, or
- heat sealing plus a snap-on plastic lid.

The cup feeding magazine contains enough cups for up to 5 min operation depending on the cup size, and Hansen (1984) described a four-lane Trepko filler which can be adjusted for four different cup sizes.

Some other features of the Trepko fillers are: (a) a safety cabinet is provided which complies with EU regulations for safety of the machine operators, (b) some models are suitable for filling twin-chamber containers, (c) sensors to detect faulty aluminium foil seals can be supplied, (d) special filling nozzles with membrane valves suitable for filling drinking yoghurt are available, and (e) the machines are designed for CIP cleaning. However, small cup filling machines are also supplied by Trepko, with the filling speed ranges between 2000 and 5000 cups hour^{-1}.

DOGAseptic series – These fully aspectic cup filling machines (Benz & Hilgers GmbH, Germany) are manufactured in different sizes to meet the demand of customers. The overall specifications are given in Table 3.5.

An illustration of such a machine is shown in Fig. 3.61, where the plastic container (before filling) and the aluminium foil lid (before heat sealing) are sterilised by H_2O_2. The sterilised packaging material is then exposed to hot air so that the sterilant is vaporised and exhausted into the atmosphere; filling of the containers takes place in a pressurised, sterile air compartment. The entire filling machine is cleaned by CIP (i.e. circulation rate 30 m^3 hour^{-1} at 0.3 MPa pressure) and the filling head can be steam sterilised at 143°C. Also, the machine is fitted with a cup leakage sensor to ensure proper closure of the containers.

Table 3.5 Overall specifications for DOGAseptic series yoghurt-filling machines

DOGAseptic model	Number of filling lanes	Output (cup hour^{-1})	Maximum cup sizes (mm)	
			Diameter	Height
42	1 × 4	9 600	115	130
61	1 × 6	14 400	95	130
62	1 × 6	14 400	75	105
81	1 × 8	19 200	75	105
81/2	1 × 8	36 500	75	105
82	1 × 8	20 000	95	130
101	1 × 10	25 200	75	120
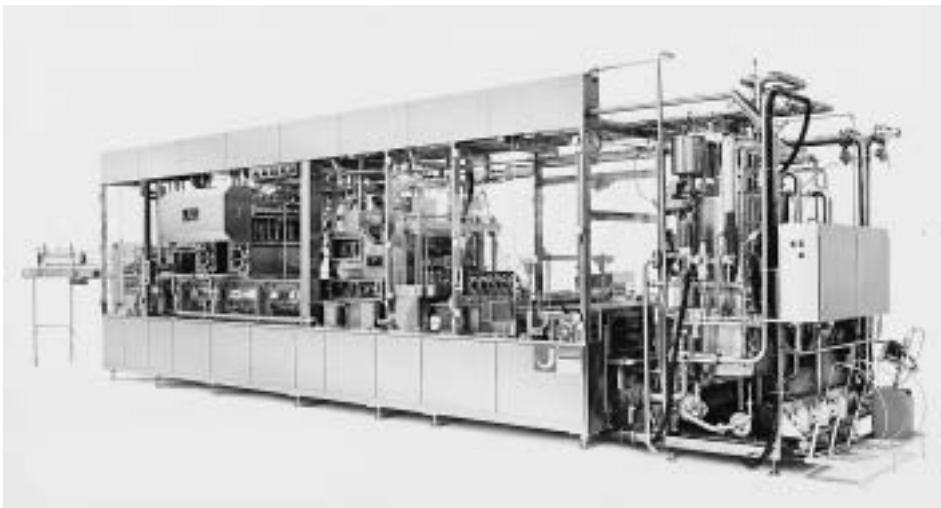
Fig. 3.61 The DOGAseptic 61 which is fully aseptic

Reproduced by courtesy of Jagenberg (London) Ltd., Purley, U.K.

The same company also provides ultraclean or aseptic versions known as SER-VOtherm and the output ranges between 38 000 and 57 000 cups hour^{-1}. These machines can fill and seal tapered cups of all kinds and shapes made of PS, PP, coated paper, coated aluminium and/or other packaging materials.

3.3.11.2 Machines for filling form-fill-seal plastic containers
The packaging material is delivered to the dairy in large reels of plastic sheet and the process of thermoforming transforms the sheet into the shapes and sizes of container required. The finished cartons are then filled with yoghurt and later heat sealed. Some examples of this type of filling machine are as follows.

Hassia THL, THM and TAS models – The thermoplastic material, such as PS, PVC or PP and/or multilayer (e.g. PS/EVOH/PE or PS/PE), is fed from a reel to the preheating section of the filling machine (Hassia Verpackungsmashinen GmbH,

Germany). In this section, contact heating plates with integrated coils are used to provide even distribution of heat; however, for PP packaging material, the pre-heating station has to be modified to obtain the required temperature for forming the cup(s). Then, mechanical, servo-driven forming plugs prestretch the plastic material to obtain a consistent distribution of the polymer over cup walls and base. The insertion depth of the forming plugs is adjustable so that different cup depths can be made on the same machine. The following stage transfers the containers to the filling head of the machine for filling with yoghurt and heat sealing. A rotary (DDA-CIP) or diaphragm (DMK-CIP) valve filling system is recommended for yoghurt packaging, whilst for two to six multiflavour yoghurt packs the flow metering (DMI) filling system is used. Some output figures for the Hassia THM models are:

Model	Cups hour^{-1}
17/28	12 000
18/42	21 600
28/48	32 400
33/80	57 600

Other features of the Hassia filling machines are:

- Numerous designs of cup opening features (e.g. unsealed area, corner break-off, raised or recessed unsealed tab) are available.
- The lid material can be heat-sealable lacquered or coated aluminium foil, PS 80–130 μm, PE or multilayer material such as kraft paper (45 g m^{-2}) or PETP (12 μm) and metalised lacquer (3 g m^{-2}).
- Labelling systems for form-fill-seal cups are available to coat one, two or three sides or all around the cup.
- For extended shelf life products, UV irradiation, pressurised sterile air tunnels or laminar flow cabinets provide a clean packaging environment, whilst steam is used in aseptic machines (see Fig. 3.62).

IIllig FS 37 and FS 51 AS – Thermoplastic material (e.g. PP and/or PS) is fed from a reel to the heating section of the filling machine and the warm sheet is formed into coherent containers after being stretched over a mould; the cup shape is obtained by forcing the plastic into the mould with compressed air. The formed containers, as well as the lid material which is also fed from a reel, enter the sterile bath of H_2O_2 and then a hot air section. All this takes place in a hermetically sealed tunnel to ensure maximum sterility (e.g. guaranteed sterility is at a level of one microbial survivor in 10 000 cartons) and the residual H_2O_2 is <0.1 μg g^{-1}, whilst the concentration of peroxide vapour is about 1 μg g^{-1}.

The Illig FS series machines (Adolf Illig Maschinenbau GmbH, Germany) are available with output capacities ranging between 2000 and 40 000 cups hour^{-1} (see Fig. 3.63). These packaging machines use a special DK 300 filler which is aseptic, in order to ensure that the yoghurt can be packed in an absolutely germ free environment. The filler is mounted independently into the filling line.

After filling the cups with yoghurt, the containers are heat sealed and labelled. For example, if there are multipacks of 1×2 or 2×2, the container labels are applied to the front and back of the set pack. Finally the containers are arranged in rows to be transferred into boxes.
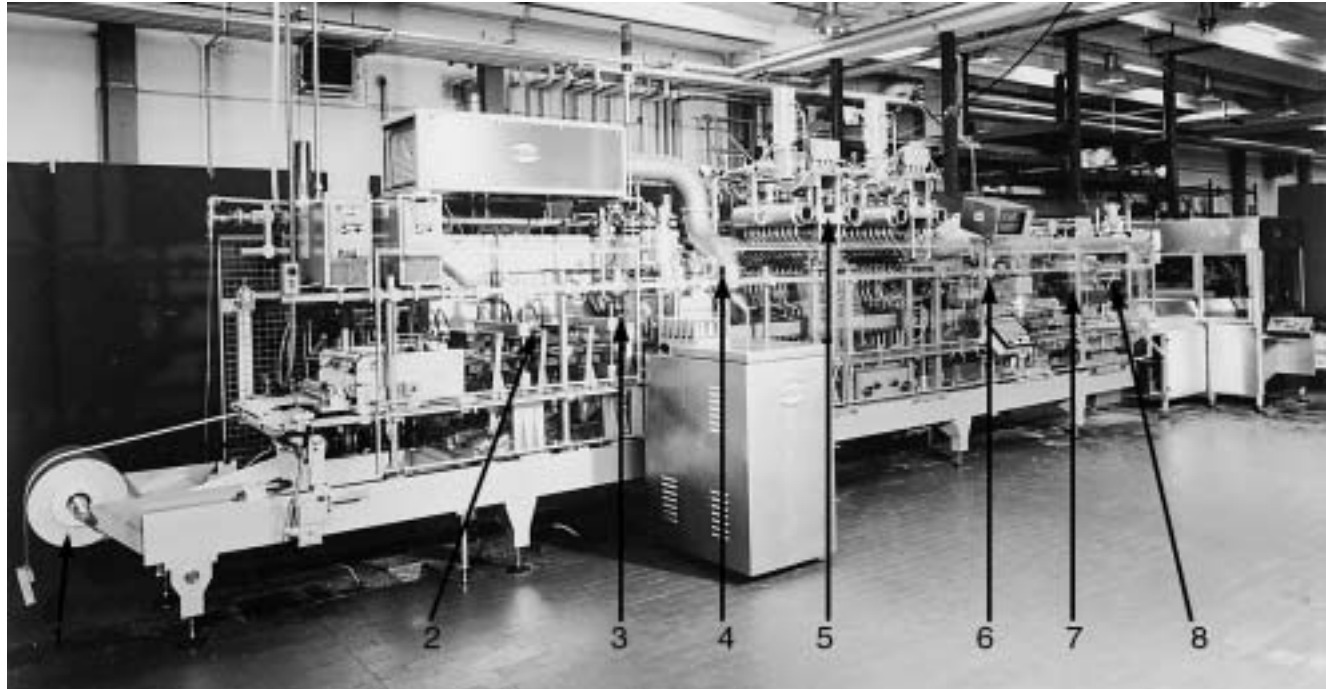
Fig. 3.62 The Hassia TAS 28/48 steam/aseptic system

1, Plastic reel; 2, preheating station; 3, thermoforming unit; 4, cup sterilisation; 5, product dosing unit; 6, steam to sterilise lid material; 7, cup sealing; 8, cup(s) punch.

Note that: (a) Saturated steam of food quality is used at 0.8 MPa pressure, (b) base materials are PS or PVC up to 700 μm thick or multilayer (e.g. PS/PVDC/PS or PS/EVAL/PS), and (c) lid materials are lacquered aluminium foil (30–40 μm thick) or aluminium laminate (PET/A1).

Reproduced by courtesy of Hassia Verpackungsmaschinen GmbH, Hessen, Germany.

Fig. 3.63   The Illig FS 51 aseptic filling machines

Reproduced by courtesy of Adolf Illig Maschinenbau GmbH, Heilbronn, Germany.

Erca-Formseal (EF) – A versatile range of form-fill-seal machines is produced by ERCA S.A. in France within the Jagenberg group. These machines are supplied in different versions such as: (a) basic (i.e. ambient condition plus a laminar flow cabinet), (b) ultraclean (e.g. as in (a) plus infrared irradiation of lids, UV irradiation or H_2O for material decontamination and/or sterilisation, and (c) fully aseptic which have similar provisions as in (b) and a Neutral Aseptic System® (NAS®) that has provision for presterilising materials using chemicals, steam or UV irradiation. Some specifications of the various EF models are:

Model	Output (125 ml cup hour^{-1})	Number of lanes
300	9 000	2×3
320	13 500	2×4
480	20 000	2×6
	36 000	4×6
600	28 000	2×8
	48 000	4×8
825	40 000	2×12

However, other features of the EF machines may include: (a) no labelling provided (only on 300 model); alternatively, the label may be partially or fully wrapped around the cup, (b) many different lidding materials can be used, and (c) integrated tray packer, slip-on-lids and rapid cutting tool (see also Anon., 1982; Parr, 1985).

Bosch – Robert Bosch GmbH in Germany manufactures a wide range of filling machines using the form-fill-seal technique. These machines utilise the concepts of ultraclean and/or aseptic technologies; also some models have very high output capacities reaching 100 000 cups hour^{-1}. Thermoforming plastics which can be used for forming the yoghurt cup are single layer of PS or multilayers consisting of PS/PVDC/PE, PS/PVDC/PS or PS/EVOH/PE. However, the lid packaging material consists of aluminium foil laminate (40 μm) which is soft, smooth, glossy and heat sealable containing lacquer of 8 g m^{-2}.

A summary of some of the machine specifications of the various Bosch fillers may include the following:

Model		Type[a]	Output (cups hour^{-1})	Number of lanes
TFC	7017	B or UC	24 000	–[b]
	7027	B or UC	36 000	–
	7033	B or UC	48 000	–
TFA	242	A	42 000	4×5
	2520 (EU)	A	42 000	4×5
	4940	A	100 000	48 cups per cycle
Servac 78		A	48 000	Flexible

[a] B: basic (open with laminar flow); UC: ultraclean (enclosed with sterilisation facilities); A: aseptic filling (enclosed where packaging material is sterilised with H_2O_2 and the filler with steam). [b] Not reported.

3.3.11.3 Machines for filling yoghurt into cartons/paper containers

Cartons coated with a layer of polyethylene are widely used in the dairy industry for packaging liquid milk. They can also be used for packaging yoghurt; a slight modification of the filling head is necessary to avoid reducing the viscosity of the yoghurt. As mentioned in Chapter 2, the containers are either formed from a reel (form-fill-seal) or from collapsed/folded preformed cartons. Some examples of carton filling machines are as follows.

Tetra Rex (TR/7 HH & ESL) – These machines are produced by Tetra Pak in Sweden. The container is formed from collapsed/folded preformed cartons (capacities range between 150 and 1130 ml). The sequence of operations for erecting the carton and filling with, for example, yoghurt is given in detail in Fig. 3.64. Some models have an output of 13 000 cartons hour^{-1} and, as an option for large size cartons, a plastic insert can be fitted that has a reclosable plastic spout. A similar gable-top carton with a Cap-Pac® spout is available from Nimco or ELL (Evergreen™) in the USA.

Elopak/Pure-Pak – Currently these gable-top carton filling machines are produced in Norway (see Wolthuys, 1986); some models, for example the UH-25, are of an aseptic type (Anon., 1997) and a screw-cap applicator can be supplied.

The Pure-Pak P-S50 filling machine handles a wide variety of cartons from 250–1000 ml and the sequence of operations could be described as follows:

- The blanks are picked from the bottom of the magazine and fed onto a set of mandrels mounted on a hub.
- The carton base is formed and sealed on the mandrels. This is done in four stages: prefolding, heating, folding and sealing.
- The cartons, which now have the base sealed, are drawn off the mandrels and placed into pockets in a conveyor chain. The conveyor is double indexing, which means that the various operations like folding, filling and sealing are done simultaneously on two cartons.
- The two cartons are filled simultaneously in a bottom-up filler specially developed for high viscous products. The filler ensures gentle handling of the product and reduces viscosity loss to a minimum.
- The tops of the cartons are heated.
- The tops are folded and pressed together with water-cooled sealer jaws.
- The filled and sealed cartons are discharged from the machine onto a conveyor and passed on for loading into transport containers.
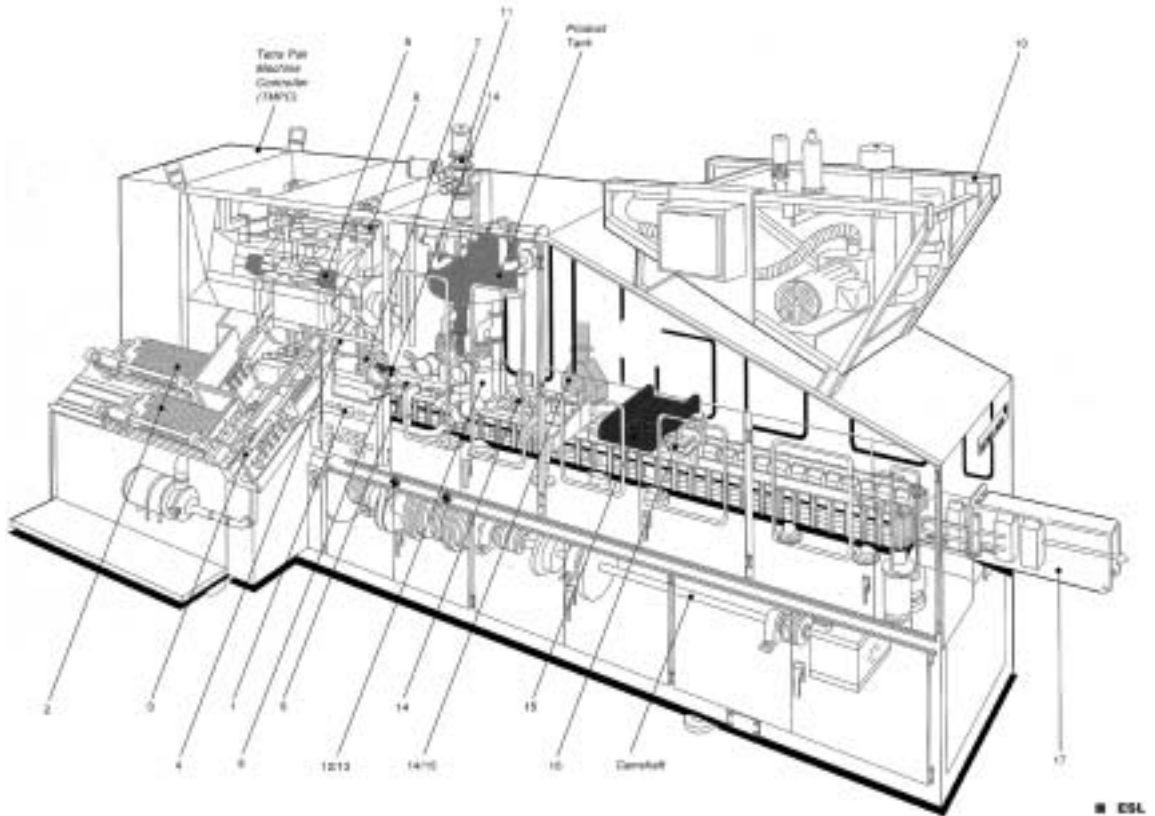
Fig. 3.64 Schematic illustration showing the packaging of yoghurt in a Tetra Rex machine

1, Operator panel; 2, cartons are fed from two horizontal magazines; 3, cartons are erected using suction cups and a pusher arm; 4, cartons are fed onto two temperature-controlled mandrel wheels and carton bottoms are prefolded; 5, prefolded bottom flaps are heated with air from electric ovens; 6, pressure pad completes the bottom seal; 7, carton unloader removes the bottom-sealed cartons from the mandrels and places them on the conveyor; 8, carton interior is sprayed with a $0.1\,g\,100\,ml^{-1}$ concentration of hydrogen peroxide; 9, cartons then pass through a germicidal, high intensity UV light chamber; 10, sterile air system (SAS) provides over pressure of sterile air to the product fill zone and product tank to prevent outside air from entering; 11, an aseptic product valve (APV) cluster allows for CIP with no break in the product line; 12, cartons pass under the product tank where metering pumps and filling nozzles operate in a sterile air environment; 13, cartons pause briefly and are filled through the bottom-up filling process; 14, moving to the sealing area, the top-sealing heater heats the prefolded carton tops with air from electric ovens; 15, top sealing is completed by pressure from water-cooled sealing jaws; 16, with the package securely sealed, a date stamp is applied; 17, packages are placed on the discharge conveyor.

Reproduced by courtesy of Tetra Pak (U.K.) Ltd., Uxbridge, U.K.

Tetra Brik – An example of a cartoning machine that forms-fills-seals the containers from a laminated paper board reel is the Tetra Brik system that comes as a basic (TB) or aseptic (TBA) version (Tetra Pak, Lund, Sweden). Detailed operation of the Tetra Brik filling machine is illustrated in Fig. 3.65. The output capacity of TAB/21 can reach 8000 cartons hour^{-1} (125–330 ml capacity) or 7000 cartons hour^{-1} (355–1136 ml capacity).
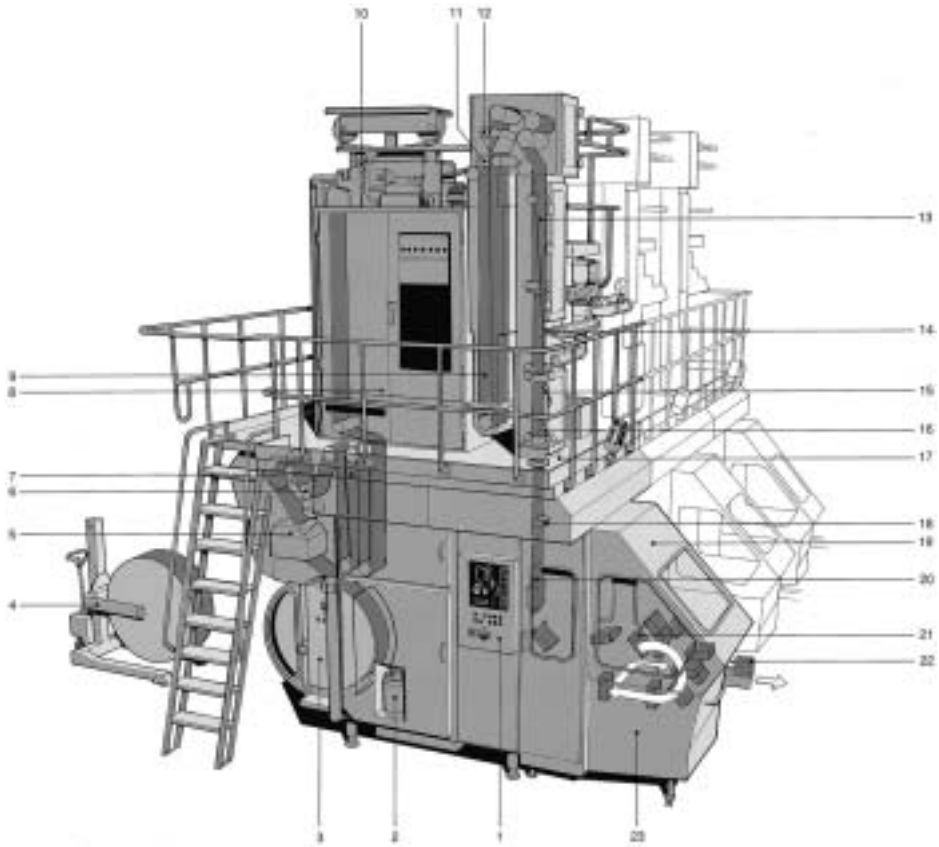
Fig. 3.65 Description of machine operation of form-fill-seal system using the Tetra Pak filling machine

1, Control panel; 2, container for H_2O_2, closed system; 3, reel of packaging material; 4, special trolley with hydraulic lift for handling packaging material; 5, automatic splicing equipment for packaging material; 6, date-stamping unit; 7, loops of packaging material, to ensure smooth, jerk-free feed and also to allow continuous production when new packaging material is spliced in; 8, most of the machine's electrical system is located here; 9, packaging material is sterilised in a deep bath of heated hydrogen peroxide; 10, strip applicator which applies a plastic strip to one edge of the packaging material. Later, at the longitudinal sealing stage, this is welded to the opposite edge. The result is a tight and durable seal; 11, rollers which remove the hydrogen peroxide from the packaging material; 12, nozzles for hot, sterile air, to dry the packaging material; 13, packaging material starts to be shaped into a tube here; 14, filling pipe; 15, element for the longitudinal seam which welds together the two edges of the packaging material; 16, short-stop element which completes the longitudinal seam when the machine restarts after any brief halt in production; 17, TBA/9 is designed so that two or more machines can be linked to form compact production units, sharing a common platform; 18, photocells, which control the machine's automatic design correction system; 19, casing which can be raised and lowered, covering the automatic external cleaning system and the final folder, where the top and bottom flaps are folded over and sealed onto the package; 20, packages are sealed beneath the surface of the liquid using induction heat. The heat comes from jaws which also shape and cut off the packages; 21, in the final folder the top and bottom flaps are sealed onto the package in two lines; 22, discharge of finished packages; 23, bath which fills with water and detergent automatically for external cleaning of the machine.

Reproduced by courtesy of Tetra Pak (U.K.) Ltd., Uxbridge, U.K.

Fig. 3.66 On-site automated handling of packaged yoghurt at the Müller factory in the U.K.

A, Nesting yoghurt cups in cardboard boxes; B, palletising the cardboard boxes; C, quick chill cooling in a tunnel; D, plastic overwrap of pallet.

Reproduced by courtesy of Molkerei Alois Müller GmbH & Co. (U.K. Production), Market Drayton, U.K.

The available methods of opening the Tetra Brik carton are: (a) cutting open, (b) opening by tearing along a performation (e.g. wave shaped or high-fin types): both systems produce a spout for poring, but the high-fin perforation makes it easy to reseal the package once opened, (c) applying a drinking straw to <500 ml cartons or pull-tab to any capacity carton, and (d) ReCap® opening (resealable plastic cap). In the latter opening system, the ReCap® lids are delivered in cardboard boxes and loaded into the magazine, which holds two boxes, equivalent to approximately two hours of production. The ReCap® lids are then automatically fed from the boxes in sheets which are cut apart in two operations. The ReCap® lids are fitted and transported by a vacuum chain to the wheel applicator, where a thin layer of hot melt is applied. The ReCap® lid is then fitted exactly over the premade pull-tab opening and held there until the glue has solidified.

3.3.11.4   Machines for filling yoghurt under controlled environment
Some packaging machines are equipped for, or have the facility for, gas flushing of the containers of fruit yoghurt before sealing. The objective is to replace the oxygen in the head space of the carton with carbon dioxide or nitrogen and so restrict the growth of yeast and moulds. Such an approach may indeed extend the shelf life of the product, but it is important not to overlook the facts. First, the packaging materials must be impermeable to these gases and second, the process of gas flushing is only effective against obligate aerobes.

3.3.12   Miscellaneous handling, chill cooling and refrigerated cold storage
The temperature of fruit flavoured stirred yoghurt after packaging in plastic containers may be about 20°C. In large factories, handling the yoghurt until it reaches the cold store may be governed by the plant design or layout and the degree of automation employed for materials handling. A highly automated example could consist of the following steps:

- The yoghurt cups are nested in cardboard trays.
- The stacked trays are palletised (see Cazanave, 1987; Anon., 1991; Hartman, 1995).
- The palletised yoghurt is chill cooled and then secured with a plastic wraparound the pallet to ensure safe handling in the cold store and during distribution and retailing.

Figure 3.66 shows how the packaged yoghurt is handled at Molkerei Alois Müller GmbH & Co. (UK Production), Market Drayton, U.K. The quick cooling of yoghurt is important if it is to retain its consistency after cold storage and the cooling tunnel which is manufactured by KTW Anlagenbau GmbH, Stuttgart in Germany is an example of this approach. According to Anon. (1996g), ten pallets of set-type yoghurt at 44°C can be cooled in one hour to 7°C (±2°C). Afterwards, the palletised yoghurt is overwrapped with a plastic sheet, transferred to the cold store and a robotic fork-lift system is used to stack the pallets in the cold store.

3.4   Mechanisation of yoghurt production and plant design

As the scale of yoghurt production increases, the use of mechanisation to handle the milk and the coagulum becomes inevitable. A wide range of equipment is

available, but the final choice is governed primarily by the method of processing adopted. Table 3.6 lists those items of equipment that might be required for the production of yoghurt from milk fortified with SMP, or alternatively from standardised milk concentrated by evaporation. It can be observed from Table 3.6, that while different equipment is required for the handling and processing of the milk, the process for the production and handling of the coagulum is broadly similar, and it is relevant that this mechanical handling can lead to structural damage to the coagulum. It is evident from the technical aspects reviewed in Chapter 2 and the effect of mechanical handling of the coagulum discussed in this chapter, that the viscosity and/or consistency of the product can be affected. One of the latest publications of the International Dairy Federation (IDF, 1998) gives details of the factors affecting the rheology of fermented milks and dairy desserts (see also Fong *et al.*, 1995; Houska *et al.*, 1996). An illustration of the effect of handling the coagulum on the viscosity of the final yoghurt is shown in Fig. 3.67 (Norling, personal communication; Bylund, 1995), and it is of note that, if the coagulum is handled carefully, the viscosity of the yoghurt recovers rapidly in cold storage, but the power to recuperate is lost when the coagulum is handled roughly.

Another important feature is, of course, the overall plant design, but the permutations available, particularly within existing buildings, means that each plant layout has to be considered in its own right (see Nicolaus, 1987). The different equipment used for the production of yoghurt should be in close proximity, for example, the distance between the fermentation tanks, the cooler and the intermediate yoghurt
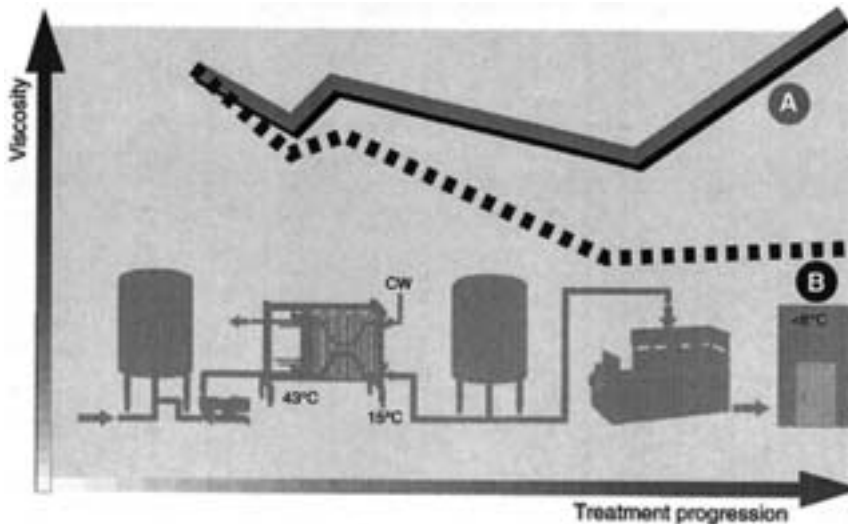
Fig. 3.67 Viscosity curve of yoghurt in relation to mechanical treatments involving sheer stress

A. Optimum plant design; B, badly designed plant.

After Norling (personnel communication) and Bylund (1995). Reproduced by courtesy of Tetra Pak (Processing Systems Division) A/B, Lund.

Table 3.6 Plant specification of a yoghurt production line – capacity 2000 l hour^{-1}

	Number required	Plant I	Number required	Plant II
Method of fortification		Addition of SMP		Concentration by evaporation
Preliminary treatment of milk	1	Centrifugal pump to circulate milk through powder mixing funnel and storage tanks.		
	1	Powder mixing funnel.		
	2	Vertical storage tanks to hold standardised/fortified milk.		
	1	Centrifugal pump to pump stored milk to balance tank.	1	as for Plant I
	1	Balance tank for intake of yoghurt milk to the plant.	1	as for Plant I
	1	Centrifugal pump for pumping fortified/standardised milk to plate heat exchanger.	1	as for Plant I
Processing of milk	1	Plate heat exchanger capacity 2000 l h^{-1} to heat treat milk and cool it to incubation temperature.	1	as for Plant I
	1	Holding tube to hold the milk at heat treatment temperature for at least 3 min.	1	as for Plant I
	1	Homogeniser (capacity 2000 l h^{-1}) to homogenise the milk at >60°C.	1	as for Plant I
	1	Hot water unit to provide thermal energy required to heat milk.	1	as for Plant I
			1	Vacuum chamber to concentrate standardised milk.
			1	Centrifugal pump to pump milk from evaporator to homogeniser, and to recirculate milk to plate heat exchanger until the desired concentration is achieved.
			1	Vacuum pump to pump the condensate from the evaporator to the regeneration section of plate heat exchanger.
			1	Vacuum pump to pump concentrated milk through plate heat exchanger.

Starter preparation/ yoghurt production	1	Viscuabator for preparation of mother and intermediate/feeder starter culture.
	2	Starter vat for the production of bulk starter culture.
	1	Positive displacement pump to pump bulk starter to the yoghurt incubation tank.
	1	Positive metering pump for continuous in-line inoculation of milk with the bulk starter culture for production of either stirred or set yoghurt.
	4–5	Vertical incubation tanks, each with a capacity of 2000 l; and/or incubation cabinets/tunnel to produce set yoghurt where the number is dependent on method adopted.
	1	Positive displacement pump to pump the yoghurt coagulum to plate cooler.
	1	Plate heat exchanger (capacity 4000 l hour^{-1}) to cool yoghurt.
	1	Cold water unit to cool yoghurt.
	1	Centrifugal pump used as a by-pass to pump recirculated water on the cold water side.
Fruit blending/ packaging	2	Vertical intermediate storage tanks, each with a capacity of 3000 l depending on production schedule.
	2	Positive displacement pumps (metering type) to pump yoghurt and fruit flavours to the blending unit.
	1	Fruit/yoghurt blending unit.
	1–2	Yoghurt filling machine(s) of total throughput 2000 l hour^{-1}.
CIP system	1	Control panel.
	3	Tanks for detergent solutions.
	4	Liquid ring pumps used as return pumps for cleaning.
	1	Plate heat exchanger to heat detergent solutions.
	1	Centrifugal pump used as feed pump for detergent solution.
	1	Filter to remove large soil particles from the CIP system.
	1	Steam controller.
Miscellaneous	1	Main control panel.
	–	Number of valves, fittings and pipes required in each of the sections mentioned above.

Data compiled from Tetra Pak A/B technical specification of yoghurt plants.
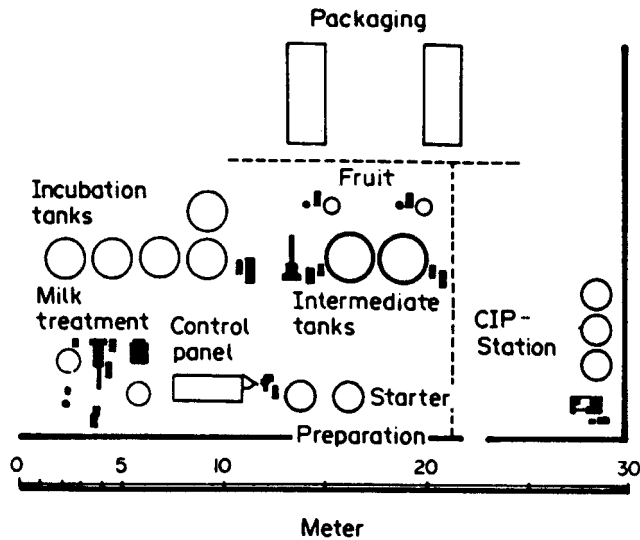
Fig. 3.68   Layout of a yoghurt plant to handle 3000–4000 l hour^{-1}

Space requirements for some Tetra Pak yoghurt plants based on the layout above; excluding the areas required for the CIP unit and the packaging machinery are:

Plant capacity (l h^{-1})	Floor area (m^2)
1000	$16 \times 10 = 160$
2000	$18 \times 11 = 198$ (200)
3000	$20 \times 12 = 240$
6000	$25 \times 12 = 300$
10 000	$26 \times 13 = 338$ (340)

Reproduced by courtesy of Tetra Pak (Processing Systems Division) A/B, Lund, Sweden.

storage tank(s) should be as short as possible. In some instances, the equipment has to be installed in an already existing building (which could be a limiting factor), but considering the recommendations mentioned earlier, the reduction in viscosity of the yoghurt could still be minimised. In an ideal situation (i.e. factory construction and plant installation carried out simultaneously) the layout of a yoghurt plant might take the form illustrated in Fig. 3.68. Notice that the flow of yoghurt from the incubation tanks to the cooler and storage tanks is virtually in a straight line and that the distance is short. The situation is similar for the transfer of yoghurt from the storage tanks to the filling machines.

Finally limited data are available on the physical damage experienced by yoghurt during transport; the effect of vibratory motion on packaged yoghurt has been examined by Richmond *et al.* (1985), who reported the following categories: (a) slight or definite, (b) cracked coagulum, and (c) complete disruption of the coagulum. The same authors also reported that the top layers of the vibrated stack (i.e. 10 high) were most damaged, and they found that overwrapping the shipping containers with polythene proved most effective in reducing synersis; <1% of containers wrapped in this manner had cartons showing any sign of synersis (see also Fig. 3.66).

3.5   Continuous yoghurt production

3.5.1   Background

In practice, the expression continuous production of set and/or stirred yoghurt is taken literally to mean the continuous flow of coagulated milk, and this can be achieved by employing a high degree of mechanisation and an appropriate plant design. For example, if a series of incubation chambers and/or fermentation tanks are used at regular intervals, the result is, in effect, a continuous production of set and/or stirred yoghurt. However, this constant flow of yoghurt should not really be termed continuous yoghurt production, since the product is still manufactured in synchronised batches and there is almost always some variability in the quality of the end product.

In theory, therefore, continuous yoghurt production should only refer to a process in which the raw material (milk) is steadily and continuously transformed into a coagulum (yoghurt). One of the earliest processes reported for the continuous production of set yoghurt was the method designed by Ueno *et al.* (1966). In this system the inoculated milk is filled into glass bottles, and the stacked crates which hold the bottles are placed on a cradle suspended from an overhead conveyor system. The distance between successive cradles is 60 cm, and the rate of production per hour is dependent on the speed of the conveyor, for example about 14 000 or about 18 500 bottles of yoghurt at speeds of 1.2 or 1.54 m min^{-1}, respectively. These cradles pass through the incubation chamber in a zig-zag manner up to five layers high and, after a certain duration (depending on the rate of acid development and the incubation temperature), the cradles pass through a chilling room (air temperature at -5°C) which cools the yoghurt to 20°C; final cooling takes place in the cold store. Hansen (1977) described a similar process used in Belgium where special trolleys (each holding 153 trays of cups filled with inoculated milk) are driven by a conveyor belt through the incubation tunnel. At pH 4.5, the yoghurt is passed to an adjacent tunnel which cools the yoghurt from 38°C to 15°C; final cooling takes place in the cold store. Incidentally, the cooling tunnel is divided into four sections and the temperature of the cold air in circulation is successively decreased, that is, starting at 8–10°C and finishing at 4–5°C. Other continuous systems have been reviewed by Rasic (1975) (see also Guyot, 1986).

A continuous process for the manufacture of stirred yoghurt is rather more complex than the systems mentioned above, but Girginov (1965) developed a semi-continuous process for the production of set yoghurt in batches and the basic principle of his technique (i.e. a two-stage fermentation) was later developed for a completely continuous process for the production of stirred yoghurt. The original Girginov method consisted of the following steps:

- Prepare the milk base, i.e. fortify, heat-treat and cool.
- Inoculate the processed milk at 46–48°C with uncooled yoghurt starter culture (42°C).
- Incubate the bulk until acidity reaches 0.23–0.27 g 100 ml^{-1} lactic acid.
- Maintain a continuous prefermentation process by the constant addition of processed milk at 46–48°C and simultaneous discharge of an equal volume; thus the volume of milk and the acidity (0.23–0.27 g 100 m^{-1} lactic acid) always remain constant.

- Cool the prefermented milk to 32–33°C, fill into containers and incubate to the desired acidity.
- Cool the yoghurt to 5–6°C, store and dispatch.

3.5.2   The NIZO process

During the early seventies, a research team at the Netherlands Institute for Dairy Research (NIZO) developed a continuous yoghurt making process based on the same two-stage fermentation, that is, the prefermentation (pH-stat) stage followed by the coagulum formation (plug-flow fermentor) stage. A flow diagram of this process and the recommended conditions for a laboratory and a pilot-scale plant operation have been well documented by Anon. (1975a) and Driessen *et al.* (1977a, b) (see also Lelieveld, 1976, 1984; Driessen, 1981). A summary of their observations and recommendations is given below:

- The yoghurt starter culture (RR) is an EPS producer that yields a viscous yoghurt; the ratio of cocci to rods in the starter culture is 1:4.
- The incubation temperature in the prefermentation tank is 45°C; this provides the optimum growth conditions for the yoghurt organisms and the pH of the milk is reduced to 5.7 within 15–20 min. At this pH, the ratio of cocci to rods is around 19:1, but this changes to 1:4 in the final product. This ratio of 19:1 is essential to provide a pH-stat, because the prefermented milk is constantly diluted and, if the specific growth rates altered, the quality of the yoghurt would be affected.
- The phenomenon of syneresis, i.e. whey separation from the yoghurt coagulum, is directly related to the degree of physical disturbance to which the network of the protein micelles is subjected, but it can also be brought about by careless processing of the milk, e.g. poor pH and temperature control during the incubation period. Disturbance of the protein micelles in a continuous process can take place at the following stages: (a) during the prefermentation, i.e. before the final network of the protein has formed, any disturbance of the coagulum below pH 5.7 could cause some damage, (b) during the coagulum formation period the network of protein is being formed and syneresis can occur if the gel is disturbed, and (c) if, after formation of the stable network, the yoghurt coagulum is stirred above pH 4.6, wheying-off can occur.

  Syneresis could, therefore, take place during the second stage of the NIZO process, as stirring of the coagulum is inevitable in a continuous process, and hence avoidance of problems is dependent on the temperature of incubation and the level of acidity. For example, if a temperature of 45°C is used throughout the process, the coagulum cannot be disturbed between pH 5.6 and 4.6 (i.e. the critical zone), while at a slightly reduced temperature of incubation, e.g. 37°C, the critical zone is between pH 5.6 and 4.8. It is for this reason that the prefermented milk is cooled to 37°C before it is transferred to the plug-flow fermentor since, at the higher pH level (pH 5.7), the coagulum can be disturbed without causing any syneresis.
- The dilution rate (e.g. the rate of addition of the milk base to the prefermentation tank) can be increased at higher pH values. This observation is based on the existence of the linear relationship between the concentration of lactic acid and the specific growth rate of the yoghurt organisms under controlled conditions.

Thus, it is recommended that fresh milk is added to the prefermentor at a rate that maintains the pH at 5.7, so ensuring the desired balance between *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* and the subsequent absence of syneresis (see also Lewis, 1967; Meyer *et al.* 1975; MacBean, 1976; Lelieveld, 1976; MacBean *et al.* 1978, 1979).

- The plug-flow fermentor unit is designed to: (a) avoid disturbance of the co-agulated milk in the fermentor during the transfer of the prefermented milk, and hence the fermentor is fitted with a special centrifugal distributor (Anon., 1975b), (b) prevent the coagulated milk adhering to the sides of the fermentor, and to this end the tank is coated with polytetrafluroethylene (PTFE) or lecithin, and (c) avoid damaging the coagulum during the stirring and removal of the coagulated milk, and hence the plug-flow fermentor is fitted with a specially designed stirring plate (Anon., 1975a).

- The residence time of the prefermented milk in the coagulum formation unit is $2\frac{1}{2}$ hours at 37°C.

- The development of this process was carried out in equipment capable of producing 250 l hour^{-1}, but the recommended throughput for a large-scale plant is around 4000 l hour^{-1} (see also van der Loo, 1981; Fig. 3.69). This industrial size was made available to yoghurt producers, but it could be argued that this technological development was too advanced for acceptance by the industry; however, continuous yoghurt production may become an acceptable process in the future.

- The alleged advantages of continuous yoghurt making are space saving, reduction in size of equipment, reduction of yoghurt losses in fermentation tanks and pipe lines, reduction in capacity of cooling and filling sections, greater flexibility in relation to total amount produced, no need for all the milk to be in stock at
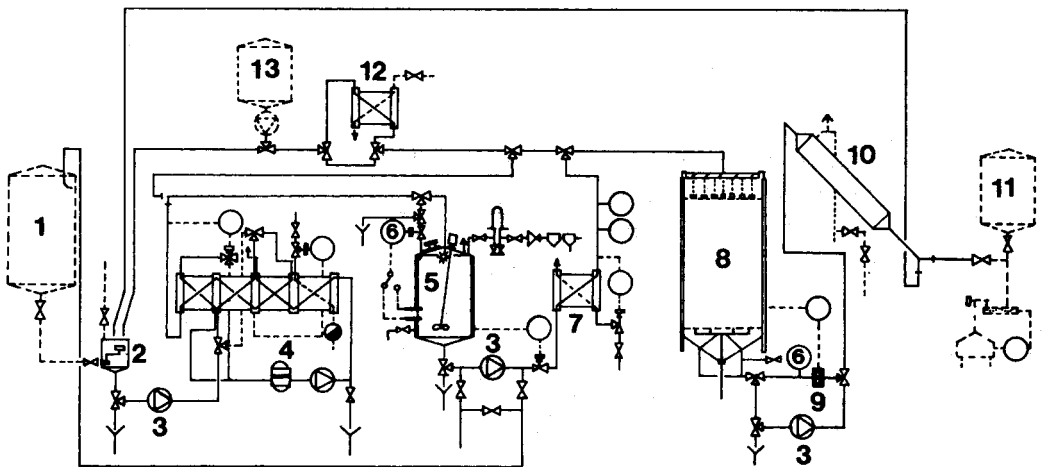
Fig. 3.69 Flow diagram showing the Stork-Amsterdam continuous process line for the production of stirred yoghurt

1, Milk storage tank; 2, balance tank; 3, centrifugal pump; 4, PHE; 5, continuous fermentation tank (pH stat-fermentor); 6, pH controller; 7, cooler; 8, coagulation tank (pH plug-fermentor); 9, positive displacement pump; 10, cooler; 11, buffer tank; 12, emergency cooler; 13, emergency buffer tank.

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the start of production, uniformity of product quality and characteristics, better control over acid development and less pressure on the cooling and packaging operations.

3.5.3   Recent developments

Recent research in the area of continuous yoghurt production mainly involves microbial growth kinetics of fed-batch fermentations (Özadali and Özilgen, 1988) and optimisation and control in fed-batch bioreactors (Shioya, 1992). In both cases the primary objective is to preferment milk in a reactor in order to accelerate yoghurt production. Prevost *et al.* (1985) entrapped the yoghurt micro-organisms on Ca-alginate beads and the rates of cell production (cfu l^{-1} hour^{-1}) for streptococci and lactobacilli were 1.8×10^{11} and 1.6×10^{11}, respectively (see Prevost and Divies, 1988a). The process ensured that a stable balance of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was liberated into the prefermented milk, and when such milk was used in yoghurt production, the incubation time was reduced by 15–20% (Prevost and Divies, 1988b).

Otten *et al.* (1995, 1996) used a fed-batch prefermentation of milk over a period of 51 hour with the same yoghurt starter culture without infection or loss of product quality. However, the same authors concluded that: (a) after one inculation of the prefermentation tank at least 20 to 30 large fermentation vessels could be inoculated, (b) by using high inoculation percentages (~15%) of the prefermented milk, the inoculation time of yoghurt production time was reduced by 50%, (c) in such a system there was greater flexibility when compared with a continuous prefermentation (see Driessen *et al.*, 1977a, b) because there was no continuous outlet flow, (d) by using high inoculation rates of prefermented milk, the yoghurt production capacity of an existing plant could be doubled with relatively low capital investment cost, and (e) to maximise profit by this method, the yoghurt manufacturer should operate 24 hours per day.

A continuous yoghurt culturing process has been studied by Ray and Raeuber (1991, 1992). From model equations and laboratory-scale experiments, they showed that constant, high stream velocity and relatively low shear gradient in a tube fermentor facilitated stable and continuous yoghurt culturing, whilst Ho and Mittal (1995) provided five different models (e.g. flow-and-hold and partial-flow-and-hold) or three continuous models (i.e. diversion, feedback and flow control) for continuous yoghurt making. In the former two systems, the flow rate was fast (8.3$l s^{-1}$) and the method required high-powered pumps compared with the other models in which the flow rate was very low (0.07$l s^{-1}$).

Continuous yoghurt production methods using two-step processes consisting of preculturing and main culturing within tube-type reactors have also been reported by Schulze and Raeuber (1993) and Steiner *et al.* (1993). All these researchers have used refractometry, optical sensors and ATR-spectroscopy to monitor continuously casein coagulation as a means of process control.

In the late 1980s, the Terlet company developed equipment for the continuous coagulaton of milk (Boer, 1987). Preacidified milk is delivered to containers (120l capacity each) that are suspended on conveyor belts which are housed in a vertical tower. The containers advance through the tower at uniform velocity at 45°C for the desired time until coagulation occurs. Afterwards the yoghurt is discharged to a cooling unit.

3.6 Automation/process control

In the past almost all the operations involved in the manufacture of yoghurt, including the cleaning stages, were carried out manually but, as processing plants have become larger and more complex (see Fig. 3.70), management and operators can have great difficulty in overseeing and controlling the process, particularly if the plant is spread over a number of process areas within the factory and/or a factory is producing yoghurt made from different ingredients for different customers. Communication between the various process areas can be difficult and support will be needed to ensure that the process is secure and manageable. This support can only be given by the use of a process control or supervisory system, supplemented by some form of management information system (MIS) (see Section 3.6.8). Such systems tend to be tailor-made for the particular process plant from proprietary components to produce a system which will allow the operators to operate the process and allow the management to control it. Periodically, the International Dairy Federation (IDF, 1973, 1985, 1991, 1995) publishes bulletins on automation in the dairy industry. The reader is referred to these publications for a more complete discussion (see also Mouchet, 1984; Lloyd, 1984; Bylund, 1995). Nevertheless, correct application of automation has many advantages such as:

- Production information for business analysis
- Product quality
- Flexible production
- Production control
- Minimising waste at start-up and shut-down
- Real time scheduling
- Plant maintenance scheduling
- CIP control
- Waste management

Taking these factors into account, the principles of automation as might be applied in a yoghurt factory are described below. The text has been cordially provided by APV (U.K.) and Tetra Pak (U.K.).

3.6.1 Levels of automation

There are various levels to which any process plant can be automated (Bird, personal communication), and these can be summarised as:

- Manual – where the operator is in sole control of the process and initiates all valve changes and tank selections by hand. A low level of automation support will be included.
- Semi-automatic – where the operator is provided with certain functions to assist in controlling the plant, such as flow plates, with proximity switches, to allow a distinct break between process and CIP; high and low level transmitters and gauges on tanks and the necessary switches; remote initiation of the CIP module.
- Fully automatic – where the operator is supported fully by an automation system and all commands are given by an operator control centre, or a number of decentralised operator interface units placed strategically around the process. These systems are usually further supported by an MIS.
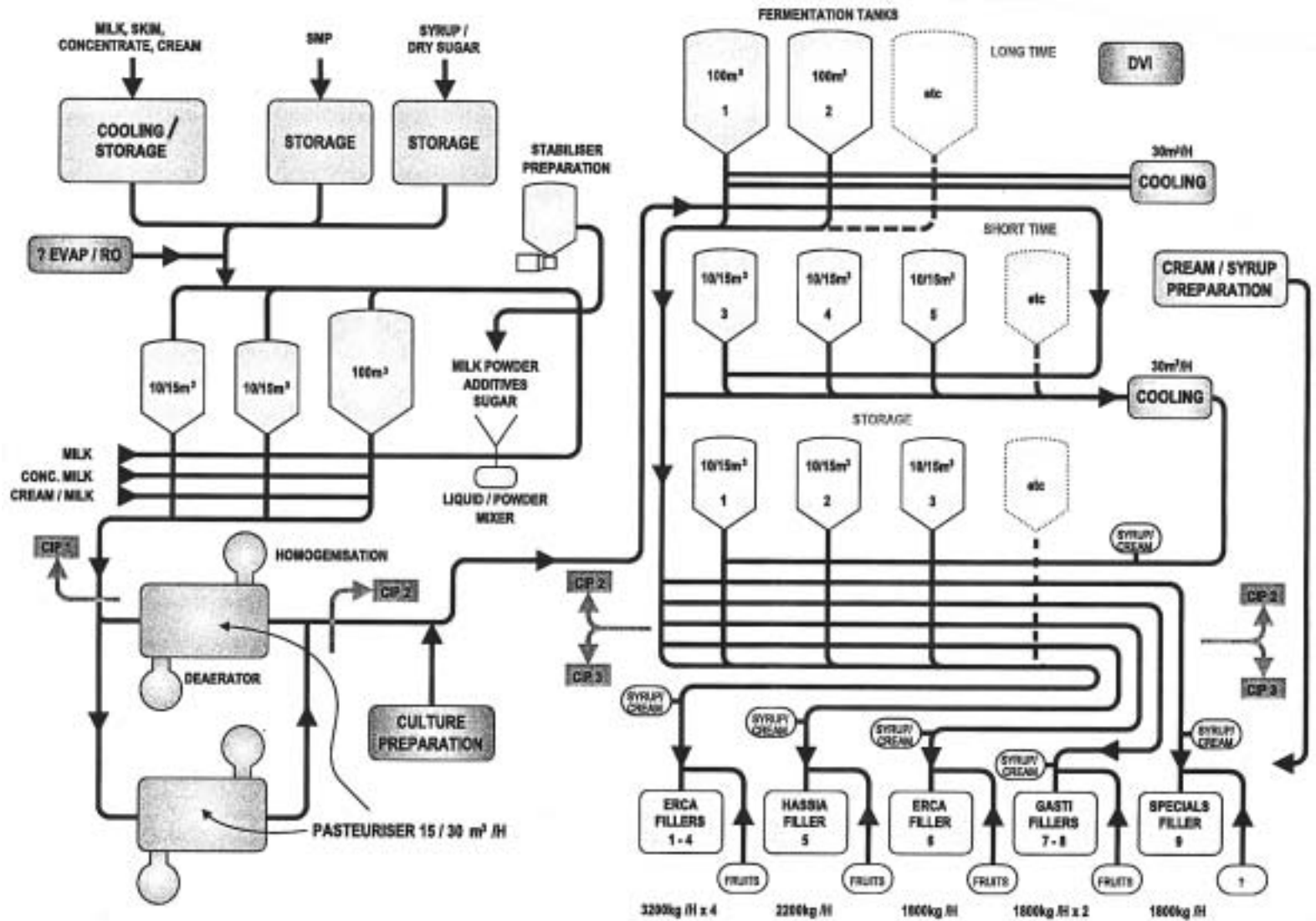
Fig. 3.70 Illustration of a typical stirred yoghurt plant capable of producing 20 000 tonnes per annum
Reproduced with courtesy of APV U.K. Co. Ltd., Crawley, U.K.

The choice of which system to install on an existing process plant is dependent on many factors. Cost is an important issue, but the level of automation required to assist the operator whilst still leaving the final control in his or her hands is also important. In other words, too much automation is just as dangerous to a process as too little, for there is a danger that a skilled operator may become complacent if suddenly confronted by a machine which does the thinking. Existing process plants can be automated to a semi-automatic level whilst still allowing all decisions affecting process functions to be in the hands of the operator.

A fully automated system is the highest level which can be installed into a site and is usually offered on new installations. In this instance, the automation concept is just as important as the process context and the design of the two systems is integral. Existing process plants may not be capable of automation to fully automated status without significant capital investment within the infrastructure of the process plant. The degree of automation in a yoghurt plant (see Fig. 3.70) is primarily dependent on capacity but, in the absence of any constraints, the processing plant is divided into different areas/departments interlinked via a central data processing unit. The choice of divisions can be subject to individual choice, but the following break-down would be quite feasible.

3.6.2 Area/department 1

In this area reception and storage of the milk takes place, together with preparation of the basic mix and automation covers handling of the liquid milk, control the flow of milk from the storage tanks, cleaning the tanks, and the selection of the dry ingredients (milk powder, sugar and stabilisers).

According to Bird (personal communication), these requirements can only be met if the process control systems are capable of monitoring and controlling the following functions:

- ingredient receipt
- recipe handling
- product routing and security
- critical process parameters
- disinfection parameters
- service(s) utilisation
- overall process plant performance.

This control is achieved by installing monitoring devices (instruments) around the process plant and taking the signals from these instruments to the control system. The control system will then compare the observed reading against the target reading and take the relevant action. The action may be, for instance, opening a steam valve to heat a CIP detergent tank to the correct temperature, or if a storage silo has reached the full level, automatically selecting the next tank in the queue to be filled.

The activation of a control instruction is normally "flagged-up" so that the operator is aware of what is happening; this is called feedback and is a most important facet of the system. If the operator does not know what is happening within the process, he or she cannot control it. Pipelines and valves are used to route product from one plant area to another, and both the control system and the operator need to be aware of the status of any transport route, particularly the valve positions.

Feedback loops from the valves allow the control system to prove that a route is available prior to allowing the operator to initiate a product or ingredient transfer.

Valves are now designed with this requirement in mind and they normally operate with compressed air driving the valve in one direction and a strong spring driving the valve in the reverse direction. All valves will revert to their rest position in the absence of compressed air and it is an important function of process plant design to ensure that valves are installed correctly. Tank outlet valves, for example, are always air actuated to the open position and spring closed – imagine what would happen if they were installed the opposite way and the compressed air supply failed!

Feedback can be just as important and valve feedback can be set at one of three levels:

- No feedback: in this instance, the valve can function but the control system cannot monitor it. This approach is only used when there is some other signal which allows the control system to monitor the effect of the valve opening. For example, the effect of a steam valve opening can be monitored by a rise in temperature at some point, but this absence of feedback is not normally recommended.
- Single feedback: in this case, the valve is monitored only in one position. When in this position there will be feedback to the control system and depending on the plant design, the feedback position could be normally-open or normally-closed.
- Double feedback: in this instance, the valve is monitored in both the actuated and rest positions. Double feedback is the most expensive system to install as two signals are required from each valve; the choice of single or double feedback requires careful consideration at the initial design stage.

3.6.3   Area/department 2

Milk standardisation, homogenisation and heat treatment take place in this section, and the operating sequence of the heat exchanger unit can be easily programmed to heat the milk to 90–95°C, hold it for the desired duration of time and then cool it to 40–45°C. To achieve this pattern, the plate heat exchanger unit will be fitted with certain controls to ensure that a repeatable and consistent performance is obtained. For example:

- The control system must ensure that the correct temperatures are achieved.
- A diversion mechanism must be in place to pass under-temperature milk back to a holding tank.
- The control system must ensure that milk leaving the heat exchanger is at the correct temperature for inoculation with the starter culture.

It is essential that this section of the plant is effectively cleaned. The CIP module may have a dedicated process control system to monitor and control its functions, such as: (a) checking and adjusting the target temperature of the detergent tank, (b) checking detergent strength measured by the conductivity of the solution and initiating the operation of the detergent dosing pump if the conductivity reading is low, (c) monitoring the return flow, temperature and conductivity of solutions used during a CIP sequence, and (d) initiating valve and pump activity when required by the programme (Bird, personal communication).

3.6.4 Area/department 3

The preparation of the starter culture is carried out here and automatic control systems are able to provide the necessary conditions for growth of the selected culture (see Chapter 8). The in-line inoculation of the process milk can be under the control of the same section.

3.6.5 Area/department 4

In this section, fermentation of the milk takes place and automation covers the control of temperature during the incubation period, monitoring the level of acidity (pH) and initiation of the cooling stage. As with the previous areas, both the operator and the control system must be aware of the status of all the vessels and valves but, equally important, the system must alert the operator if an observed parameter is outside of the target parameter. It has been suggested by Bird (personal communication) that it is usual for the control system to print out the alarm on a printer attached to the system. This will give two levels of information: in the first instance, immediate notification to the operator that something is not correct and that the system is taking remedial action or that the operator should make adjustments to the process, and second, tabulation of a hard-copy of all alarms for future reference. For emergency situations, audible alarms are essential, but in order to anticipate possible problems, operators can derive great benefit from visual displays of the entire process within their section; a number of options are available.

A diagrammatic visual display of the plant is useful so that the operator can observe the status of the process quickly. Such displays may take one of the following forms:

- Mimic panel: this is a diagrammatic display of the whole plant showing process blocks and transport routes. Lights illuminate to indicate to the operator when a component is running or activated, such as a pump or high-level switch on a tank. These displays look impressive to visitors to the plant, but their ability to inform the operator is questionable due to the amount of information displayed. They are also inflexible and cannot be easily updated when changes to the process occur.
- Matrix panel: this is a panel of lights set in a form that will light up when a route is in operation. The object is normally on the horizontal axis and the status – empty, full or under CIP – is on the vertical axis. The status of, say, a tank under CIP will be indicated by a light displayed at the conjunction of the tank reference and plant status. Differing coloured lights can indicate status – green for process, brown for CIP and red for alarms; a flashing light can indicate a queued situation.
- Colour graphics: this is similar to a mimic panel, but the plant is displayed in pages. The initial page will display the whole plant with all ingredient reception lines, tanks, pasteurisation plants and CIP modules. Selecting a plant area will allow the operator to assess the next page where that section of the plant is identified in greater detail. Selecting a plant item will allow the operator to display the status of that item. In the case of a tank, it will indicate the product definition, the extent of fill by a coloured level and whether the tank is filling or emptying. It will also state the volume in the tank and the temperature. The information can be displayed at the click of a mouse.

3.6.6 Area/department 5

In this section, blending of the fruit with white base takes place and since a factory may be manufacturing 15–20 different varieties of yoghurt in a week, automatic monitoring is essential. Thus, there will be different types of fruit/flavours (e.g. strawberry, banana, black cherry and many more), there be different qualities of each fruit/flavour to meet the specifications set by different retailers and there may be separate formulations of white base for branded or own-label lines. In addition, each variety will require a specific form of packaging. Ensuring that all the possible permutations are covered depends increasingly on computer control.

What these aspects highlight is that the manufacturer is no longer able to operate in isolation and that market forces often have a considerable impact on production. Consequently, the scheduling of different batches of yoghurt has become central to production planning and, since many individual recipe formulations may be produced in parallel within a single process line, there are likely to be many discrete batches passing through a particular plant at any one time. The sizing of batches and their routing through the plant is, therefore, a complex task which can have a major effect on the optimisation of resources and, ultimately, on the profitability of a manufacturing facility (Chester, personal communication). The difficulty of optimising batch scheduling has been exacerbated by the demands of modern sales and marketing requirements and there have been two distinct trends which have had a major impact on processors, namely extension of product ranges and "just in time" delivery.

3.6.6.1 Extended product range

In anticipation of market demands, multiple versions of the same generic products have been developed. These versions exhibit particular features designed to appeal to different market segments and to stand apart from competitors' products. Typical factors upon which products are discriminated include:

- Perceived quality (i.e. high price for high quality and, conversely, supermarket "value" ranges at reduced prices)
- Appeal to different age groups (e.g. cartoon/film related packaging and flavours to appeal to children)
- Perceived health benefits (e.g. low fat versions, different yoghurt cultures)
- Dietary requirements (e.g. vegetarian yoghurts, with no added gelatine)
- Shelf life (e.g. extended shelf life (ESL) and UHT versions)

Owing to their perishable nature, different types of yoghurt must be manufactured frequently. Most factories will manufacture each product several times per week and depending on shelf life, in some instances every day. Thus, where a wide product range is supported, a large number of different batches must be processed simultaneously. In order to accommodate a large number of batches, manufacturing facilities must be equipped with numerous and variously sized storage vessels and a vastly increased number of process routes. The resultant increase in routeing permutations and storage options greatly complicates batch planning and scheduling and, since equipment resources are limited, there is great pressure on the production manager to optimise batch scheduling to maximise their use whilst, at the same time, meeting production demands.

3.6.6.2 Just in time delivery

Driven by the desire to extend the shelf life of goods bought by the consumer (so increasing sales) and reduce the stock holding of both the producer and retailer (so increasing turnover), the time allowed for producing and delivering food products, in particular, dairy products, has been greatly reduced. Increased consumer mobility has additionally led to greater fluctuations in demand for particular products. When taken together, these factors mean that dairy producers often receive orders for goods only hours before they are required on the shelf (Chester, personal communication).

3.6.6.3 Production schedules

The combined demands of more products and shorter deliveries have placed great pressures on production managers. They are required to make more decisions (since more products mean more ingredients and more routes) and make them rapidly. In effect, this means that a large amount of batch data must be interpreted in a short time.

Consequently, graphical representations of processes are often used to aid production planning. In particular, Gantt charts – production schedule diagrams based on the project management tool – are used to display details of product batches as they are conveyed through the process (see Fig. 3.71). Since every batch may be displayed on a single chart, production data, such as equipment utilisation and delivery times, can be rapidly appraised and used to determine future action. Since production schedules display the flow of ingredients through the plant and give details about what time individual processing units are utilised, they embody the essence of the manufacturing process.

3.6.6.4 Batch planning

Since batch schedules encompass a huge range of process data, they can be extremely time consuming to generate manually. This problem means that it is difficult for a production schedule to show current process information, so that it is often not possible to use them to plan manufacturing. According to Chester (personal communication), preparation of schedules requires detailed knowledge of:

- the throughputs of individual process units
- the recipes required to make different products
- the quantities of products to be manufactured.

Where production requirements are relatively simple and vary little, schedules can be produced sufficiently rapidly using pencil and paper. This approach is often found where plants produce only one or two products and where production takes place in a few predictable stages. However, where a large number of products are supported and where reduced delivery times require rapid planning, the preparation of production schedules may be automated by use of spreadsheet packages or, more appropriately, batch scheduling software.

3.6.6.5 Batch scheduling software

This software consists typically of a database coupled to a set of scheduling algorithms (Chester, personal communication). The database contains an imprint of the plant equipment and routeing, as well as details of the product types and recipes.
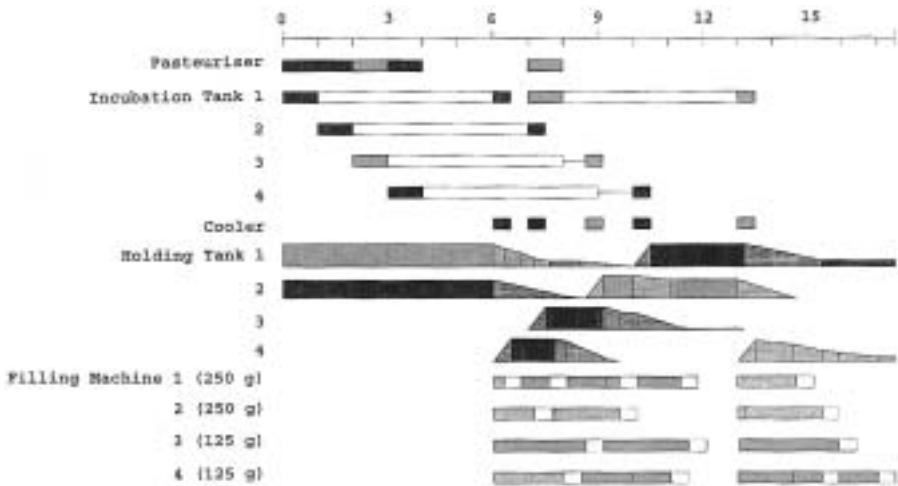
Fig. 3.71   A Gantt chart illustrates details of yoghurt batches as conveyed through the process

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The scheduling algorithms encapsulate the logic rules by which the schedule is constructed. Typically these include physical restrictions, such as "different products must be processed separately", and scheduling rules, which embody best practice for managing the process, for example "fill into the first tank which becomes available". To generate a batch schedule to meet particular production requirements, details of production orders and delivery requirements must be input into the scheduling package. This information is combined with recipe data, equipment throughput and plant connectivity to generate information on the number and size of the various material batches required to meet each product order. The scheduling rules are then applied to generate a production schedule that provides details of the timings of individual batches.

Scheduling may be done semi-automatically, with the user being prompted to confirm the size and equipment utilised by each batch, or fully automatically with user involvement being required only where the scheduling rules fail to determine a single course of action. Once generated, production schedules can be quickly manipulated to meet the varying demands placed on the manufacturing facility. Factors which have an impact on planning production include:

• achievement of customer orders
• requirement to clean equipment
• need to match operator shift patterns
• need to minimise equipment start-ups and shut-downs (thereby causing limited wastage of product and time).

3.6.7   Area/department 6
In this section the cleaning-in-place (CIP) station is located, and for further details refer to Chapter 4.

Up until now, interest has centred on the ability of the control system to monitor and control the operation of the process, but sections like filling and packaging bring about an interface with management, because if someone forgets to buy the necessary cartons, then no yoghurt can be packaged anyway. Consequently, it is vital that control systems can be expanded to collate information that will allow the management to gain access to information which is necessary to monitor the interaction of outside supplier/buyers with the company and to control the financial performance of the process plant.

3.6.8   Management information system

The large volumes of data generated by the various automation systems controlling a manufacturing process may be passed to a management information system (MIS). The MIS harnesses the incoming data to provide meaningful high level information, thus allowing rapid evaluation of the state of the process. This enables factory management to respond to changing conditions thereby improving manufacturing effectiveness (Chester, personal communication; Bird, personal communication). Typically, the MIS provides a means of integrating data from the following areas:

• Raw ingredients reception (e.g. weigh bridges for raw milk tanks)
• Process control systems
• Stock control and dispatch systems
• Packaging machines and palletisers
• Boilerhouse and other service providers.

On receipt, the MIS collates and stores data within a structured database, which may then be interrogated to provide critical information about the process. Data are displayed typically in a tabular or graphical format which elicits rapid intake. By undertaking the extraction and presentation of data, the MIS performs much of the interpretation stage which translates data into information. Thus, management information systems can provide data on inventory levels, product batching, cleaning usage and integrity, services loadings and maintenance requirements.

Management level information can be of interest not only to the processor, but also to its customers. The MIS may, therefore, provide benefits in terms of attracting large customers such as supermarket chains. For example, archiving of CIP data (e.g. temperatures, flow rates, clean duration) is used to police the rigorous hygiene standards demanded by both the producer and its major clients. By rapidly and automatically interpreting process data, the MIS equips management with the information required to take action to improve the process. Personnel are thereby empowered to manage their process more effectively; the MIS provides the conduit through which data flow from the sensor to the boardroom.

3.6.9   System architecture

The ability of the automation system to monitor and control the process and to collate and display management information requires very careful design since the demands of the two functions may be different. The balance between control and management must, according to Bird (personal communication), be decided very early in the process design, and the requirements of both the process plant

operator and the management must be balanced against both the complexity and the cost of the system. Simple plants need simple systems, but there are a few golden rules which apply to the design of a totally automated system:

- It must effectively allow the operator to control the process.
- It must effectively allow the plant manager to manage the process.
- Don't spend a pound to save a penny! (don't over-automate).
- The system must reflect the complexity of the process.

The design concept is the first stage in defining the system architecture and the wishes of all parties must be considered and weighed. Systems available in the marketplace are numerous and specialist assistance may be required in the initial process design. Most of the process plant suppliers and contractors have access to specialists who can advise on a suitable automation system once the process has been defined. The process is paramount – the automation system is there to support the process. Many good installations have been spoilt because the process has been compromised to take advantage of a cheap automation system.

The block flow chart shown in Fig. 3.72 illustrates a total automation concept based on a decentralised process control system with integral MIS. Decentralised operator interface panels allow the operator to have local control of the process, whilst an MIS system monitors and displays the relevant management information which can be displayed anywhere on the site using repeater panels.

3.6.10   System security

An MIS is generally regarded as a common database with selected access according to personal status or function. The more access that there is, the greater the danger of corrupting the system, so access must be on a strict need-to-know basis. The system will need to be protected by a password system which should be changed at frequent intervals. The passwords can be personal, or based on a personal/function basis and, in this way, access frequency can be logged against individuals.

For example, the process operator will require access to the operator interface unit to control the plant, and he/she will need both the relevant area password and a personal password. On no account can he/she be allowed to gain access to the process software. The next level of access may be the production management, who can access operator interface unit and also certain MIS elements. The extent to which specified individuals have access to the software will need to be defined. The site accountant should not normally require access to the operator interface unit, but will require access to the MIS for ingredient usage, recipe frequency, type of packaging and services utilisation. The engineering department will require access to the planned maintenance files and the services utilisation information, but, possibly, little more. The site manager may require access to all the above information, but will have restricted access to the software, whilst the system manager, who manages and maintains the whole system, will require access to every facet of the system including the software. Changes to software can only be made with his collaboration, and then only when the changes have been fully agreed and documented.

It is important also to remember that automation systems are site specific so that, while these general guidelines should give an indication of the advantages of automation, the introduction of a system needs specialist advice at every stage.
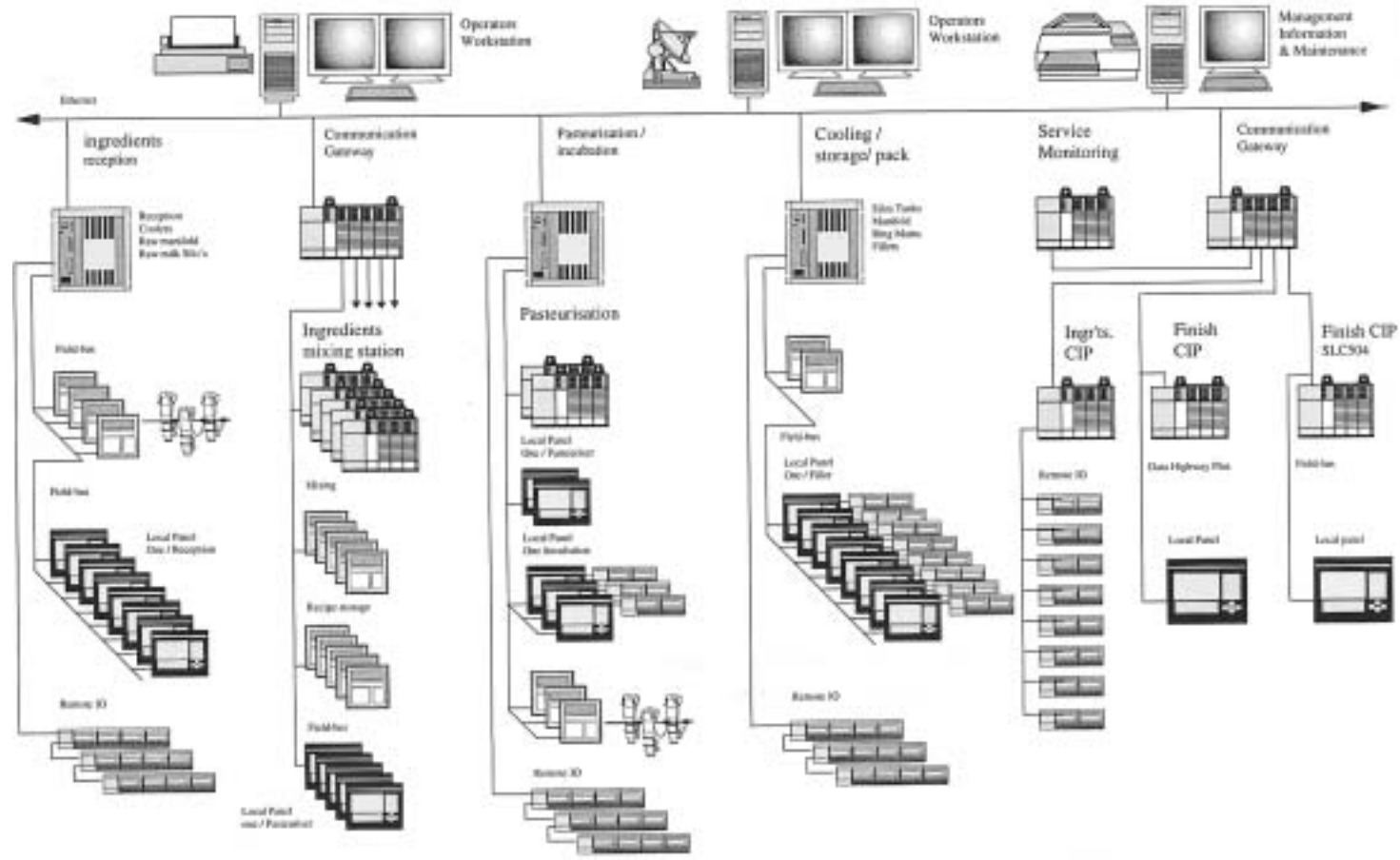
Fig. 3.72 Illustration of an automated system in a yoghurt process plant

Reproduced with courtesy of Tetra Pak (U.K.), London, U.K.

Fig. 3.73 Process control in a modern factory for the production of yoghurt and strained yoghurt

Reproduced by courtesy of Delta Dairy S.A., Athens, Greece.

Figure 3.73, for example, illustrates automation and process control for the production of yoghurt and strained yoghurt in a modern factory in Greece (see also Mortensen, 1995).

3.7 Building design, maintenance and services

3.7.1 General background and introduction

As mentioned elsewhere, the International Dairy Federation has published many documents on different aspects of dairy hygiene which include the manufacture of dairy products, processing equipment, cleaning and disinfection, storage and distribution. The latest document in this area is a manual that provides guidelines for *Hygienic Design and Maintenance of Dairy Buildings and Services* (IDF, 1997), and which includes recommendations for the plant designer of a factory that has hygienic barriers between raw materials and manufactured products. Similar manuals are also published by Campden & Chorleywood Food Research Association providing guidelines on the construction of ceilings, walls, floors and services for food production areas (Timperley, 1993, 1994) (see also Brolchain, 1993; Jolly, 1993). This approach ensures, therefore, that if a total quality management system (e.g. ISO 9000 and 9001) or hazard analysis critical control point (HACCP) system is being implemented, certification and inspection procedures for the premises are easily accommodated (see also Sowry, 1988; EU, 1992; Shapton and Shapton, 1994).

3.7.2 Location of a dairy plant

The different factors involved in locating a factory site are summarised in Table 3.7. Nevertheless, according to Timperley (1993, 1994) and IDF (1997), the layout of a dairy production unit is referred to as an operational layout which comprises dif-

Table 3.7 Summary of factors involved in selecting a location for building a new dairy plant

Main items	Sub-items
Positioning the dairy factory in the landscape	Topography Landscape Soil quality Foundation of the building
Climatic conditions	Sunshine Wind Precipitation in the form of rain or snow due to surrounding hills, vegetation and water areas Vegetation Floods Air humidity
Surrounding community	Adjacent industries Crop fields Water supply Power supply Utilisation of site area may be regulated by local authority
Society and environmental issues	Environmental legislation Public development plans Waste water disposal Noise, smoke and dust Availability of manpower
Milk supply	Delivered directly from farms and/or milk collection centre Minimise transportation time to maintain good microbiological quality of the raw milk
Preparation for extension	U-flow of production line provides: (a) space saving, and (b) extension possibilities in three directions, whilst a disadvantage makes it difficult for extension areas located innermost Straight line of production has the following advantages: (a) delivery and discharge are clearly separated, (b) easy to divide into zones (see text) to provide minimum hygiene risks, and (c) extension of most functions is only possible in two directions; the only disadvantage is that more floor area is required

Adapted from IDF (1997).

ferent departments or units. For example, in a yoghurt factory the layout consists of: (a) milk and ingredients reception, (b) milk preparation, (c) yoghurt production (including starter culture preparation/handling), (d) product packaging, (e) materials and stores, (f) cold stores, and (g) quality control laboratories. This type of layout ensures that the following aspects can be taken into consideration:

- Different but related products can be manufactured on the same site
- Equipment is expensive and stationary
- Processing times vary according to the operation
- Volumes of product sales vary.

Taking these aspects into account, it is recommended that the layout team consist of a manager, a technologist, a microbiologist, an architect and a plant operations expert and/or engineer. Furthermore, in any dairy layout where milk reception and processing take place within one enclosure or building, it is advisable that the reception and processing areas are separated. In general, the systematic approach to the design of any dairy building constitutes the "10 steps" procedure as detailed by IDF (1997).

3.7.3 Layout of a dairy plant

If a linear flow scheme has been chosen for the manufacture of yoghurt, a key aspect in hygiene design is the division of the factory into risk zones which are identified as follows:

- *Green zones* – These are areas where there is no risk of contaminating manufactured products, or areas where contamination is of minor importance. Some examples of these areas include raw milk reception, cleaning facilities for returnable containers, toilets, CIP equipment and power generators; however, these areas should be isolated from each other and in particular CIP of raw milk equipment from the rest of processing plants, toilets from raw milk reception and wet areas from dry areas.
- *Yellow zones* – These zones are regarded as areas where microbiological preventative measures are carried out. In these areas the risk of exposing the product to a contaminated environment is limited, but they are located or border a high risk red zone. Examples of yellow zones in a yoghurt factory are the store for packaging materials, laboratories, and milk processing area(s); wet and dry areas should be also separated in this zone.
- *Red zone* – In this area the strictest hygiene is required to minimise the risk of contaminating the product from the environment (e.g. air, machinery and equipment, pipes, rooms, drains and/or personnel). These areas in a yoghurt factory are identified as the bulk starter production area, yoghurt incubation tanks, processed fruit/flavours handling and yoghurt filling or packaging.

In the yellow and red zones, the following aspects should be included as criteria for verification by HACCP:

- buildings
- flow of product(s)
- personnel.

3.7.4 Design and construction of dairy buildings

Based on past experience within the dairy industry, the materials used for construction have met all criteria of durability and cleanability; however, during the construction of the wall boarding into a wall system, the hygienic conditions have been difficult to achieve at such interfaces (Timperley, 1993, 1994; IDF, 1997). However, the hygiene risk is not from the actual building structure(s), but from contamination from outside sources entering the building by various mechanisms.

In principle, the dairy building should provide a safe environment. It should:

- Protect the processing environment from extraneous matter and contamination by micro-organisms
- Provide a safe and pleasant environment and protect workers from the external environment
- Be cost effective with minimum maintenance

To achieve these aims, the building must be large enough to allow ready access between the building fabric and the equipment and sufficient space between individual items of equipment. The design of the building should provide that first, the positioning of the equipment and location of services should be away from walls in order to allow easy access for maintenance of the building, and second, the servicing, CIP and maintenance of the processing equipment should not have any detrimental effect on the fabric of the building.

Recommendations for dairy buildings have been given in detail by IDF (1997), including diagrammatic illustrations showing adequate or preferred designs in contrast to structures to be avoided. A summary of some of these recommendations might include the following.

3.7.4.1 Number of storeys
If possible, the processing area should be designed on one level, since stairs in a multilevel building are difficult to clean and permit liquids to transfer from one area to another; in a multistorey dairy, retain the main processing operations on one level.

3.7.4.2 Roofs
The roof should be fully sealed against water, rodents and birds, and some examples are: (a) if flashing is used to provide a seal, it should not form cavities, (b) the ridge points or changes in direction should be flashed, (c) the roof should be self draining towards the gutters to prevent the occurrence of ponding, (d) the roof should be sealed to the walls to prevent the backflow of water into the building, (e) exhaust fans or refrigeration plant should be mounted well clear of the roof surface to allow for run-off of liquids and space for cleaning under the equipment, and (f) cracks should be sealed and, if coating or sealant materials are used, they should be resistant to chemicals (e.g. acids or CIP vapours) and ultraviolet rays. In addition, a roof pitch >10° eliminates the possibility of liquid ingress through the joints of tiles or other covering.

3.7.4.3 Gutters
Locate the gutters beyond the wall claddings and extend the roof part way down the wall before termination in the gutter in order to minimise the ingress of contaminants as a result of changing wind pressures on the walls and roof. Avoid using internal gutters because contaminants may enter the building due to blockages.

3.7.4.4 Ceilings
Ceilings should provide a barrier against dust and moisture. Some recommendations for construction are: (a) joints (i.e. on the upper and lower surfaces of the ceiling lining) should be adequately sealed, (b) ensure minimum 10° slope and proper insulation to reduce condensation, (c) provide purpose-made flashing to reduce the incidence of cracks due to thermal expansion, (d) the underside of the ceiling should be smooth, and (e) the cavity between roof and ceiling should be

accessible from outside the processing area, otherwise the access from inside should be designed with an airlock system.

3.7.4.5 Walls

Both internal and external walls should be designed and constructed to prevent the ingress of contamination, to protect against vermin, to be insulated and to ensure an absence of cracks. If sheet wall-cladding materials are used, they should be sealed at all joints and laps. It is generally recommended that (a) exposed structural membrane should be flashed and sloped to provide free draining and prevent roosting, (b) the voids in the cores of concrete blocks should be filled, otherwise cracks in the mortar joint could be a source of contamination, (c) inner surfaces should be coated with a flexible membrane coating which can be easily cleaned, and (d) mortar joints should have a 12 mm radius for easy cleaning rather than being straight.

Surface finishes of internal walls should be of materials that prevent blistering and mould growth, are resistant to milk, acids and CIP chemicals and are easily cleaned.

3.7.4.6 Access

Windows and doors (i.e. internal and external), airlocks and removable panels should be properly constructed. Some illustrations of preferred structures have been provided by IDF (1997).

3.7.4.7 Floors

Floors should be constructed to withstand heavy loads and vibration from equipment, be properly sealed and provide adequate drainage. Floor finishing materials are critical and should be easily cleaned, withstand CIP solutions including acids and be non-slippery. Epoxy resins, for example, are widely used. For further details the reader should consult Cattell (1988), Jackson (1997) and Weatherburn (1997).

3.7.4.8 Services

These include electrical wiring, ventilation ducting, drains, lighting, pressure-relief ducting, decks and platforms, stairs and piping. Specifications for such installations have been reported by IDF (1997).

3.8 Conclusion

It is evident that a multitude of factors can influence the rheological properties of yoghurt (see Chapter 2) including the mechanical handling of the coagulum (i.e. factors discussed in this chapter). Shear stress can reduce the viscosity/consistency of yoghurt, but the phenomena associated with improved firmness of the product after 24 hours storage at $<5°C$ are still not well established (see IDF, 1998). Hence, it is possible to suggest that future developments in yoghurt science and technology may include:

- Greater understanding of the physical behaviour of the coagulum after being subjected to shear stress and cooling.

- Improved milk solids formulations of the milk base (e.g. combination of SMP and WPC) and possible homogenising of the milk after heat treatment rather than before at 60–70°C.
- Provision of wider microbial blends of the starter culture to meet the requirements of the consumer and enhance the functional characteristics of yoghurt and its related products.
- Greater reliance on automation especially in large centralised factories where yoghurt is produced and improved on-line testing and monitoring of the product(s) during manufacture.
- Resurrection of the NIZO process for yoghurt production using the continuous method.

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4

Plant cleaning, hygiene and effluent treatment

Cleaning aspects

4.1   Primary objectives

The keeping quality of yoghurt is governed by a multiplicity of interrelated factors such as:

- The hygienic quality of the product which, in turn, is dependent on the effective heat treatment of the milk base, the purity of the starter culture, the microbiological quality of added fruit/flavours and other ingredients and the care which is exercised during storage, handling and distribution of the yoghurt (see Chapter 10).
- The cleanliness of surfaces coming into contact with the yoghurt, e.g. processing equipment, filling machines and packaging materials.
- Miscellaneous, i.e. the hygienic manufacture of dairy products, the attitudes of the dairy personnel and the hygienic condition of the buildings/building site (IDF, 1980, 1984, 1985, 1987, 1991, 1992, 1994, 1996, 1997a,b; Wainess, 1982).

Factors related to some aspects mentioned above are discussed elsewhere, so that to achieve the primary objective, that is, an excellent yoghurt with good keeping quality, the remaining essential factor is the provision of hygienic processing equipment and packaging materials. The nature of contaminants from surfaces coming into contact with any food product, including yoghurt, could be physical, chemical or biological, and contamination from these sources can be minimised by the following approach (Swartling, 1959; Dunsmore *et al.,* 1981a, b; Dunsmore, 1983):

- removal of residues (milk, yoghurt and other additives) which can provide nutrients for micro-organisms remaining on the surfaces of equipment;
- cleaning and sanitisation/sterilisation of equipment by removal and destruction of micro-organisms which survived the removal of residues step;

Table 4.1 Soil characteristics of a yoghurt plant

Soil component on the surface to be cleaned	Solubility in water	Alkaline	Acids	Ease of removal during cleaning		Comments
				Without alteration by heat[a]	Effect of alteration by heat[b]	
I. Dairy						
Lactose	G	–	–	Good	Carmelisation/browning; more difficult to clean	Unlikely to take place during heat treatment of yoghurt milk
Fat	P (in solutions without surface active agents)	P	P	Good with surface active solutions	Polymerisation; more difficult to clean	Unlikely to take place during heat treatment of yoghurt milk since most types of yoghurt produced in the U.K. are low fat varieties; this condition may not arise
Protein	P	G	Av.	Poor with water; better with alkaline solutions	Denaturation; difficult to clean	This effect is most likely to take place during prolonged heating of milk, e.g. HTLT (see Table 2.15) or preparation of starter culture milk
Mineral salts	G-P	–	G	Reasonably good	Precipitation; difficult to clean	This effect is most likely to take place during prolonged heating of milk, e.g. HTLT (see Table 2.15) or preparation of starter culture milk

II.	*Dairy additives*					
	Sweetening agent	G	–	–	Good	Caramelisation/browning; more difficult to clean	Condition may arise in the case of lactose hydrolysed milk heated at 85–90°C for >45 min, or if a high percentage of sugar is added to the milk base before heat treatment
	Fruit	G-P	–	–	Good	Caramelisation/browning; more difficult to clean	This effect takes place in dairies that pasteurise their own fruit base before addition to yoghurt
	Colouring or flavouring matter	G	–	–	Good	NA	
	Stabilisers	c			c	NA	

(G) good; (Av) average: (P) poor; (NA) not applicable. HTLT, high temperature long time.

[a] Possible places of identification are: milk reception area, preparation of basic mix, yoghurt incubation tanks and/or yoghurt filling section. [b] Possible places of identification are: heat treatment section, multipurpose tanks, fruit processing equipment and/or bulk starter tanks. [c] Refer to text in Chapter 2.

Adapted from IDF (1979), Tamplin (1980) and Romney (1990).

- storage of equipment under conditions that limit microbial growth/survival when the equipment is not in use;
- removal of residual cleaning compounds which may contaminate the yoghurt.

The efficiency of plant hygiene/sanitation is, therefore, dependent on the performance of the cleaning and sanitation/sterilisation operations.

In the commercial situation, cleaning is the removal of yoghurt "soil" (Table 4.1) from the surface of the processing equipment, and this is followed by sanitation/sterilisation, that is, the destruction of most (sanitation) or all (sterilisation) of the residual micro-organisms; these aspects will be discussed in relation to the type of equipment used in the production of yoghurt and the degree of automation.

4.2   Principles of the cleaning process

The processing of milk during the manufacture of yoghurt forms different types of soiling matter on the surfaces of equipment (Table 4.1) and this soil consists of organic compounds (e.g. protein, fat, lactose and other non-dairy ingredients) and inorganic salts. The degree of deposition of the soil on the processing surfaces is governed by many factors, but is directly proportional to the amount of heat applied, which results in more denaturation of the milk proteins and more precipitation of the organic salts (from milk and water). Hence, soil resulting from the heating of milk is more difficult to remove than soiling matter from unheated milk. The factors that can affect fouling of processing equipment, including the cleaning of fouled surfaces have been reported by Grandison (1988), Fryer (1989), Bott (1990), Kessler and Lund (1990), de Jong *et al*. (1992), Jeurnink and Birkman (1994), Kastanas *et al*. (1995), Fryer *et al*. (1996), IDF (1997c), Tuthill *et al*. (1997) and Parchal (1997).

It is important that the processing equipment, including the pipelines, is properly emptied from yoghurt residues before commencing the cleaning programme. This approach ensures:

- reduced product losses (i.e. recovery >90%),
- minimum cleaning cycles,
- minimum milk solids discharge in effluents (i.e. environmental pollution) and reduce effluent costs,
- reduced costs of detergents/sterilisers and improved cleaning efficiency.

Recovery of the product from the process plant can be achieved by purging water through the installation, but this may lead to dilution of the yoghurt. Alternatively, purging a scraper through an automated plant before the cleaning cycle can achieve the same advantages. One example is the "pig" pipe scraper which is marketed by the Tuchenhagen company in Germany (Anon., 1993). The pig itself is made from an inert flexible moulding material that is wear resistant and compatible with both the product and the cleaning chemicals. The leading and trailing edges of the pig are equipped with scrapers that nest closely to the inside diameter of the pipe and, as a result, provide maximum scraping efficiency. A permanent magnet is moulded into the core of the pig and as the flux extends beyond the pipe wall, its location can be detected.

At either end of the process, a station is provided for the launch and recovery of the pig. The launching station has one air inlet and one exhaust valve situated behind

the normal parking position. In addition, the pig has a special cleaning location where it is retained by four pins. After removing the yoghurt from the pipelines, the cleaning fluid is introduced and the pins ensure turbulent distribution around the pig, thus leaving no dead pockets to harbour bacteria. The system is available with one or two pig scrapers for uni- or bidirectional use, respectively. Figure 4.1 illustrates the concept in more detail (see Bird, 1996).

The cleaning process necessitates the use of certain compounds referred to as detergents, which are available in liquid or powder forms. The basic functions of the detergents are:

- establishing intimate contact with the soiling matter through their wetting and/or penetrating properties;
- displacement of the soil, for example: by melting/emulsifying the fat by wetting, soaking, penetrating and peptising the proteins and by dissolving the mineral salts;
- dispersion or displacement of the undissolved soil by defloculation and/or emulsification;
- preventing redeposition of the soil by maintaining the properties of the above factors, and by ensuring good rinsing;
- miscellaneous, i.e. to be non-corrosive, to have no odour nor taste, to be non-toxic and non-irritable to skin.

In order to achieve the above properties/functions of a detergent different formulations are used. Table 4.2 illustrates some compounds, and their properties, that can be employed in the manufacture of a proprietary detergent.

1 Drain
2 Sanitation Purge valve
3 Pig divert valve
4 Tank valve
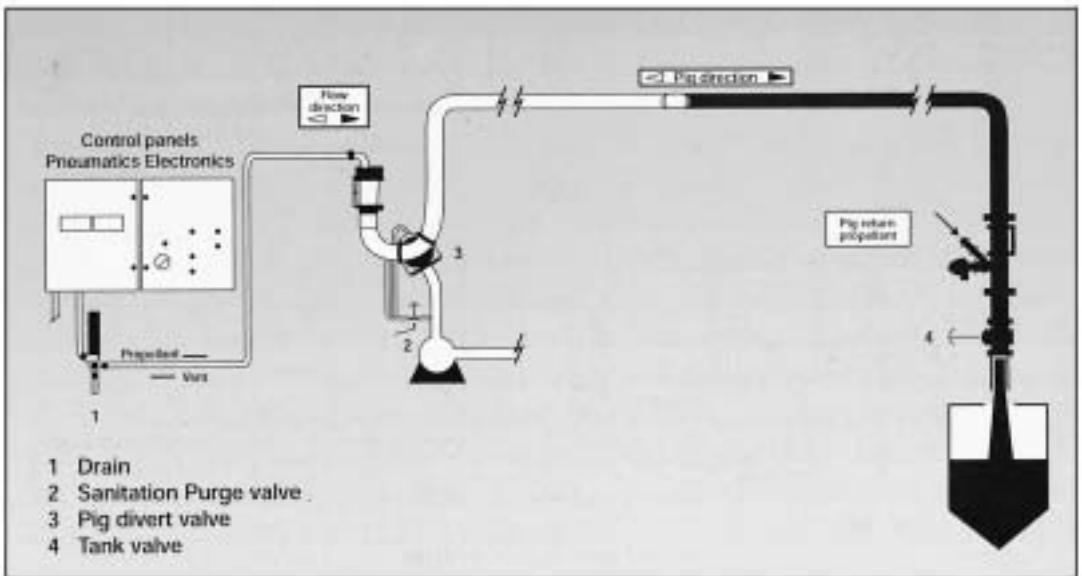
Fig. 4.1 "Pigging" system that reduces wastage during start-ups, shut-downs and product changeovers

Reproduced by courtesy of APV U.K. Co. Ltd., West Sussex, U.K.

Table 4.2 Functional properties and characteristics of detergent constituents

Type	Detergent components		I	II	III	IV	V	VI	VII	General comments
Inorganic alkalis	1. Sodium hydroxide		E	P	P	P			E	These compounds can affect degree of alkalinity, buffering action and rinsing power of a detergent.
	2. Sodium orthosilicate		G	F	F	P			G	
	3. Sodium metasilicate		G	G-P	VG	G			F	For high alkalinity preparations use alkalis (1) and (2) which can cause skin irritation; therefore, handle them with care.
	4. Trisodium phosphate		F	F-P	VG	G			F	
	5. Sodium carbonate		F	P	P	P			P	For removing heavy soil, alkalis (2), (3) and (4) are very effective.
	6. Sodium bicarbonate		G	P	G	F			F	For low alkalinity, i.e. mild or hand detergents, use alkalis (5) and (6).
Acids	Inorganic	Nitric acid								Acids are normally used for the removal of tenacious soil, e.g. in UHT plants.
		Phosphoric acid								
		Sulphuric acid								These materials are corrosive and can cause severe skin burns; therefore handle them with care, and if incorporated in a detergent formulation they may have to be used with corrosion inhibitors.
	Organic	Hydroxy acetic acid	G			G				
		Gluconic acid								
		Citric acid								
Surface-active agents	Anionic	Sodium alkyl aryl sulphonate								The classification is dependent upon how these compounds dissociate in aqueous solution, e.g. surface-active anions, cations, etc.
		Sodium primary alkyl sulphate								Some of these compounds are also used as emulsifying agents.
		Sodium alkyl ether sulphate		E	E	E				Non-ionic agents do not ionise in solution.
	Non-ionic	Polyethenory compounds								
	Cationic	Quaternary ammonium compounds (QAC)	(see sterilising agents below)							Surfactants tend to reduce surface tension of the aqueous medium and promote good liquid/soil/surface interfaces.
	Amphoteric	Alkyamino carboxylic								

Sequestering and chelating agents										Notes
Sequestering and chelating agents	Sodium polyphosphates	F	P	G	F	G				They prevent water hardness precipitation, are heat stable and are used for formulation of combined detergent/steriliser compounds.
	Ethylenediamine tetra acetic acid (EDTA) and its salts			VG	E	E	E	G		Their inclusion in formulations is to "hold" calcium ions in alkali solution and prevent reprecipitation.
	Gluconic acid and its salts					E	E			The bacteriostatic property of EDTA is achieved by withdrawing trace metals from bacterial cellular membranes. Gluconic acid is a stronger chelating agent than EDTA in alkali solutions (2–5% strength).
Sterilising agents	Chlorine — Chlorinated trisodium orthophosphate							E		Their inclusion provides a balanced product for cleaning (i.e. detergent) and sterilisation (e.g. hypochlorous acid, QAC, iodine or peroxide).
	Chlorine — Dichlorodimethyl hydration							E		Consult list of brands, approved by the authorities concerned, that can be used as detergent/sterilisers as an alternative to steam or boiling water for the sterilisation of dairy equipment.
	Chlorine — Sodium dichloro-isocyanurate									
	Chlorine — Sodium hypochlorite							E		
	QAC — Cetyl trimethyl ammonium bromide			VG	VG	VG		E		
	QAC — Benzalkonium chloride			VG	VG	VG		E		
	Iodine — Iodophors	G	G			G		E		
	Peroxide — Peracetic acid									
	Peroxide — Hydrogen peroxide									
Miscellaneous inhibitors	Sodium sulphite									These inhibitors minimise corrosive attacks by acids and alkalis on metal. The sulphites protect tinned surfaces, and silicates protect aluminium and its alloys from attack by alkalis.
	Sodium silicate									

Table 4.2 *Continued*

Type	Detergent components	I	II	III	IV	V	VI	VII	General comments
Antifoaming agents									Antifoaming agents are sometimes incorporated in a detergent formulation to prevent foam formation which could be generated by pumping/jetting action during detergent recirculation. Fats and alkalis may form soaps by saponification and these antifoaming agents prevent foam formation.
Suspending agents									Sodium carboxymethyl cellulose or starch assist in maintaining undissolved soiling matter in suspension, thus referred to as suspending agents.
Phosphates	{ Orthophosphates			G		G	G		Some of the polyphosphate compounds hydrolyse to orthophosphates in aqueous solution at high temperature, but presence of alkalis reduces the rate of hydrolysis.
	{ Polyphosphates			G		G	G		
Water softening									Precipitation of calcium and magnesium ions from hard water in order to avoid water-scale deposition on surfaces of equipment especially for the last rinsing step after cleaning.

(E) Excellent, (VG) Very good, (G) Good, (F) Fair, (P) Poor.
I, Organic dissolving; II, wetting; III, dispensing suspending; IV, rinsing; V, sequestering; VI, chelating; VII, bacteriocidal.
Data compiled from IDF (1979), Tamplin (1980), BSI (1970, 1977, 1984).

4.3 Factors involved in the selection and performance of a detergent

There are many different types of detergent available on the market and most of them have been developed for a specialised cleaning purpose.

4.3.1 Type/range of detergents used in the yoghurt industry

Different types of processing equipment are used during manufacture and the type of detergent is chosen in relation to its cleaning function, such as:

- *Mild detergents* are employed for manual washing operations.
- *Combined mild detergents/sterilisers* are similar to the detergent mentioned above, but with improved properties of sanitation.
- *Detergents for cleaning-in-place (CIP)* are extensive in number and they are divided into two basic categories, where no heating is applied and where heating is involved.

  Examples of milk processing equipment where no heating is applied are:
  - milk reception area
  - storage tanks and silos
  - equipment used for preparation of the milk base
  - incubation tanks
  - plate/tubular coolers
  - intermediate yoghurt tanks
  - filling machines
  - ultrafiltration (UF) or reverse osmosis (RO) plants

  Examples of cleaning equipment involved in the heat treatment of milk are:
  - heat exchangers
  - evaporators
  - bulk starter culture tanks
  - equipment for processing fruit.
- *Bulk liquid detergents* are similar to those mentioned above and are normally used by large dairies using automatically controlled CIP systems. They are in liquid form, since it is easier to dispense liquid into the cleaning cycle and control the concentration of the detergent.
- *Detergents for bottle washers* in the yoghurt industry are very limited, since most products are packaged in single-trip containers.
- *Detergents for churn washers* are used to protect certain metals (e.g. aluminium) since they tend to reduce the problem of corrosion and/or oxidation (i.e. dark or black discoloration of the aluminium surface).

Hence the choice of a detergent for a specific cleaning purpose and/or particular item of yoghurt processing equipment is directly related to its functional properties. Some suggested formulations are shown in Table 4.3.

4.3.2 Type of soiling matter

The soiling matter produced during the manufacture of yoghurt (Table 4.1) may be of two types, a soil which is easy to remove (for example milk and yoghurt) and a more difficult type of soil (for example, heated milk and/or fruit). It is obvious that

Table 4.3 Some examples of suggested detergent formulations (composition %)

Cleaning duty	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Bottle soak		14		68		6			8	4					
Bottle washer				95	5										
Dairy equipment cleaner (manual)						10	51	25				10		4[a]	
Milk can washing (machine)		20				12	26	40						2	
Milk can washing						10	51	25				10		4	
Pipeline cleaner		30				10	32	25						3	
Heavy duty CIP				95	5										
Dairy cleaner		7	18			8		15		46				6	
Vat cleaner	6	6						20		30	38				
Acid descaler													30	0.3	69.7
Acid cleaner												2	26		72
Acid cleaner (milkstone remover)													50	10	40

1, Sodium bicarbonate; 2, sodium carbonate; 3, sodium chloride; 4, sodium hydroxide; 5, sodium gluconate; 6, sodium metasilicate; 7, sodium sulphate; 8, sodium tripolyphosphate; 9, tetrasodium pyrophosphate; 10, trisodium phosphate; 11, metasilicate (crystals); 12, dodecylbenzene sodium sulphonate (LAS is 50% acidic); 13, phosphoric acid; 14, surfactant; 15, water.

[a] Non-ionic.

Data compiled from Cutler and Davis (1972), IDF (1979) and Tamplin (1980).

the choice of certain compounds to be incorporated into a detergent must take heed of the nature of these differing residues.

4.3.3 Water hardness and quality

Water is used during all the cleaning cycles in a processing plant and it is essential that two factors are considered. First, good quality potable water must be used (Table 4.4 illustrates some suggested standards for chemical specification and bacteriological quality), and second, the degree of hardness must be taken into account. This latter aspect is important, since detergents are formulated in relation to the degree of water hardness and the presence of excess inorganic salts, mainly calcium and magnesium, can reduce their effectiveness. In addition, these salts can leave deposits on the surfaces of equipment which are difficult to remove.

Water hardness may be of two types: temporary or permanent.

Temporary or carbonate hardness is due to the carbonates and bicarbonates of calcium and magnesium. These salts are easily precipitated or removed by heating; a typical example is scale formation on the inside of a kettle. In a yoghurt plant the same situation may arise in the evaporator and heat exchangers, since these sections are normally sterilised by circulating hot water (e.g. 85°C for 30–45 min). Deposits of calcium and magnesium salts on the surfaces of such equipment not only reduce the overall heat transfer efficiency of the plant, but can also provide a nucleus for other soil depositions to take place.

Permanent or non-carbonate hardness is due to formation of other types of calcium and magnesium salts (e.g. sulphates and chlorides). Their conversion into insoluble deposits is due to the presence of certain alkalis and, for this reason, specific constituents are incorporated into a detergent to minimise the precipitation.

Table 4.4 Some suggested chemical and bacteriological standards for water used in food processing plants

Chemical specifications		Bacteriological standards	
Total hardness (as CaCO$_3$)	≤50 µg g^{-1}	(1)	Throughout any year, 95% of samples should not contain any coliform organisms or *Escherchia coli* in 100 ml.
Chloride (as NaCl)	≤50 µg g^{-1}	(2)	No sample should contain more than 10 coliform organisms per 100 ml.
Chloride (elementary)	≤1 µg g^{-1}	(3)	No sample should contain more than 2 cells of *E. coli* per 100 ml.
pH	6.5–7.5	(4)	No sample should contain >1 or 2 cells of *E. coli* per 100 ml in conjunction with a total coliform count of 3 or more per 100 ml.
Iron (as Fe)	1 µg g^{-1}	(5)	Coliform organisms should not be detectable in 100 ml of any two consecutive samples.
Manganese (as Mn)	0.5 µg g^{-1}		
Suspended solids	Substantially free		

After Anon. (1969) and IDF (1979). Reprinted with permission of HMSO, London and International Dairy Federation, Brussels, respectively.

Table 4.5 Units for hardness of water and equivalent in degrees of hardness

Units	Earth alkali ions m val l^{-1}	Equipment in degrees of hardness			
		German°	English°	French°	US°
1 m val l^{-1} of alkali earth ions	1.00	2.80	3.5	5.0	50.0
German 1° = 10 mg CaO l^{-1} or 7.19 mg MgO l^{-1}	0.38	1.00	1.3	1.8	17.9
English 1° = 10 mg CaCO$_3$ 0.7 l^{-1}	0.27	0.80	1.0	1.4	14.3
French 1° = 10 mg CaCO$_3$ l^{-1}	0.20	0.56	0.7	1.0	10.0
US 1° = 1 mg CaCO$_3$ kg^{-1}	0.02	0.06	0.1	0.1	1.0

The expression val l^{-1} (g equivalent l^{-1}) is an alternative for equivalent weight l^{-1}, so that m val l^{-1} ≅ m EW l^{-1}; e.g. if the EW l^{-1} of CaCO$_3$ = 50 g l^{-1}, an m val l^{-1} = 0.05 g l^{-1}.
After IDF (1979). Reprinted with permission of International Dairy Federation, Brussels, Belgium.

The degree of water hardness is a measure of the mass of dissolved calcium and magnesium salts in the water, and according to Anon. (1967) and IDF (1979), the United States Geological survey classified water supplies as soft, moderately hard, hard and very hard if the total hardness (expressed as 1° = 1 mg CaCO$_3$ kg^{-1}; see Table 4.5) was 0–60, 60–120, 120–180 and over 180, respectively. However, water hardness is sometimes expressed in different terms/units/degrees in different countries, and Table 4.5 gives a comparison of the units used in Germany, the United Kingdom, France and the United States.

4.3.4 Miscellaneous factors

The formulation of a dairy detergent is also influenced by such factors as method of cleaning adopted and the materials used for the construction of the equipment, plant and other utensils; use of materials is discussed in Chapter 3.

4.4 Cleaning methods

The cleaning of any part of a yoghurt processing plant may involve one of the following methods: manual cleaning, cleaning-in-place (CIP) and miscellaneous cleaning methods. The basic steps involved in any of the above methods are somewhat similar. In principle they consist of the following operations.

In the *preliminary rinse* the processing plant, including starter culture equipment, filling machines and churns are rinsed with water to remove the bulk of the milk residues, yoghurt and/or fruit from the equipment. For conservation purposes, the final rinse (see below) is recovered, especially in large plants and used for this preliminary rinse.

In the *detergent wash* alkali compounds are usually used (refer to Tables 4.2 and 4.3 for specific applications) and during this stage the aim is to remove any adhering soil.

The *intermediate rinse* is to remove any detergent residues from the equipment prior to the operations acid wash and/or sterilisation/sanitation) that follow.

The *acid wash* cleaning operation is optional and may be performed only once a week to clean the heat processing equipment. It is important to point out that acids are harmful to the skin and hence an acid wash is normally used in CIP. Inorganic (nitric and phosphoric) and/or organic (acetic, gluconic, oxyacetic) acids may be used, since they have the ability to dissolve milkstone and remove hard water scale. Although phosphoric is only a medium-strong acid, both mineral acids are corrosive to certain metals (e.g. tinned steel). However, the organic acids do not pose the same problem, even at high concentrations. Nitric and other acids have good sanitising properties (Zall, 1990; Dunsmore, 1981; Dunsmore and Thompson, 1981; Lück *et al.*, 1981; Dunsmore *et al.*, 1981a, b; Wei *et al.*, 1985; Lück and Gavron, 1990; Wildbret and Sauerer, 1990).

The *intermediate rinse* is to remove any acid residues from the equipment prior to the sterilisation/sanitation treatment.

Sterilisation/sanitation treatment of the plant and processing utensils must be effected before commencing production and this aim is achieved using one of the following:

- nitric acid
- chemical compounds (QAC, chlorine, chloramine to achieve sanitisation)
- heat (live steam is limited in its application, but hot water circulation at 85–90°C for 15–30 min is a valuable procedure; the temperature must be maintained on the return side of the plant and at the product outlet points) can produce sterile plant
- miscellaneous (refer to section on sterilisation).

In the *final rinse* good quality potable water is used to remove the sterilant residues from the processing plant. If hot water circulation is used for sterilisation, this stage

is obviously omitted, but the plant must be properly drained before production commences.

4.4.1 Manual cleaning

Some parts of a processing plant (e.g. utensils and filling machines) can only be cleaned by hand, while others, such as homogenisers and separators, if not designed to be cleaned-in-place, have to be dismantled and cleaned-out-of-place (COP) as indicated by Custer (1982). The sequence of hand cleaning is as follows: (a) disconnect and dismantle the equipment, (b) prerinse with potable tepid water at around 20–30°C, (c) prepare the mild/hand detergent solution at the appropriate concentration in water at 40–50°C, (d) brush/wash the various parts, (e) intermediate rinse with tap water, (f) sterilise using chemical agent, and (g) final rinse with water.

Factors which may influence the results of hand cleaning are:

- the human element which may manifest itself in the form of low detergent concentrations or inefficient scrubbing action;
- the temperature of the detergent solution may not be high enough;
- since chemical sterilisation is dependent on concentration and contact time, operators may overlook one or other of these factors.

Proper management, supervision and personnel training can all help to achieve the desired aims and discussion with the detergent manufacturer can also ensure that correct cleaning procedures are introduced.

Manual cleaning can also be improved by providing a cleaning-out-of-place (COP) tank, so that the cleaning operations are: (a) place the dismantled and prerinsed parts in the tank, (b) fill the tank with hot water, (c) add the correct amount of detergent, and circulate the hot detergent solution for up to 30 min, (d) drain detergent to waste or collect for other cleaning purposes, (e) rinse parts with continuous circulation of mains water, and (f) drain and sanitise/sterilise by submerging all parts in hot water or chemical sterilant.

The COP method could also be used for cleaning pipelines in a small dairy, or in those parts of a factory where it may be difficult to provide a proper CIP system.

4.4.2 Cleaning-in-place

This system of cleaning is engineered to clean processing equipment without dismantling and reassembling the different units and, in addition to minimising manual operations, the CIP system has proved beneficial in respect of:

- improved hygiene, possibly through a combination of the chemical action of the detergent and the physical action of the circulating solution(s);
- better plant utilisation;
- increased savings (of detergent, steam and sterilising agents);
- greater safety.

In order to make use of a CIP system, it is essential to have a closed circuit through which the cleaning solution(s) can be circulated. A typical basic unit is illustrated in Fig. 4.2. The design of any CIP system is tailormade for a specific cleaning objective, but the principal methods of CIP cleaning are classified into three basic
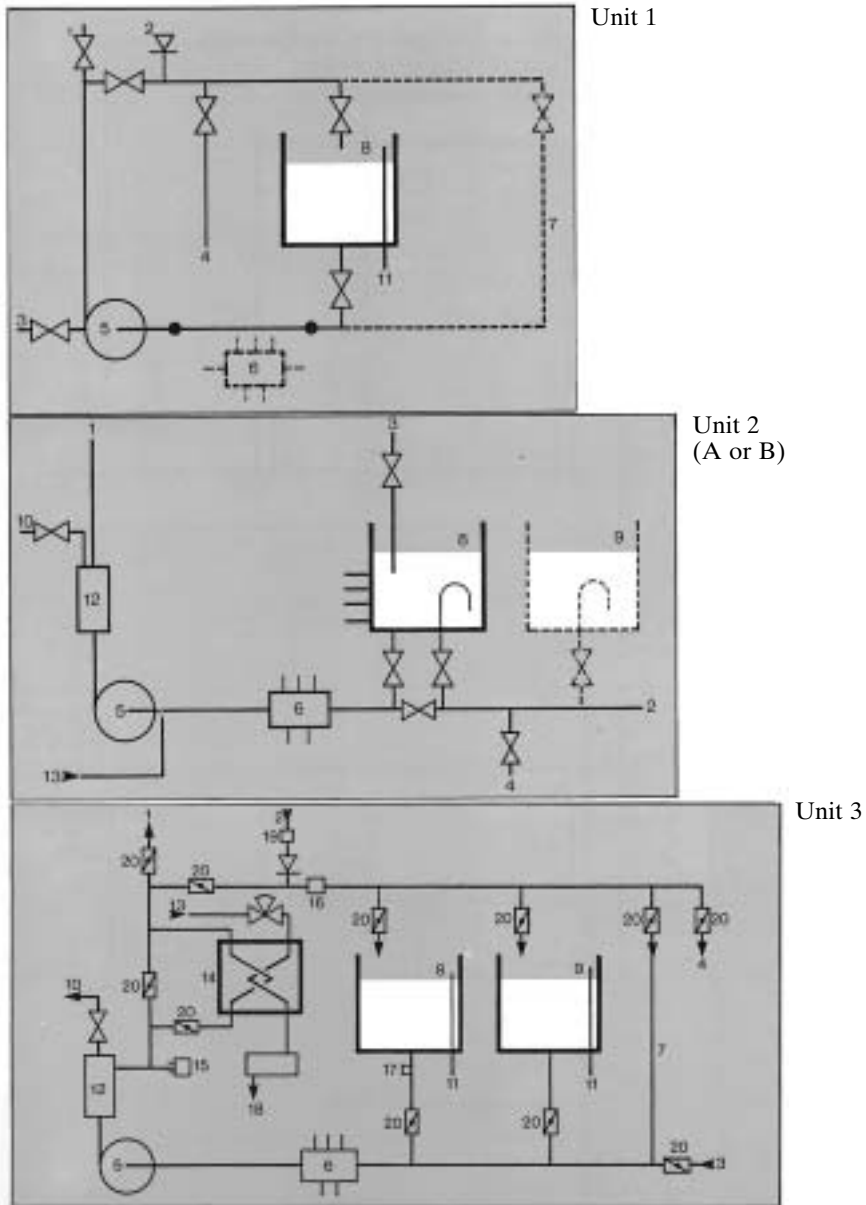
Fig. 4.2 Schematic illustration of APV Paraclean CIP systems

Unit 1, basic model; Unit 2, (A) single use package or (B) limited recovery option; Unit 3, multi-use system

1, CIP feed; 2, CIP return; 3, water inlet; 4, drain; 5, puma pump; 6, injection sleeve; 7, recirculating loop; 8, detergent tank; 9, water recovery; 10, sample cock; 11. overflow; 12, filter; 13, steam in; 14, paraflow heat exchanger; 15, temperature probe; 16, soluvisor; 17, conductivity probe; 18, condensate; 19, no-flow probe; 20, butterfly valve.

After Anon. (1997). Reproduced by courtesy of APV U.K. Co. Ltd., West Sussex, U.K.

systems: the single-use system, the re-use system and a combination of the two systems known as the multi-use system.

4.4.2.1 Single-use system

Unit 1 (Fig. 4.2) is basically small and is normally situated as close as possible to the equipment being cleaned. In a single-use cleaning system, the detergent is only used once and the washing solution is run to waste; this system is ideal for small plants.

The disposal of the detergent solution could be a disadvantage, especially if the strength and the functional properties of the solution are still available; however, after cleaning heavily soiled equipment in a large plant, it is the normal practice to discard the detergent solution after use because it has lost its strength. Such a system could be employed in a yoghurt plant, for example, for the cleaning of the bulk starter vats and/or multipurpose yoghurt tanks.

Figure 4.2 (Unit 2A) illustrates the basic components and the overall principle of the single-use system. However, these units can be supplied for automatic or manual operation, and can be further modified, that is, with the addition of a recovery tank (e.g. dotted tank in Fig. 4.2; Unit 2B), so that the wash solution and water rinse are recovered for the next preliminary water rinse; it is then known as the single-use system with a limited recovery option.

4.4.2.2 Re-use system

In this system, the detergent and/or acid solutions are recovered and re-used as many times as possible, especially in parts of the yoghurt plant where the equipment is not heavily soiled, for example in the milk reception area, the fortification/standardisation tanks and/or the yoghurt fermentation tanks. Thus, the preliminary rinse of such equipment removes a high percentage of the soil and, as the detergent solution circulated during the wash cycle is not heavily polluted, it can be re-used many times.

The re-use CIP system can be described as having these essential components: the detergent (Lye) tank(s), acid tank, water tank, water recovery tank and heating system, all interconnected with a system of pipework fitted with CIP feeds and return pumps. The concentration of the acid and lye solutions is regulated via feeds from tanks containing the corresponding compounds in a concentrated form, and the unit is also fitted with neutralisation tanks in which the lye and/or acid solutions are neutralised prior to their disposal into the effluent system. Furthermore, Tamplin (1980) and Romney (1990) pointed out that water consumption in a re-use system can be optimised by providing a recirculation facility for the hot water and the use of a return water tank. The on-site application of this system may be modified so that a low concentration of lye solution (0.5–1.0% caustic) is used for cleaning cold milk handling equipment, yoghurt fermentation tanks and the interconnected pipelines, while another lye tank contains up to 2% caustic for circulation during the cleaning of the milk processing plant.

Tamplin (1980) also pointed out that in a dairy operating 15–20 individual cleaning circuits per day, this CIP system becomes more efficient if another CIP feed pump is incorporated, so that two circuits can be cleaned simultaneously. However, any extension of the re-use CIP system is limited, since the tank capacity is defined in advance by the circuit volume, temperature requirements and desired cleaning

programmes; the latter aspect is fully automated in most processing plants, and the cleaning circuits are operated from a remote control panel.

4.4.2.3   Multi-use system

This system of CIP cleaning attempts to combine all the most desirable features of the single and re-use systems. The system is illustrated in Fig. 4.2 (Unit 3), and has the following features: (a) automatically controlled programmes for maximum flexibility, (b) not all cleaning liquids and/or solutions need be included in every cleaning programme (i.e. modular adaptability), and (c) economic features are low running cost, low water consumption and minimum effluent discharge (see Barron, 1987, 1988; Stack, 1997).

It can be observed that any of the above three CIP systems could be used for cleaning the yoghurt processing equipment (see Fig. 4.3 and 4.4; Jørgensen, 1993; Lyons, 1997), but the final selection of any one CIP system is governed primarily by factors such as:

- capital available for investment,
- desired degree of automation,
- estimated volume of yoghurt to be produced,

and hence, the final design may well be something of a compromise.

4.4.3   Miscellaneous cleaning methods

Alternative cleaning methods can be applied to suit special purposes and some examples of these have been reported by Haverland (1981), Chamberlain (1983) and Potthoff *et al.* (1997).

4.4.3.1   Soaking

Processing equipment and/or fittings are immersed in a cleaning solution at high temperature and after a soaking period of 15–20 min, the equipment is cleaned manually or mechanically. Unfortunately, no information has been given regarding the composition of the soaking agent, but it is possible that effective cleaning relies heavily on a digestion of the soil followed by a scrubbing action.

4.4.3.2   Ultrasonic treatment

This method of cleaning is a recent development on the soaking method discussed above. The equipment, utensils and fittings are immersed in a cleaning solution and any soil is lifted from the surfaces by the scrubbing action of microscopic bubbles generated by high frequency vibrations.

4.4.3.3   Spray method

This method of cleaning is widely used in the industry and involves spraying hot water or steam onto equipment surfaces *in situ*. The cleaning solution is sprayed from special units (portable or fixed) and its function is to remove as much heavily soiled matter from processing equipment surfaces as possible, before they are cleaned using one of the conventional methods.

4.4.3.4   Enzyme-based treatment

This method of cleaning does not employ conventional strong solutions of alkaline and/or acid components, but uses enzymes, surfactants, a buffer and complexing
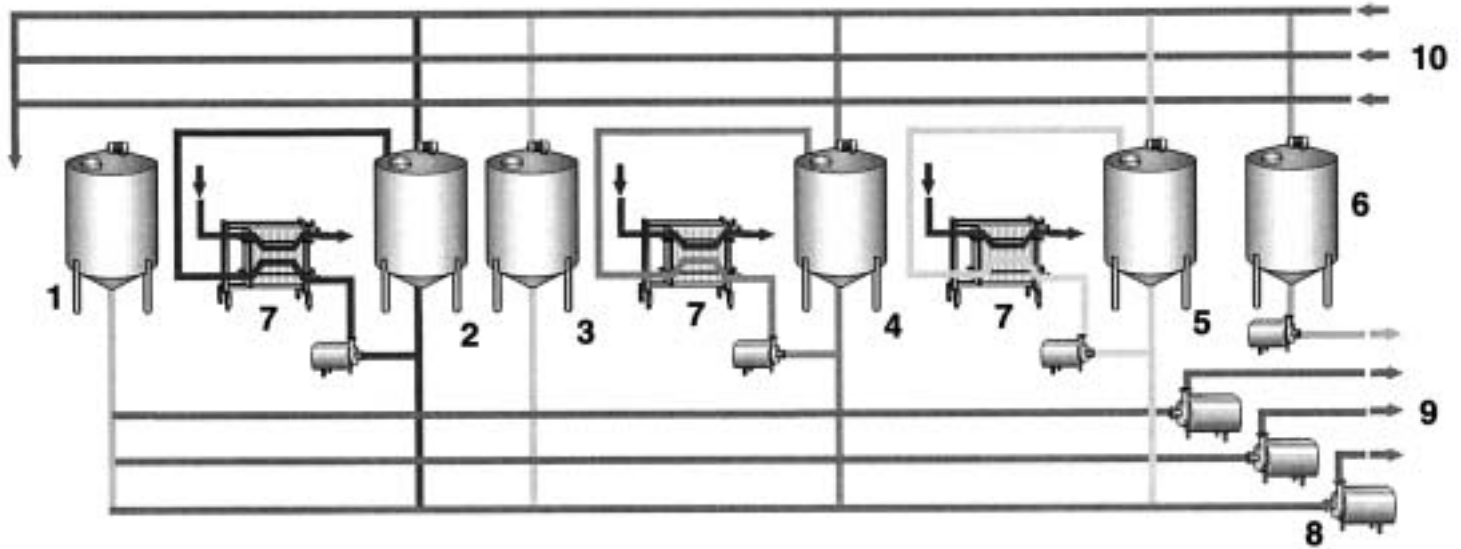
Fig. 4.3 Illustration showing the general design of a central CIP station

1, Cold water tank; 2, hot water tank; 3, rinse water tank; 4, alkaline detergent tank; 5, acid detergent tank; 6, rinse milk tank; 7, plate heat exchanger for heating; 8, CIP pressure pumps; 9, CIP pressure lines; 10, CIP return lines.

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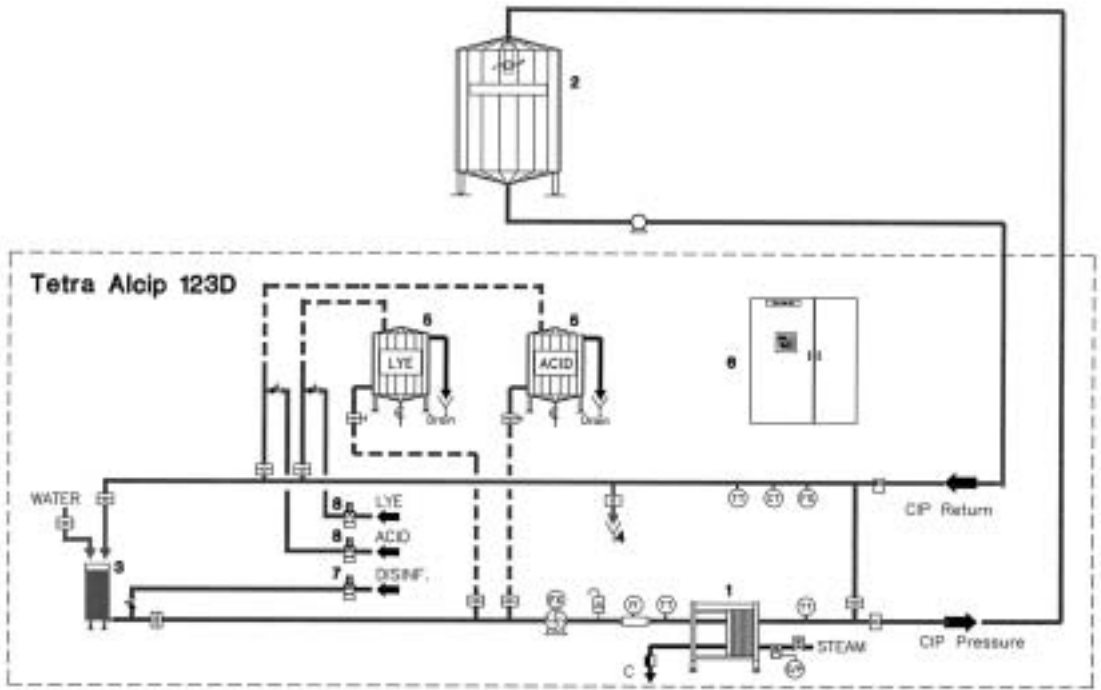
Fig. 4.4. Illustration of a CIP system for tanks and pipelines

1, Plate heat exchanger; 2, cleaning circuit (e.g. tank); 3, circulation tank; 4, drain; 5, detergent solution tank; 6, control panel; 7, metering pump for disinfectant; 8, metering pump for detergent concentrate.

TT: temperature transmitter; FS: flow switch; CT: conductivity transmitter; FX: frequency control; FT: flow transmitter.

Stage	Programme for ripening tanks	Time (min)	Temperature (°C)
1	Prerinse with water	3	ambient
2	Lye wash–1% caustic soda solution with complexing agent additive, to prevent scale precipitation and for improved dispersion	10	75
3	Intermediate rinse	1	ambient
4	Acid wash – 1% nitric acid solution	5	65
5	Final rinse with water, goes to rinse water recovery tank	5	ambient
6	Hot water disinfection, water goes to drain	6	>86

After Anon. (1995). Reproduced by courtesy of Tetra Pak (Processing Systems Division) A/B, Sweden.

agents with specific characteristics to remove soil from dairy processing equipment. Hence, the cleaning process takes place at a reduced temperature of 50–55°C, a high pH of 8.5–9.5 and at a low concentration of reagents (e.g. P3-Paradigm® is applied at 0.09% concentration; see Potthoff *et al*., 1997). The enzyme hydrolyses any protein attached to the equipment surfaces and, as a consequence, the detached material will be evacuated with the main CIP flow. The buffer stabilises the pH, whilst the surfactant removes the fat and the complexing agent prevents scale build-up on the surface of the equipment. In addition, the final rinse contains a sanitiser

(e.g. P3-ParaDES®) that inhibits the growth of micro-organisms. Since such cleaning solutions are used at low concentrations, these components add very little to the biological oxygen demand (BOD) or chemical oxygen demand (COD) of the CIP discharge to the effluent from the dairy. Incidentally, one such cleaning agent has been developed by Henkel-Ecolab in Germany.

4.5   Factors influencing the efficiency of cleaning

The result of cleaning any type of processing equipment is dependent on a multiplicity of factors and some of these have been discussed by Milledge and Jowitt (1980), Haverland (1981), Bodyfelt (1981), Simard and Tastayre (1985), Sharp (1985), Timperley (1989), Ball (1990), Flagg and Thompson (1990), Romney (1990), Anon. (1992), Floh and Eng (1993), Timperley *et al.* (1994) and Bylund (1995).

4.5.1   Type of soil
Residues from milk that has been heat treated are more difficult to remove than those left behind by cold milk, and similarly residues from heat-treated fruits can adhere tenaciously to metal surfaces.

4.5.2   Method of cleaning adopted
Certain factors can be controlled much more effectively under the CIP system, for example, concentration and temperature of detergent, and hence the CIP system is more reliable and efficient, provided that the system is well maintained.

4.5.3   Contact time
Effective cleaning is time dependent, that is, the longer the contact time between the detergent and the soil matter, the cleaner the equipment will be after the cleaning cycle. However, the type of soil must not be overlooked. For example, 10 min is long enough (according to Anon., 1995) for a solution of 1% caustic soda at 75°C to clean the yoghurt fermentation tanks and pipelines (i.e. the soil is milk components), but the time has to be increased to 25 min when cleaning an ordinary milk pasteuriser (i.e. the soil of heated milk). Thus, contact time is important, since the functional properties of a detergent, for example, wetting, penetration, dissolving and suspending of soil, have a longer time to act.

4.5.4   Concentration of detergent solution
The concentration of the detergent solution used for manual cleaning is limited, since at high concentrations it may cause skin irritation, but in a CIP system, elective cleaning is improved with high detergent concentrations, although the law of diminishing returns comes into effect above a certain level. This ceiling

concentration, as applied to cleaning yoghurt processing equipment, would be in the region of 2–3%, since, as reported elsewhere, a caustic soda solution about 1% is sufficient for cleaning storage tanks, pipelines and yoghurt fermentation tanks; 1–<2% is recommended for cleaning multipurpose tanks and plate heat exchangers, and 2–3% for cleaning UHT plants.

It is important to monitor the strength of the detergent solution, especially in a re-use or multi-use system, but high detergent concentrations (i.e. above 2–3%) are not economic in a yoghurt processing plant. However, up to 5% may be necessary to clean a conventional evaporator if this approach is used to raise the total solids in the mix.

Acid solutions are normally used in the region of <1%, since at higher concentrations corrosion of metal surfaces may occur (see Fig. 4.4). However, with a bench-scale tubular heat exchanger (i.e. heating milk to 72°C) the use of a single-stage detergent system has been shown to produce clean surfaces both physically and chemically in half the time taken by a two-stage (i.e. alkali-acid) procedure which did not remove mineral deposits (Timperley and Smeulders, 1987, 1988). Ultimately under industrial operation, the choice of the cleaning system may differ when the yoghurt milk is heated to higher temperatures and held for longer periods.

4.5.5 Temperature

In general, the higher the temperature of the detergent solution, the more effective its cleaning action, so that while manual cleaning has to be carried out at around 45–50°C, the major sections of a yoghurt plant will be cleaned at 85–90°C using CIP; higher temperatures (e.g. 100–105°C) are used during the alkaline wash of a UHT plant. Acid treatments are usually carried out at around 60–70°C. Nevertheless, under certain conditions, for example, the use of enzyme preparation for cleaning purposes, the temperature of the CIP solution is ≤55°C (see Section 4.4.3).

4.5.6 Flow rate or velocity

The flow characteristics of a liquid in a pipe can be either laminar or turbulent and these configurations are influenced by such factors as pipe diameter, fluid momentum and fluid viscosity. A numerical presentation of the degree of turbulence in the fluid is referred to as its Reynolds number (e.g. Re 2000 = laminar, Re 2000–4000 = transitional and $Re > 4000$ = turbulent) and the higher the number, the more disturbed the flow. Thus, the physical scrubbing action in a CIP system is greatly influenced by the flow rate of the fluid, and effectiveness of the cleaning operation is greatly improved by increasing the velocity of the solution. Although the presence of any obstruction affects the flow rate of liquid through a plant, the mean velocity can still be calculated and Timperly and Lawson (1980) have substantiated that the residual bacteria on a surface are reduced to a minimum if the mean flow rate is maintained at $1.5 \, m \, s^{-1}$, or as Kessler (1981) suggested $Re > 10^4$. However, the design and construction of any milk processing plant can affect the flow rate of liquids (i.e. milk base, yoghurt or detergents) and the mathematical equations used to measure these losses have been detailed by Romney (1990).

Silos and large storage tanks are cleaned using a CIP system and such equipment can be fitted with either sprayballs (Fig. 4.5) or rotating jets (Fig. 4.6)

Fig. 4.5   Some different types of spray ball
Reproduced by courtesy of APV U.K. Co. Ltd., West Sussex, U.K.

which help in distributing the CIP fluids. Tamplin (1980) compared these two basic types. Flow rates tend to be higher using sprayballs rather than rotating jets; this aspect could be important for achieving good results in cleaning. Romney (1990) has also detailed the various aspects involved in tank cleaning and currently the systems have been categorised according to their performance as follows:

- Category 1 – high pressure and low volume systems which tend to be used for tank cleaning; the heads have two nozzles as opposed to the four or eight available on large heads. The operating pressures range between 0.4 and 1 MPa, with corresponding flow rates from 3000–8000 l hour^{-1}.
- Category 2 – high pressure and high volume systems which are based on category 1 and are suitable for larger units; the operating pressures are between 0.6 and 1.5 MPa, and the flow rates from 8000 to 35 000 l hour^{-1}.
- Category 3 – low pressure and low volume systems. This category covers small fixed sprayballs and fixed jets, but not the rotating types; their application in dairies is restricted to those places where a very light cleaning duty is required.
- Category 4 – low pressure and high volume systems include the majority of tank cleaning heads, such as for milk silos and process buffer tanks; large flow, fixed spray balls and rotating spinner-type heads which use the reaction force of the jet to rotate the head are placed in this category.

4.5.7   Acid wash

Effective cleaning can also be dependent on the constituents of the detergent (see Table 4.2), and the acid wash is a supplementary cleaning process for the removal of milk stone and other types of soil. Whether or not the latter wash is conducted

Fig. 4.6 Spray turbine for tank cleaning

The spray turbine consists of two rotating nozzles on the same pipe. One rotates in the horizontal plane and the other in the vertical. Rotation is produced by jet reaction from the backward-curved nozzles.

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regularly on a daily basis or once a week is subject to plant quality control and the final decision is based on microbiological tests.

4.5.8 Plant design

Any type of food processing plant, including a yoghurt plant, is constructed from a variety of vessels, pipelines, elbows, pipecouplings, valves and pumps. These components cannot be relied upon to be free from bacterial infection, and hence the efficiency of a cleaning programme may be dependent on plant design. Numerous factors are involved and according to the recent reviews of Lelieveld (1976), Milledge (1981), Timperley (1981), Timperley and Lawson (1980) and Romney (1990), the relevant factors could be summarised as follows: (a) corrosiveness of the stainless steel, (b) surface finish and surface grain (e.g. 80 μm average diameter grit had the effect of harbouring bacteria), (c) pipe couplings – the ring joint type (RJT) is unsuitable for CIP and the international sanitary standard (ISS) type can result in crevices that are difficult to clean, (d) good orbital welding is normally used for CIP circuits, but does not facilitate proper inspection, (e) dead pockets must be avoided, but if "T" pieces cannot be ruled out, the length must be kept short, (f) pumps are difficult to clean, especially reciprocating and positive displacement types, (g) valves are of three types – plug cock, plug and stem, and membrane; the latter two can be cleaned easily and sterilised by CIP, but not the plug cock type, and (h) plant layout.

Microbial attachment to milk contact surfaces has also been studied by Zoltay *et al*. (1981), Stone and Zottola (1985) and Bellon-Fontaine *et al*. (1990) using scanning electron microscopy. They confirmed that adhesion is influenced by such factors as metal roughness, surface treatments, welded seams, and the nature of any

rubber/plastic joints, and each can affect the efficiency of cleaning and sanitation of a processing plant.

4.5.9 Chemical composition of a detergent

It is often difficult to obtain the exact chemical composition of a given detergent, but some general data are given in Tables 4.2 and 4.3; however, according to Tamplin (personal communication), some typical commercial detergents have the following chemical composition.

(A) Detergent for cleaning silos and milk storage tanks ($g\,100\,g^{-1}$):

EDTA	25.0
Sequestering agents	2.5
Emulsifiers	1.0
Antifoam agent	0.5
Soaps	5.0
Water	66.0

The solution is used at a level of 0.2–$0.5\,ml$ or $g\,100\,ml^{-1}$ along with a level of 1–$2.5\,g\,100\,ml^{-1}$ caustic soda at 60–$90°C$. Alternatively, if a blended product is used the composition ($g\,100\,g^{-1}$) is as follows:

Caustic soda	44
Sequestering agents	1
Water	55

and depending on the level of soiling, the concentration used would be 0.7–$4\,ml$ $100\,ml^{-1}$ circulated at 65–$90°C$.

(B) Detergent for cleaning a plate heat exchanger (PHE):

• Powder detergent ($g\,100\,g^{-1}$)

NaOH	30–50
EDTA	8–15
Trisodium phosphate	15–25

plus bulking agent (soda ash) and alkalinity booster (silicates), whilst the wetting agents are generally produced *in situ* by saponification; if required, a low foaming, non-ionic agent can be added; the recommended strength is 1–$2\,g$ $100\,g^{-1}$.

• Liquid detergent ($ml\,100\,ml^{-1}$)

NaOH (100° Tw. solution)	40–60
EDTA (30% concentration)	20–30
Silicates	55–15

plus other minor ingredients; such a detergent is used at a strength of 1.5–$3\,ml$ $100\,ml^{-1}$, and due to limited solubility of EDTA in NaOH (e.g. >50% NaOH), it is recommended that Na-gluconate be added as an organic sequestrant. The above detergent formulations are used in single-stage cleaning cycles,

but for two-stage cleaning (i.e. detergent/acid), the following might be more suitable.

- Powder detergent $(g\,100\,g^{-1})$

NaOH	60–80
EDTA	2–10
Phosphates	2–10

plus filler (soda ash) or liquid alkali [NaOH (100° Tw. solution) 85–95 ml $100\,m^{-1}$] and Na-gluconate $(5–15\,g\,100\,g^{-1})$, whilst the acid could be $1\,ml\,100\,ml^{-1}$ phosphoric acid.

(C) Combined detergent/sanitiser:
This type of product might include: Na-tripolyphosphates or calgon, Na-isocyanurate, chlorinated trisodium phosphate, silicate or soda ash for bulking (70%), and a chlorate tracer (a typical formulation (%) for use at 0.5% would be: phosphates 15, silicates 5 and soda ash 25–30). The latter compound ensures an available chlorine level of $250\,\mu g\,g^{-1}$ at the maximum operating temperature of 50°C.

(D) High caustic EDTA blend:
This formulation is similar to the detergent described above for cleaning silos and milk storage tanks, but the chosen causticity and operating temperature is dependent upon water hardness (e.g. for a hard water condition, the phosphate concentration would be increased to 10–15%). Such a detergent would be used at a rate of 0.5–1%, and the microbiological "kill" is achieved by the combined action of causticity and temperature.

(E) Non-caustic alkaline detergent (%) followed by a sanitiser:

Soda ash	50–70
Silicate	30
Phosphate	5–12
EDTA	5–10

plus a low foam wetting agent; this non-caustic detergent treatment is normally followed by sanitisation with sodium hypochlorite at ambient temperature and a concentration of about $100–150\,\mu g\,g^{-1}$ available chlorine.

(F) Acid detergent (%):

Phosphoric acid (81% conc.)	20–50
Non-ionic wetter	3–8

Formulations (C), (D), (E) and (F) could be used for cleaning yoghurt incubation tanks and/or silos or milk storage tanks and the recommended detergent is the "high caustic with EDTA" blend. Although the above detergent formulations may be out-of-date, in theory, the principle(s) and/or efficacy of cleaning dairy equipment may still be applicable to present day detergent formulations. Additional information has been reported by Wirtanen *et al*. (1997) who studied on-site efficiency of sanitisation in large dairy factories in different Scandinavian countries using different commercial detergent compounds.

4.6 Specific cleaning and sterilisation operations of yoghurt processing equipment and utensils

A comprehensive account of the cleaning and sterilisation of dairy plant and equipment has been published by British Standards Institution (BSI, 1970, 1977, 1984), and the relevant data, which are applicable to a yoghurt processing line, are illustrated in Table 4.6. Certain processing equipment, for example different types of heat exchanger, is used for the heat treatment of milk and the procedures of cleaning and sterilising ordinary high temperature short time (HTST) and ultra high temperature (UHT) units including yoghurt plants operating between 90°C and 110°C are shown in Table 4.7. However, membrane filtration plants (i.e. UF and RO) necessitate a different approach to cleaning. Table 4.8 illustrates the cleaning and sanitation procedures for those plants that are used in the dairy industry.

In general, the CIP system is used to clean the major sections of a yoghurt processing line and CIP programmes can be either manually operated or fully automated. Automatic control has been achieved during the past few decades using computers and microprocessors and, as a result, the process has become more efficient with better detergent recovery, a reduction in energy consumption and a reduction in the scope for human error. Many different types of computer are available on the market, but a review of these systems is unnecessary, since the layout, design and programme of a CIP system is basically tailormade to suit individual yoghurt plants.

However, CIP control systems offered by different manufacturers have certain advantages and the overall choice is governed by the level of capital expenditure and the degree of automation required. The programme of a CIP system may include up to 30 different functions for cleaning a tank or other processing unit, and the same programmes may also allow a prolonged cleaning operation to be introduced at certain times. Another feature which is common to these CIP control systems is the safeguard against power failure, and this precautionary factor is important, especially to accommodate a power failure taking place in the middle of a cleaning programme, otherwise the programmed function would be terminated.

Although the flexibility of any CIP controller system is assessed prior to making the final decision about which unit to install, some general points might be considered.

First, there must be no risk of the product becoming contaminated with the detergent and/or sterilant solutions. This safeguard can be achieved using one of the following systems:

- flow selector plate
- manual "key pieces" or "security flow pipes"
- use of special valves.

The former two systems are suitable for small plant operations and, as a further precautionary measure, interlock switches are often incorporated. The use of key pieces also offers a high degree of security, in that, for example, if installed at two places in a tank installation (bottom fed), the first will be positioned at the bottom when the product is being handled, while the second will be positioned at the top (i.e. above spray ball(s)) during the operation of the CIP programme. Alternatively, different types of mixproof valve could be used. A single-seat valve with external cleaning has one seat and two valves mounted on the same plug. The area between the

Table 4.6 Recommended methods for cleaning and sterilisation of yoghurt processing equipment

Equipment/utensils	Cleaning method	Sterilisation method

1. Milk cans/churns	*Manual wash*
	(a) Combined detergent/steriliser
	  Rinse can with tepid water.
	  Add 5 l detergent/steriliser solution at 40–50°C.
	  Scrub thoroughly inside/outside surfaces of the can including
	    neck and lid.
	  Place can on its side and roll for $\frac{1}{2}$ min.
	  Allow at least 2 min contact time.
	  Empty, rinse with clean water and invert to drain on a rack.
	  Wash detachable can lids separately in a trough.
	(b) Detergent only
	  Use same steps mentioned above.
		(a) Chemical sterilisation
		  Follow recommendations of detergent's manufacturer.
		(b) Steam
		  (i) Steam chest = 96°C for 30 min.
		  (ii) Steam jet – not less than 2 min.

	Machine wash (rotary or tunnel) Drainage stage for liquid milk residues. Prerinse with water (cold or at 40–50°C). Drainage stage(s). External wash with water at 40–50°C. Drainage stage. Jetting with solution of detergent at 70–80°C. Drainage stage(s). Rinse with water at 85°C (minimum). Live steam injection. Hot air drying at 95–115°C.	No sterilisation required

2. Weighing bowls and receiving tanks	Hose the bowls and receiving tanks with cold water and then
	  with water at 50–60°C.
	Close outlet and add suitable volume of solution of general
	  purpose detergent.
	Brush the internal and external surfaces, covers and strainers
	  with suitable brush and as solution is drained from tank, scrub
	  outlet valves and fittings.
	Hose tank and fittings with clean water, reassemble, the
	  equipment is then ready for sterilisation.
		(a) Combined detergent/steriliser
		  Equipment is ready to be used immediately after final rinsing
		    stage, or if this is not possible, resterilise immediately before
		    use
		(b) Chemical sterilisation
		  Prepare solution of sterilising agent.
		  Partially fill weighing bowl and receiving tanks with solution
		    of sterilising agent.
		  Brush in same manner as indicated during "Cleaning
		    method" using a brush reserved for this purpose.
		  Rinse residues of sterilising agent from equipment by
		    hosing with cold water and use equipment immediately; if
		    this is not possible resterilise immediately prior to use.

3. Pumps and pipelines

CIP system (refer to Table 4.7)

Manual wash
Rinse with cold water.
Dismantle and wash parts in trough filled with detergent solution.
Brush all surfaces coming into contact with milk, and for pipes use long handled brush; brush pipes from both ends.

For CIP use
(a) Hot water circulation for not less than 15 min measured from the time that all parts of circuit reach a temperature not less than 85°C.
(b) Chemical sterilising agent – circulate solution, for example sodium hypochlorite, i.e. 50–100 μg ml^{-1} of available chlorine at 20–40°C for contact period of 10–20 min; discharge and rinse with clean water and use immediately, if this is not suitable, resterilise prior to use.

For Manual use:
(a) Form pipework into closed circulation circuit and sterilise by one of the methods mentioned above (hot water or chemical sterilising agent).
(b) Sterilise dismantled pipelines and fittings using steam for a period of 15 min.
(c) Soak dismantled parts in solution of sterilising agent, rinse with cold water, reassemble immediately taking precautions to avoid recontamination; if equipment is not used immediately, resterilise immediately before use.

4. Milk/yoghurt processing plants

HTST and UHT plants (Refer to Table 4.7)

Batch type holding plants
       or   (Milk is heated up to 95°C and held for 5 min)
Yoghurt multipurpose tank
  Remove as much product as possible from the vessel.
  Fill with solution of sodium hydroxide-based detergent which may contain sequestering agent.
  Heat solution to 75–85°C by passing steam through the jacket, start the stirring mechanism and maintain temperature for 30 min.
  Drain cleaning solution from vessel.
  Rinse well with cold clean water.
  Sterilise.
Note: should milk stone have accumulated in the vessel, treatment with a suitable acid (phosphoric acid of B.P. quality – 100 ml in 5 l water at 40–50°C after the detergent wash has been rinsed) may become necessary.

Refer to Table 4.7

Sterilise using one of the following methods:
(a) *Steam*
Connect low pressure steam supply to the outlet pipe of the vessel by means of screw couplings as a safeguard against accidents; using trailing hoses are dangerous and should not be used.
Steam for a period of not less than 10 min after condensate temperature has reached temperature of 85°C.
(b) *Alkaline solution*
By means of CIP equipment, use 1% caustic solution at a temperature of not less than 75°C for minimum contact of 10 min.
Rinse with cold clean water.
(c) *Chemical sterilising agent*
Use solution of sterilising agent as mentioned above.

Table 4.6 *Continued*

Equipment/utensils	Cleaning method	Sterilisation method
5. Homogenisers	*Part of the processing plant* (Refer to item 4 above)	(Refer to item 4 above)
	Separate unit Form all associated pipework including the homogeniser into a closed circuit. Reduce pressure from the homogeniser valve. Start up the homogeniser and rinse out circuit with water to remove loose milk residues; allow rinse water to go to waste. Pressure gauges, suction valves and inlet and outlet manifolds should be removed, cleaned and rinsed manually, and reassembled for sterilisation. Add sufficient detergent of type used on the main plant, or any specialised product for cleaning homogenisers, to about 90 l of water. Introduce detergent solution to the homogeniser and circulate for about 30 min at 70–80°C. Apply pressure of about 0.6 MPa. Leave all by-passes slightly open to allow passages of rinse water and detergent solution. Rinse with clean cold water to waste. Note: In prerinsing and final rinsing, the time of circulation should be kept to a minimum owing to the poor lubrication properties of water on the piston rods and hoses. No special precautions other than those mentioned are necessary when alkaline detergents are used, as these provide adequate lubrication.	Release pressure from the homogenising valves and introduce hot clean water. Continue circulation for a period of not less than 15 min after the return water has reached a temperature of 85°C. Note: (a) Ensure that all drain valves, pressure gauge line, etc., are raised to temperatures of 85°C for not less than a period of 15 min by bleeding the lines throughout the sterilising period. (b) The large mass of metal in the homogeniser blocks necessitates a long heating up period.
6. Filling machines	*Manual wash* At the end of the filling period rinse through with cold water, and wash away any product which has been rinsed on to the tracks. Dismantle removable parts. Rinse component thoroughly with cold water or at temperature 40–50°C. Clean all components manually with solution of a suitable detergent at 40–50°C. Rinse all components thoroughly with cold water until free from detergent. Reassemble the machine which is now ready for sterilisation. *CIP wash* If applicable, consult machine manufacturer for a recommended wash cycle.	(a) Combined detergent/steriliser. (b) Chemical sterilisation agent, e.g. sodium hypochlorite 50–100 μg ml^{-1} of available chlorine at ambient temperature for contact period of not less than 10 min. (c) Hot water circulation for a period of not less than 15 min measured from the time effluent water reaches a temperature of 85°C. (d) Steam (not widely practised). Items (c) & (d) may not be applicable to all machines, therefore, before using any of these methods consult machine manufacturer. As described in (a) to (d) immediately above. Follow the recommendations of the machine manufacturer.

7.	Starter culture tanks	*Manual wash*
		 (a) Small size utensils of starter culture equipment can be washed by hand as described in item (1).
		 (b) Vessels not equipped for CIP.
		     Dismantle all removable parts and wash separately.
		     Hose out the residual starter with cold water as soon as vessel is empty.
		     Scrub surfaces with solution of mild alkaline detergent or detergent/steriliser at 40–50°C.
		     Rinse with cold clean water and reassemble, the vessel is then ready for sterilisation.
			Use one of the methods mentioned in item (4).

		CIP wash
		     Start cleaning operation as soon as the vessel is empty, i.e. before the starter dries on to the surfaces.
		     Carry out CIP using a suitable alkaline detergent or detergent/steriliser solution; pay particular attention to outlet valve.
		     Rinse with clean water in accordance with the starter vessel manufacturer's instructions.
		     The vessel is then ready for sterilisation.
			Use one of the methods mentioned in item (4).

8.	Vessels for bulking fruit	These may be used in large-scale yoghurt production, and the cleaning cycle may comprise:
		     Rinse thoroughly with water at 40–45°C.
		     Scrub with milk detergent.
		     Rinse with cold clean water.
		     The vessel is ready for sterilisation.
			Use one of the methods mentioned in item (4).

9.	Miscellaneous	(a) Glass bottles and crates.
		     Follow recommendations provided for washing/sterilisation of returnable glass milk bottles.
		 (b) Membrane (UF & RO) machines.
		 (c) Single effect evaporator
		     Follow instructions of equipment manufacturer; one such unit is used as illustrated in Chapter 3 and the evaporator is cleaned with the rest of the processing equipment.
			
			 See Table 4.8
			 See Table 4.7

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Table 4.7 Cleaning and sterilisation method for milk and yoghurt processing plants[a]

Types of processing plant	CIP programme	
	Detergent wash	Sterilisation
HTST[b] (pasteurisers)	Rinse with cold water for 15 min. Circulate detergent solution at 70–80°C for 20 min. Rinse with cold water. Note: Change flow from "forward" to "diversion" during the detergent wash. Plates may be opened, brushed and hosed with water. An occasional acid wash is carried out after the alkali wash, since a straightforward acid wash may cause corrosive damage to stainless steel.	(a) Hot water circulation (not less than 15 min from the time that all sections of the plant reach temperature not less than 85°C – operate flow diversion valve frequently during the circulation period). (b) Chemical sterilising agent (see Table 4.10).
UHT[b]	Rinse with cold water for 15 min *Alkali wash* • Primary stage Circulate 3% solution of mixed alkali for 30 min at 100–105°C; change flow from "forward" to "diversion" at intervals; flush out alkali solution and rinse with water. • Secondary stage Circulate 2% solution of alkali containing a high proportion of a calcium sequestering agent for 30 min at 100–105°C; flush alkali solution and rinse with water. • Alternative method Circulate higher strength of detergent solution containing high proportion of calcium sequestering agent. *Acid wash* Circulation of 0.5% acid solution for 30 min at 75–80°C. Rinse with clean cold water. Plates may be opened, brushed down and hosed with cold water.	UHT plants are frequently sterilised automatically. Alternatively, circulate pressurised hot water at temperature not less than 140°C and not more than 150°C for a period of not less than 15 min and use plant immediately; or steam under pressure. Note: Ensure that all sections of the plant are within temperature range from 140–150°C; Temperatures greater than 150°C may cause rapid deterioration of rubber joints; Chemical sterilisation agents are not suitable.

Yoghurt/equipment	*First example* (Time/temperature relationship is 85°C for 6–10 min). Rinse with cold water for 20 min; open the holding tube and scrub by hand, and finally rinse with water for 5 min. Detergent wash (2% caustic for 30 min at 85–90°C). Flush out detergent and rinse with cold clean water for 20 min; open holding tubes for visual inspection. Once a week carry out acid wash (1% phosphoric acid) following the detergent wash at 85–90°C for 30 min; also once a week open the plates and check.	Hot water circulation for 30 min at 85–90°C; or steam under pressure for UHT yoghurt plants.
	Second example (Time/temperature relationship is 90°C for 2–5 min). Preliminary cold water rinse for 3–5 min. Detergent wash (1% concentration for 6 min at 65–75°C). Final cold water rinse for 6 min. Note: Perhaps once a week use an acid rinse (1% concentration) for 6 min carried out after flushing out the detergent and rinsing with cold water; also carry out water rinse after the acid wash.	Hot water circulation for 20 min at 90–95°C.
	Third example (Time/temperature relationship is 115°C for 3 s). Preliminary cold water rinse for 5 min. Detergent solution (2% caustic) with circulation for 45 min at 85°C. Intermediate water rinse for 5 min. Acid solution ($1\frac{1}{2}$–2% phosphoric acid) circulation for 45 min at 70°C. Final rinsing with cold water for 5 min.	Hot water circulation for 20 min at 85°C.

[a] These are plate heat exchanger plants; batch processing plant is discussed in Table 4.6.

[b] Data compiled from BSI (1970, 1977, 1984). Reprinted with permission of British Standards Institution, London, UK.

Table 4.8 Recommended procedure for the cleaning and disinfection of UF and RO plants

Equipment	Cleaning and disinfection programme
UF plant fitted with spiral wound membranes used for processing skimmed milk	Rinse/flush the plant with water (5–15 min) until all the product has been removed.
	Detergent wash by recirculating a solution of 1.2% P3-Ultrasil 11 (Henkel-Ecolab) at 50°C for 30 min.
	Rinse/flush the plant with water (5–15 min) until the detergent has been removed.
	Acid clean by circulating 0.3% nitric acid solution (P3-Ultrasil 75) at 50°C for 20 min.
	Rinse/flush the plant with water (5–15 min) until acid has been removed.
	Detergent cleaning in which solution of 1.5% P3-Ultrasil 141 and 150 $\mu g\,g^{-1}$ sodium hypochlorite at 50°C is circulated for up to 40 min.
	Rinse/flush the plant with water (5–15 min) until detergent has been removed.
	Disinfection by circulating a solution of 2.5 mg g^{-1} sodium metabisulphite or 1.5% P3-Ultrasil 73 at 25°C for 15 min.
	Stop the plant.
	Rinse/flush the plant with water (5–15 min) before next production.
RO plant with spiral wound membranes	Rinse the plant with water until product has been removed.
	Detergent wash by recirculating a solution of 1% Divos 100 (Diversey Lever) at 50°C for 30 min.
	Acid clean by circulating 1% Divos 2 at 50°C for 20 min.
	Rinse/flush the plant with water or RO permeate.
	Enzymatic cleaning in which solution of 1% Divos 98PE is circulated at 50°C for 1 h.
	Rinse/flush the plant with water or RO permeate.
	Disinfection by circulating a solution of 0.6% Divos 2 at 50°C for 20 min.
	Stop the plant.
	Rinse/flush the plant with water before next production.

After Kønigsfeldt (personal communication).

two seals is open to the atmosphere and this leakage drain chamber is closed by a small shut-off valve before the seat valve is activated; an external CIP line is connected to the drainage line via the small valves (see Fig. 4.7 and Bylund, 1995). A double-seated valve (with external cleaning or seat-lift cleaning) has two independent seals separating the two liquids and a drainage chamber in between (see Fig. 4.7 and 4.8). This chamber must be open to the atmosphere to ensure full mixproof safety in case either of the two seals should leak. When a double-seated mixproof valve is activated, the chamber between the upper and lower body is closed and then the valve opens to connect the upper and lower pipelines. When the valve is closed, first the upper plug seals and then the leakage chamber is opened to the atmosphere. This gives very small product losses during operation. It is important that the lower plug should be hydraulically balanced to prevent pressure shocks from opening the valve and allowing products to mix. During cleaning, one of the plugs lifts, or an external CIP line is connected to the leakage chamber. Some valves can be connected to an external cleaning source for cleaning those parts of the plugs which have been in contact with the product.
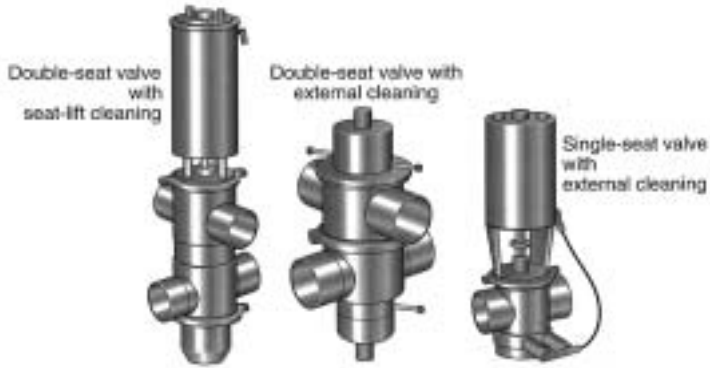
Fig. 4.7 Illustration of three types of mixproof valve

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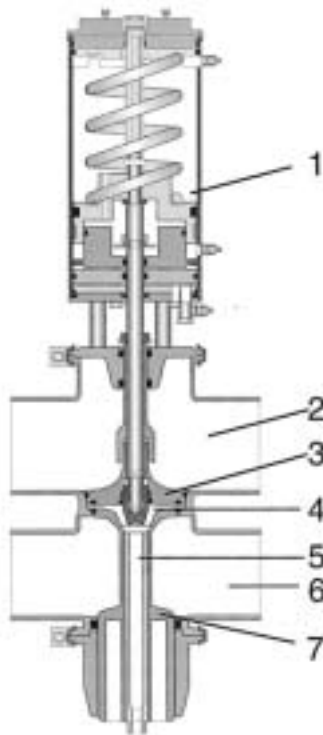
Fig. 4.8 Detailed illustration showing the assembly of a double-seat mixproof valve with balanced plug and built-in seat lift

1, Actuator; 2, upper port; 3, upper plug; 4, leakage chamber with drainage; 5, hollow spindle to atmosphere; 6, lower port; 7, lower plug with balancer.

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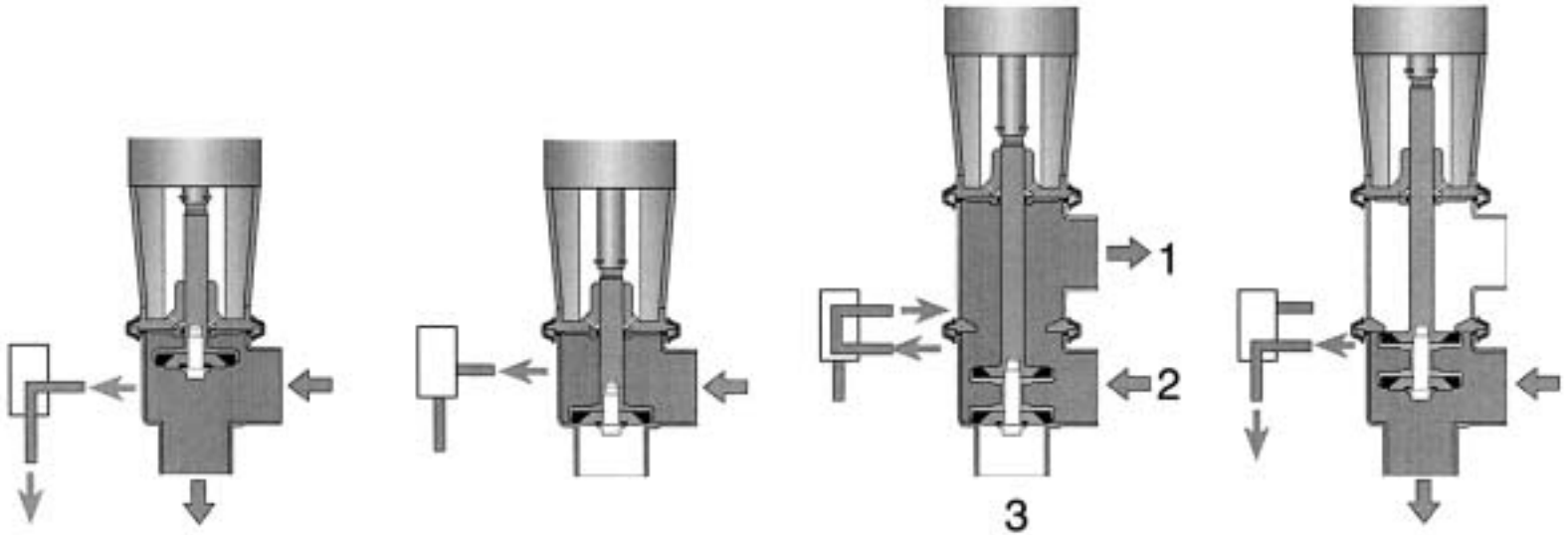
Fig. 4.9 Shut-off and change-over valves with plug in different positions

This valve has between three and five ports; when the plug is lowered the liquid flows from inlet 2 to outlet 1, and when the plug is lifted to the upper seat, the flow is directed through outlet 3 (see right hand drawing).

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The three-way valve is a single valve which is arranged in such a way that, in the closed position, one part is open to the atmosphere and any leakage of the CIP solutions will fall outside the vessel; thus contamination of the product is prevented. However, a double-valve system with electric interlocks has been developed which ensures total isolation of the circuit being cleaned from the adjacent section where product could be flowing (Fig. 4.9).

Second, a good drainage system must be in place so that the product and/or cleaning solutions can be quickly removed from the plant to prevent intermixing. Therefore, sound design of a plant is essential and the piping layout must have the following features:

- self-drainage capability,
- no parallel flow (i.e. the detergent flows in the opposite direction to the product),
- no dead ends.

Third, in modern yoghurt installations, the "pigging" system is employed to purge the product from the pipelines in order to improve cleaning efficiency (see Fig. 4.10). However, in older installations air purging is used to purge the yoghurt. A blast of oil-free compressed air is forced into tanks and pipelines as a convenient method of evacuating residual product (e.g. milk or yoghurt) from the plant; the volume of air delivered and the duration of purge is calculated to empty the pipelines
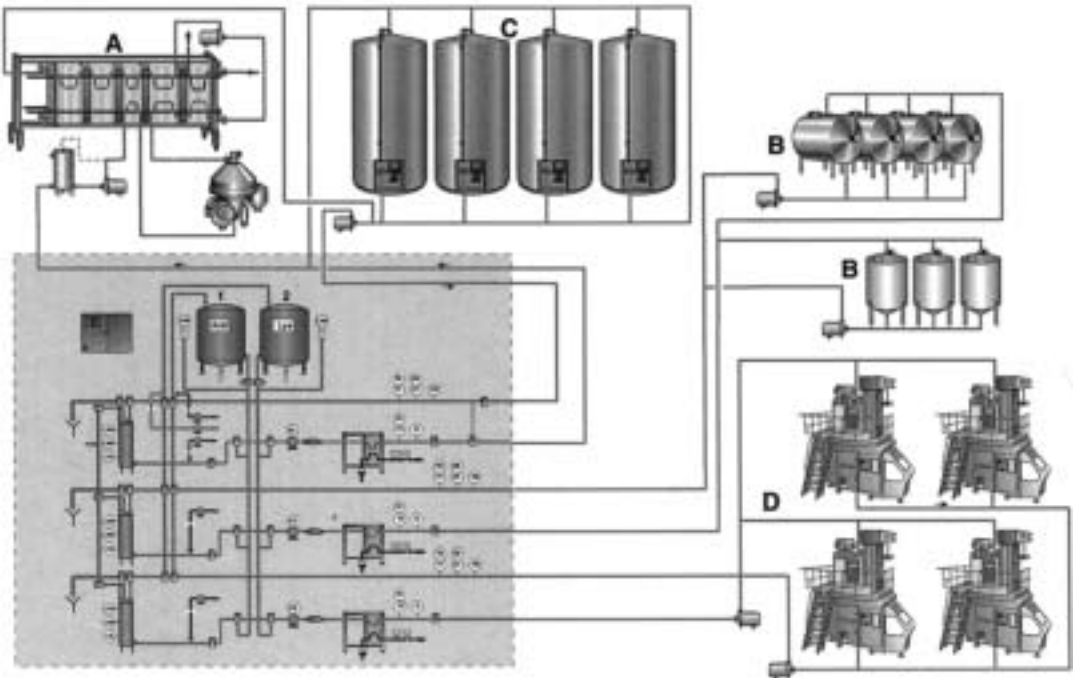
Fig. 4.10 The centralised CIP system

A. Milk treatment; B, series of tanks; C, silos; D, filling machines.
1, Acid detergent tank; 2, alkaline detergent tank.
Central CIP station is located within the dotted lines in the figure.

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effectively. The result is improved product recovery, minimum soiling matter to be removed and less rinsing water required, and better utilisation of detergent since elective concentrations can be maintained for a number of runs. Incidentally, although the air purging system is mainly operated before the cleaning cycle commences, it is also used to evacuate residual rinsing water during and/or after cleaning (e.g. the preliminary rinse at the beginning of the cleaning cycle).

Fourth, in large plants the CIP system itself will need to be cleaned occasionally, and the usual approach is to install a separate CIP system for cleaning the main installation. The main problem arises from the precipitation of milk protein in the detergent tanks.

Fifth, specific data regarding the CIP of yoghurt filling machines are not available, but Langeveld *et al.* (1982) evaluated the efficiency of a CIP system in removing secondary contamination from a Hamba-2000 filling machine; they concluded that the CIP programme was satisfactory. This particular CIP programme included a prerinse with water, circulation of an alkaline solution at 70°C for 20 min, and finally rinsing with water containing 1 μg of free chlorine ml^{-1}.

Sixth, the design of the CIP station is determined by many factors (Bylund, 1995) such as:

- How many individual CIP circuits are to be served from the CIP station?
- Are the milk base and/or yoghurt rinses to be collected, and/or processed for re-use or discharge?
- What method of sterilisation of the equipment to be used (i.e. chemical, steam or hot water)?
- What method of detergent system is to be used – single or multistage?
- What is the demand for steam for cleaning and sterilisation purposes?

Thus, two types of CIP systems can be used, centralised cleaning and decentralised cleaning. The former system (see Fig. 4.10) is normally used in small dairy plants with relatively short CIP pipelines. The detergent solutions and hot water are kept hot in insulated tanks and the required temperatures are maintained by heat exchangers. The final rinse water is collected in the rinse-water tank and is used as prerinsing water in the next cleaning programme; the milk/water mixture from the first rinse is collected in the rinse-milk tank. The detergent solution must be discharged when it has become dirty after repeated use, and the storage tanks must then be cleaned and refilled with fresh solutions. It is also important to empty and clean the water tanks, especially the rinse-water tank, at regular intervals to avoid the risk of infecting an otherwise clean process line.

A station of this type is usually highly automated. The tanks have electrodes for high and low level monitoring and the quality of the returning cleaning solutions is controlled by conductivity transmitters. The conductivity is proportional to the concentration of the active ingredient and at the phase of flushing with water, the concentration of the detergent solution becomes lower. At a preset value, a changeover valve routes the liquid to drain instead of to the relevant detergent tank. CIP programmes are controlled by a computerised sequence controller and large CIP stations can be equipped with multiple tanks to provide the necessary capacity.

Decentralised CIP is an attractive alternative for large dairies where the distance between a centrally located CIP station and peripheral CIP circuits would be extremely long (see Fig. 4.11). The large CIP station is replaced by a number of smaller units located close to the specific groups of process equipment in the dairy,
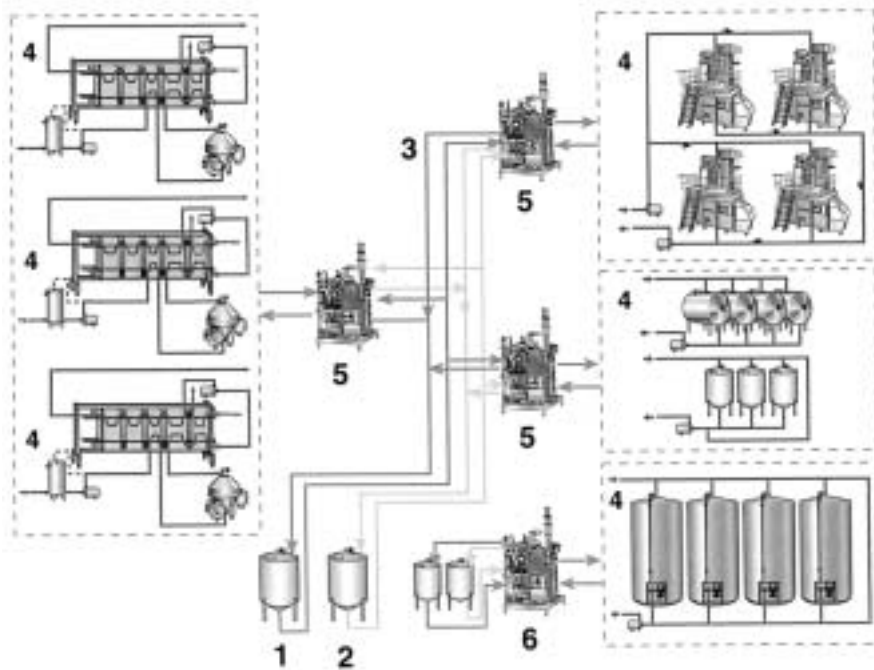
Fig. 4.11 Illustration of decentralised or satellite CIP system

1, Alkaline storage tank; 2, acid storage tank; 3, pipelines for detergents; 4, equipment to be cleaned; 5, satellite CIP units; 6, decentralised CIP system with its own detergent tanks.

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but there is still a central station for storage of the alkaline and acid detergents which are distributed to the individual or satellite CIP units. The supply and heating of rinsing water (and acid detergent when required) is arranged locally at the satellite stations. These stations operate on the principle that the various stages of the cleaning programme are carried out with a carefully measured minimum volume of liquid – just enough to fill the circuit to be cleaned. A powerful circulation pump is used to force the detergent through the circuit at a high flow rate.

The principle of circulating small batches of cleaning solutions has many advantages. Water and steam consumption, both momentary and total, can be greatly reduced. Milk residues from the first rinse are obtained in a more concentrated form and are, therefore, easier to handle and cheaper to evaporate. Decentralised CIP reduces the load on sewage systems compared to centralised CIP, which uses large volumes of liquid.

The concept of single use detergents has been introduced in conjunction with decentralised CIP, as opposed to the standard practice of detergent recycling in centralised systems. The one time concept is based on the assumption that the composition of the detergent solution can be optimised for a certain circuit. The solution is considered spent after having been used once. In some cases it may, however, be used for prerinsing in a subsequent programme.

Sterilisation aspects

4.7   Fundamentals of the sterilisation process

Milk and/or yoghurt soiling matter on the surfaces of processing equipment is usually contaminated with micro-organisms and, as indicated elsewhere, the cleaning stage should (in theory) remove all soil. Thus, any residual matter is an excellent medium in which micro-organisms can grow and multiply and sanitisation of the process equipment becomes a necessity in order to destroy such organisms, otherwise the keeping quality of yoghurt produced on subsequent days could be reduced. The effectiveness of the sterilisation process (using heat or chemical agents) is mainly dependent on the efficiency of the cleaning cycle. For example, any residual soil can become baked onto the contact surface to the extent that it becomes difficult to penetrate the soil in order to destroy the micro-organisms. Furthermore, the residual soil affects the subsequent cleaning process in the first instance because the active concentration of any chemical sanitising agent will be reduced and disinfection becomes less effective, and second, because it is possible that large numbers of micro-organisms may survive the sanitation stage and multiply in the soil; in such cases, infrequently used equipment may become heavily contaminated.

Effective sanitisation of processing plant is therefore directly governed by observation of the following points:

• Maintain the correct cleaning cycle prior to the sanitisation stage.
• Follow the recommendations laid down for the sanitisation method adopted, e.g. strength of the chemical solution, correct contact time and temperature.
• Usually the processing equipment is sanitised directly before use, and hence after the cleaning stage the equipment must be properly drained or purged with air, otherwise the moist condition, in the presence of any residual "soil", can encourage micro-organisms to multiply; if sanitised equipment is not used within a few hours, it is recommended that it should be resanitised before use.
• Any yoghurt plant has joints, valves, dead ends and rubber gaskets into which traces of soil and micro-organisms can penetrate and hence frequent dismantling of these components is essential; furthermore, heat sterilisation is more effective than chemical disinfection for reaching "blind" areas where micro-organisms could have penetrated.
• The hygienic condition of any yoghurt plant is governed by the rigour of the cleaning and/or sanitisation stages. For example, in a UHT plant, the aim is to render the equipment sterile before use, but for other types of plant, a "good sanitary" condition is acceptable by health authorities in many parts of the world. In fact, Zall (1990) differentiated between sanitisation and sterilisation as follows: "both treatments are aimed towards the destruction of micro-organisms, and the former aspect is more easily achieved as compared with sterilisation, which is more a rigorous and difficult procedure".
• The use of chemical disinfection agent(s) and/or compound(s) is subject to approval of the legal authorities concerned and in the United Kingdom a cumulative list is provided periodically by the Ministry of Agriculture, Fisheries and Food.

4.8 Methods of sanitisation and/or sterilisation

The methods which can be employed to achieve either sterilisation or sanitisation include the following.

4.8.1 Heat

Heat is normally applied as dry heat or moist heat. A hot oven used for the sterilisation of laboratory glassware at a temperature above 150°C for not less than 2h provides an example of the use of dry heat. For practical reasons, dry heat is not used to sanitise yoghurt processing equipment, but moist heat is widely used, for example:

- autoclaving (steam under pressure)
- steaming or tyndallisation
- hot water
- steam (free flowing).

The first two methods are used for sterilising microbiological growth media and/or the medium for propagation of the starter cultures (e.g. the mother or feeder stage). The principles of these two methods are discussed in detail by Meynell and Meynell (1970). In an autoclave, steam under pressure is used and the recommended working condition is 121°C for 10–15 min (under a pressure of 0.1 MPa). However, the steaming method, which was introduced by Tyndall in the 1870s, consists of heating liquids up to 100°C for a few minutes so that all the vegetative microbial cells are destroyed. The liquid is cooled to ambient or 30°C to induce the spores to germinate and, after a few hours, the steaming/cooling cycle is repeated again. Further repetition of the heat treatment ensures destruction of all the viable spores in the liquid.

The steaming of milk is also practised in laboratories for the propagation of feeder starter cultures in flasks up to three litres capacity, but in this case only one heating operation is required.

Processing plant can be sterilised or sanitised using hot water or steam and the efficiency of the process is primarily dependent on three factors, the time–temperature combination (i.e. the temperature reached and the time for which the temperature is maintained), the humidity and the pressure.

The on-site applications of hot water circulation or steam (free flowing) for sanitising yoghurt equipment are illustrated in Table 4.9. Hot water circulation is most widely used. The limited application of free flowing steam is because (a) there are heat stresses generated that can cause pipelines to buckle or crack, (b) the intense heat generated can result in cracks in welded seams and can damage rubber gaskets, (c) since steam cannot be recirculated, its generation is a waste of energy, (d) the process is very noisy, and (e) the use of steam may pose a hazard to personnel. However, steam under pressure may be used to sterilise plants for the manufacture of UHT yoghurt.

However, a mixture of hot air and steam (*c*. 250°C) can be injected to sterilise yoghurt containers before filling and the process has been patented in Germany (Ammann, 1981). Such a process would, of course, be limited to certain materials due to the high temperature used, but unfortunately no specific type of container has been mentioned.

Table 4.9 Effectiveness of sterilising/sanitising yoghurt equipment using different methods of heating

Type of heating	Working application	Comments
Dry heat		
Hot air in a dry oven	>150°C for a least 2 h	Inactivates bacterial spores and is normally used to sterilise glassware.
Moist heat		
1. Pasteurisation	72°C for 15 s	Inactivates mesophilic micro-organisms including pathogens, psychotrophic bacteria yeast and mould (some mould spores are heat resistant). This method is not practised for the sanitisation of processing equipment.
2. Hot water	85°C for 15–20 min	Inactivates all vegetative cells (including thermoduric bacteria) with the exception of spores and bacteriophages; this method is recommended for sanitising processing plants.
3. Boiling water	100°C	As (2) above. Limited in its application but it is used for disinfection purposes; bacteriophages are inactivated.
4. Steaming	100°C for 10 min (2–3 cycles)	As (2) above. Efficiency is dependent on spore germination; it is not used for plant disinfection.
5. Steam (free flowing)	100°C	Not more effective than boiling water and bacterial spores are not inactivated. Used to sterilise milk churns for 1–2 min, or storage vessels until the condensate reaches 85°C – 10 min treatment.
6. Steam under pressure	121°C for 10–15 min (about 0.1 MPa)	Achieves proper sterilisation, but this method can only be used to sterilise growth medium, e.g. starter culture milk or agar.

Data compiled from BSI (1977), Meynell and Meynell (1970) and Zall (1990).

4.8.2 Chemical agents

Many chemical preparations can be used as sterilising agents and such compounds are used either alone (i.e. as sterilant) or combined with other chemicals (e.g. detergent/sterilisers). The former type is more widely used in the yoghurt industry and the efficiency of these chemical agents is influenced by the following factors:

• concentration of the chemical compound(s) in the sterilising solution,
• contact time between the chemical solution and the surfaces of the processing equipment,
• temperature and pH of the chemical disinfectant,
• amount of residual soiling matter in the processing equipment,
• type(s) of micro-organisms being inactivated,
• hardness of the water,
• inactivation by combination with residual detergent.

According to BSI (1977, 1984), chemical disinfectants which are commonly used in the dairy industry are as follows.

4.8.2.1 Chlorine

The most common source of chlorine is hypochlorite (sodium or calcium). These chemical compounds may be obtained in liquid or powder form and their bacteriocidal effect is due to the release of chlorine which is normally in the range 50–250 μg ml^{-1} depending on the application.

Chlorine compounds in the undiluted form are corrosive to equipment and can be hazardous to health and should always be handled with care and at the correct concentrations. The following aspects may also be considered:

- Rinse the equipment thoroughly after the detergent wash, i.e. before circulating the hypochlorite solution.
- If an acid wash is incorporated into the cleaning cycle, the post-acid programme will be: (a) rinse with water, (b) rinse/wash with alkaline solution to remove all residues of acid, (c) rinse with water, and (d) sanitise with hypochlorite solution.
- Owing to the corrosive nature of chlorine, the sterilisation of utensils and equipment is often carried out immediately before use.
- The recommended working concentration is 200–250 μg ml^{-1} at 40°C for 10 min or for 15 min at ambient; at higher temperatures, the chlorine volatilises and loses its bacteriocidal effect.
- The concentration of a sterilising solution of hypochlorite must always be checked to maintain its bacteriocidal power.

Although not normally used in the dairy industry, other forms of chlorine which could be used for sterilising purposes are elemental chlorine (available in a gas cylinder) and chloramine-T; the latter compound has a slow acting bacteriocidal effect compared with the inorganic sterilising agents. The combined detergent/sterilisers contain chlorine in the form of dichlorodimethyl hydantoin and/or sodium dichloroisocyanurate and the upper working temperature is around 70°C.

4.8.2.2 Quaternary ammonium compounds (QACs)

These compounds are basically cationic, surface-active bacteriocidal agents, for example alkyldimethylbenzyl ammonium chloride (benzalkonium chloride). QACs are sometimes used as detergent/sterilisers but, as the formulation is dictated by the needs of the manufacturer rather than the user, it should be noted that certain alkaline compounds (anionic wetting agents) can reduce the bacteriocidal action of QACs. It should also be noted, regarding QACs, that:

- They are stable in concentrated form and have a long shelf life.
- In concentrated form they are much safer to handle than hypochlorite solutions and they are relatively non-corrosive to metals.
- Owing to their high surface activity, excessive foam can be produced during circulation through the plant and hence QACs are sometimes difficult to rinse away.
- Factors that can impair their bacteriocidal effectiveness are the presence of organic matter, water hardness which can reduce their activity and the type of organism; that is, Gram-negative bacteria like coliforms and psychotrophic organisms may be less affected, especially at low concentrations (e.g. at <50 μg ml^{-1} of QAC at 10°C), than Gram-positive bacteria (e.g. staphylococci and streptococci) and a buildup of organisms resistant to QACs may develop in the plant.

- Recommended concentrations vary from 150 to 250 $\mu g\,ml^{-1}$ of QAC at >40°C for a contact time of not less than 2 min.

4.8.2.3 Iodophores

The bacteriocidal compound is iodine which has been combined with a suitable non-ionic surfactant to provide a usable product; the iodine complex is acidified with, for example, phosphoric acid for better stability and improved bacteriocidal effect. Iodophores are often considered as detergent/sterilisers due to the presence of surface-active agents together with the acid, and in general:

- The recommended level in solutions is 50–70 $\mu g\,ml^{-1}$ of free iodine in water of moderate hardness and the pH of solution should be around 3; hard water can neutralise the acid in the iodophore.
- Iodophores have a good shelf life at ambient temperatures, but some iodine may vaporise; however, excessive loss occurs at temperatures above 50°C.
- Some plastic materials, e.g. gaskets, can react with iodine and the product can acquire an iodine taint.
- Iodine stains any residual soiling matter on the surfaces of equipment and visual inspection of the plant can indicate the standard of hygiene.
- Milk residues can inactivate the iodine and an early indication of this loss is the fading of the amber colour; therefore, always check the strength of the iodophore, especially if the solution is recirculated.

4.8.2.4 Miscellaneous sterilising agents

Amphoteric (ampholytic) surface-active agents are known to have good detergent/steriliser properties, but due to their high foaming characteristics, they are not recommended for CIP. However, they are used for manual cleaning, since they are non-corrosive and non-irritant to skin.

Acidic sterilising agents are formulations that consist mainly of inorganic acids (e.g. phosphoric acid) and an anionic surfactant. They are used as combined detergent/sterilisers, or as sterilising agents per se. The latter type has a strong bacteriocidal action, albeit generally slower than hypochlorite and the sterilising effect is due to the highly acidic conditions produced at normal concentrations (e.g. pH 2). However, this low pH may be corrosive to metals, since it is equivalent to the acid wash employed to remove milkstone.

Sodium hydroxide (caustic soda) has a bacteriocidal effect due to its high alkalinity. Concentrations of 15–20 $g\,l^{-1}$ at 45°C for 2 min are sufficient to inactivate non-spore forming organisms. An improved sterilising action is achieved at higher temperature (e.g. >70°C) and may be used for washing glass bottles.

Mixed halogen compounds containing chlorine and bromine can be employed and due to the synergestic effect, these halogens can be employed as sterilants at lower concentrations than the individual elements.

Formaldehyde is used for sterilising and/or storage of membrane plants.

Hydrogen peroxide (H_2O_2) is used in some parts of the world for the chemical sterilisation of milk. Although a large number of vegetative bacterial cells are destroyed, spore formers (aerobic and anaerobic types) survive. However, in the present context, hydrogen peroxide can be used for the sterilisation of packaging material (e.g. aseptic types from Tetra Pak, Gasti and Pure Pak). Either the packaging material is passed through a bath of H_2O_2 solution (e.g. Tetra Pak) or the

finished carton is "fogged" with a mist of H_2O_2 (e.g. Pure Pak). The solution contains a 15% concentration of H_2O_2 and the carton is then heated (i.e. hot air 80–90°C) to remove any remaining H_2O_2; however, concentrations up to 30% have been reported by Hahn (1981) for sterilising plastic yoghurt cups and up to $1500 \mu g \, ml^{-1}$ is recommended for sterilising RO plants. Since H_2O_2 is a strong oxidising agent and potentially explosive, it is advisable to handle it with extreme care.

Non-acceptable types of sterilising agents are used for general disinfection and/or sterilisation purposes, but are not normally used in the dairy industry; their inclusion in this section is for information only. Examples of these compounds are:

- lysol and other phenolic compounds,
- heavy metals (e.g. mercury, zinc, silver, lead and copper),
- volatile disinfectants (e.g. liquid ethylene oxide, β-propiolactone or chloroform),
- alcohols are of limited application (i.e. for sterilising laboratory utensils).

4.8.3 Filtration

The sterilisation of liquids can be achieved by filtration, but it is the treatment of air which is of real significance in the yoghurt industry. Air filters are normally fitted in a starter culture laboratory, so that the air is cleaned of the majority of dust particles, bacteria, yeast and fungal spores. Special filters are also available to trap airborne bacteriophages. The sterility of the culture propagation room is further maintained by having the pressure of the filtered air in the room slightly above atmospheric so that, on opening the starter room or the yoghurt processing and packaging areas, pressurised air passes outward, so preventing unfiltered air from entering the sterile area.

4.8.4 Irradiation

Irradiation can be used in the laboratory and the processing area to maintain a clean atmosphere and ultraviolet (UV) radiation, in particular, has been used with success. The wavelength of UV has to be less than 400 nm and more than 180 nm (*c.* 260 nm), to be effective; the latter figure (180 nm) is critical, since below 180 nm the radiation is absorbed by atmospheric oxygen. The effect of UV radiation on microorganisms is either inactivation or destruction, mutation, or the induction of phage growth in lysogenic bacteria.

Some practical applications in the dairy industry are sterilising the air entering a laboratory, starter culture room and/or processing area and sterilising packaging materials before filling (Anon., 1979). It must be emphasised that it is important to protect the eyes from UV radiation, because the microbiological wavelengths can cause damage.

4.8.5 Spraying, fogging or fumigation

Solutions containing active chlorine or formaldehyde can be sprayed/fogged into the atmosphere of an enclosed room with the objective of destroying aerial contamination in the form of bacteriophage particles and/or mould spores. However, excessive use of chlorine-based chemicals may result in severe rusting of exposed metal objects (e.g. window frames or steel beams), and fumigation with formalin

may be hazardous, especially when used in mixtures with potassium permanganate (BSI, 1977, 1984); as a precaution, always add formalin after a permanganate treatment and not before. It must also be stressed that the inhalation of low levels of active chlorine or other fumigants over a long period of time could lead to pulmonary damage in susceptible individuals and hence application of the technique must be carefully monitored.

There are, therefore, many different methods which could be employed for the sterilisation/sanitisation of yoghurt processing equipment, but by far the most popular methods are:

- sodium hypochlorite to sanitise milk storage tanks and yoghurt incubation/fermentation tanks;
- hot water circulation to sanitise the milk processing equipment and, possibly, the bulk starter tanks;
- chemical solutions to sanitise yoghurt filling machines;
- H_2O_2 or UV radiation to sanitise packaging materials;
- autoclaving to sterilise laboratory utensils and bacteriological media; glassware may be treated in a hot oven.

The ultimate choice of any method of sterilisation/sanitisation is governed mainly by the recommendations of the equipment manufacturer supported by the degree of hygiene required by the quality controller manager. Obviously variations exist between one yoghurt plant and another in respect of procedures for cleaning and/or sterilisation, but a summary of some recommended methods for sterilisation is provided in Table 4.10.

4.9   Kinetics and mechanisms of microbial destruction

The growth of micro-organisms is governed by such factors as moisture content of the growth medium, availability of nutrients including trace elements, the presence or absence of oxygen; pH and temperature. However, manipulation of these factors is used by dairy scientists and/or processors to control microbial growth in the manufactured product and achieve their destruction/inactivation during sterilisation processes, that is, the product or equipment.

The temperature range over which micro-organisms can survive runs from as low as –250°C to as high as 150°C or above, but in practice the limits are less extreme. The thermal death point varies from one bacterial species to another and the spore-forming bacteria are the most heat resistant; their destruction relies not only on the level of heat applied, but also on various intrinsic and extrinsic factors, such as the age and thermal resistance of the organisms, as well as the water activity, pH and type of substrate. The protective action of a substrate is especially important and it is for this reason that processing equipment must be free from any soiling matter in order to achieve an effective sterilisation.

The criterion of death of a micro-organism is usually equated with the loss of its ability to reproduce, including the inactivation of spores. Thus, as the temperature is gradually increased above the optimum growth condition of the organism, cell injury or stress starts to occur and these changes can ultimately lead to death. It is important to note, however, that although some injured cells may be unable to reproduce, they can become viable again once the damage has been repaired. This

Table 4.10 Guide to procedures recommended for sterilising/sanitising yoghurt plant and equipment

	When sterilising is carried out separately following cleaning						When cleaning and sterilisation are carried out as a combined operation using a detergent/steriliser							
	Heat		Chemical agents				Chlorine-based products				QACs		Iodophores	
							Alkaline		Neutral					
	Steam	Hot/ boiling water	Sodium hypochlorite	Iodophors	QACs	Amphoterics	Low foam	Average foam	Low foam	Average foam	Low foam	Average foam	Low foam	Average foam
Milk storage vessels	A	C	A	A	A	B	A	B	A	B	A	B	A	B
Heat exchangers	C	A	B	C	C	C	B	C	C	C	C	C	C	C
Homogenisers	C	A	C	C	C	C	C	B	C	C	C	C	C	C
Culture, fermenting and fruiting vessels	A	C	A	A	A	B	A	B	A	B	A	B	A	B
Cans and lids	A	C	C	C	C	C	A	A	A	A	A	A	B	B
Pipelines and pumps	B	A	A	A	A	B	A	A	A	A	A	A	B	B
Yoghurt bottle and carton filling and capping machines	B	B	A	A	A	B	A	A	A	A	A	A	A	A

A: Suitable. B: May be suitable; investigate thoroughly before using. C: Not suitable or not normally used.

After BSI (1977). Reprinted with permission of British Standards Institution, London, U.K.
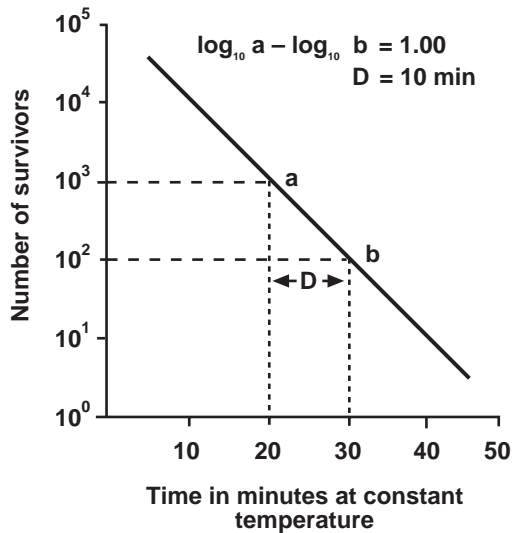
Fig. 4.12 Illustration of a hypothetical survivor plot
$Log_{10}a - log_{10}b = 1.00$; $D = 10$ min.

type of unpredictable behaviour highlights the complexity of thermoprocessing and although a few general points are discussed below, it is advisable that the field should be explored further (for example, Brown and Melling, 1971; Nickerson and Sinskey, 1972; Stumbo, 1973; Fellows, 1988; Burton, 1988; Pflug and Holcomb, 1991; Russell *et al.*, 1992; Pettersson *et al.*, 1996).

In pure culture and under ideal conditions, the death rate of micro-organisms is considered to be logarithmic. If the number of viable cells is plotted against time of exposure at a given temperature, a straight line will be obtained (see Fig. 4.12). From such a survivor curve the decimal reduction time D value can be calculated and according to Stumbo (1973), it is defined as follows:

> D value is the time required at any temperature to destroy 90% of the spores or vegetative cells of a given organism; numerically, equal to the number of minutes required for the survivor curve to traverse one log cycle; mathematically, equal to the reciprocal of the slope of the survivor curve.

It is important when D values are quoted that the temperature should be stated also. For example, if the temperature of exposure is 90°C then the D value is expressed as $D_{90} = 10$ min (see Fig. 4.12). According to Olson and Nottingham (1980), the straight line of Fig. 4.12 extends, in theory, below the base line, that is, into the area of negative logarithms, but in practice, of course, the number of organisms is rarely reduced to zero and hence there is always the probability of survivors. Thus a heat treatment (e.g. during the processing of food or the cleaning/sterilisation of equipment) is predetermined in order to obtain an acceptable level of microbial destruction.

Thermal death times are a measure of relative resistance of micro-organisms to different lethal temperatures. Figure 4.13 illustrates a hypothetical example. The
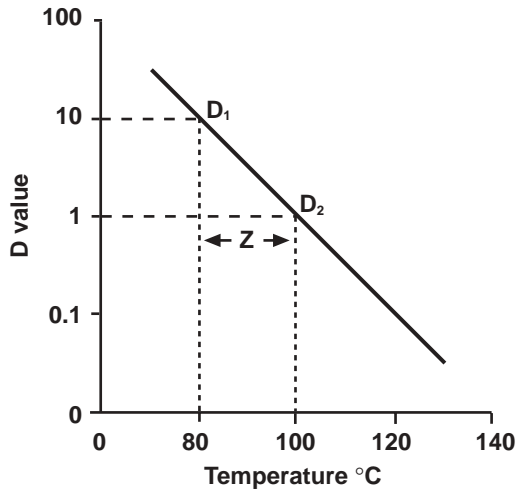
Fig. 4.13   Illustration of a hypothetical thermal death time plot
$\text{Log}_{10}D_1 - \text{log}_{10}D_2 = 1.00; Z = 20.$

slope of the curve is referred to as the Z value and it is defined by Stumbo (1973) as follows:

> Number of degrees Celsius or Fahrenheit required for the thermal destruction curve to traverse one log cycle. Mathematically, equal to the reciprocal of the slope of thermal death curve.

Hence, both D and Z values can be used during the calculation of a heat process and the sterilisation effect is expressed as the F value which is defined by Stumbo (1973) as follows:

> The equivalent, in minutes at 121.1°C, of all heat considered, with respect to its capacity to destroy spores and vegetative cells of a particular organism.

An illustrated example of F value is the time in minutes required to destroy a specified number of spores at 121.1°C when $Z = 10$.

Another value which is sometimes considered in heat processing is Q_{10}, which is the ratio of the rate of one temperature to that at a temperature 10°C below it. Therefore the gradient $1/Z = \log Q_{10}/10$. The kinetic relationship between Q_{10} and Z is discussed in detail by Stumbo (1973). However, the mechanisms involved in the inactivation of micro-organisms by heat are considered to be chemical in nature. Rahn (1945) considered the logarithmic order of death of micro-organisms to be due to a loss of reproductive power. Since moist heat is more effective than dry heat, it is suggested that the heat energy results in extensive molecular disorganisation in the microbial cell and denaturation of the protein constituents and, in particular, the deoxyribonucleic acid (DNA) units responsible for cell reproduction. By contrast, mechanisms of inactivation by dry heat are not well established, but as reported by Meynell and Meynell (1970), could be due to a mutagenic action which gives rise to multiple lesions in the DNA.

Table 4.11 Mode of action of chemical sterilising agents against micro-organisms

Type of compound	Mode of action
Alcohol group	Possible actions are: • denaturation of proteins, or • interference with cell metabolism, or • lytic action
Phenol	Cause physical damage to the cell wall of organisms
Chlorine	Possible mechanisms are: • hypochlorous acid combines with the protein in cell membranes to produce certain compounds which interfere with cell metabolism, or • chlorine inhibits certain enzymatic reactions
Iodine	Possible mechanisms are similar to chlorine above

Bacteriocidal effect of chemical sterilisers is also affected by other parameters, e.g. pH, solvent composition and the presence of electrolytes. Data compiled from Zall (1990).

The biocidal mechanisms of each chemical sterilising agent are rather different and some relevant information is illustrated in Table 4.11.

4.10 Means of assessing the sanitary condition of a processing plant

Inspection of a yoghurt processing plant is a routine exercise which must be carried out in order to ensure that the cleaning and sterilisation operations are properly conducted. Different methods and/or techniques have been devised by quality controllers to monitor the sanitary condition of the plant, thus maintaining a good keeping quality of the manufactured yoghurt and at the same time meeting the requirements of the health authorities. The available methods of inspection are divided into the following categories.

4.10.1 Physical examination

This technique may involve the use of sight, feel or smell. The former two approaches can be useful to confirm the presence or absence of soil, since the absence of soil indicates that the plant has been adequately cleaned. However, the use of certain chemicals, a buildup of milkstone on plant surfaces, or merely wear and tear can affect the original shine of stainless steel. An acid clean can remove the layer of milkstone and leave a bright shiny surface, but not in the other cases. Although iodophores are not widely used, they offer one advantage in that any residual soil on plant surfaces becomes an amber colour and can then be detected by sight.

By contrast, unclean odours are indicative of inadequate cleaning and yoghurt incubation tanks that have not been properly sanitised can often be detected by smell alone.

A UV light (black lamp) can be useful to detect soil on clean plant surfaces, since where the rays are absorbed by inorganic and organic substances (e.g. calcium salts and casein) light is given off (Zall, 1990); the use of a "black lamp" should be complementary to other types of test performed by quality controllers.

4.10.2 Chemical examination

If detergent and sterilising agents are used for cleaning and sanitising purposes it is imperative that chemical tests of rinsing water are carried out to detect residues of these compounds. Thus, the presence of detergent and/or sterilant compounds could be directly related to a faulty CIP programme, because the final rinsing stage is either too short or not performed properly. Alternatively, it could reflect some fault in plant design, so that the cleaning and sanitising compounds are not being drained completely. The nature of the tests depends on the type of detergent and/or sterilising agent used. An example of one such test is the use of bromo thymol blue indicator on both the rinsing/drainage water and normal plant water (i.e. control). A colour change to yellow of the sample water indicates the presence of acid and a blue colour is due to alkali compounds. It is debatable whether or not pH measurement is reliable enough to detect traces of acid or alkali, but rinses containing high concentrations of these compounds can be detected easily using a pH meter.

4.10.3 Bacteriological examination

Microbial counts of plant surfaces, processing equipment and packaging materials are direct evidence of the hygienic quality of the plant. Different methods have been described for bacteriological examination of equipment, and examples of these can be found in BSI (1991) and APHA (1993).

Enumeration of total counts of bacteria, coliforms and yeast and moulds are the most popular microbiological examinations carried out and the types of microorganisms present reflect, to some extent, the standard of plant hygiene. The examination of processing equipment, packaging containers and other utensils for microbiological purposes can involve the swab technique, the rinsing method (or a combination of both) and/or agar impression plates (Lück and Gavron, 1990).

Swabs can be prepared either in the laboratory or purchased ready made from different suppliers; alternatively, agar contact slides could be used. In the rinsing method, a processing tank, glass bottle, milk churn or yoghurt container is rinsed with sterile water or Ringer's solution and the sample is analysed for total bacterial numbers or the presence of different types of organism.

In cases where the volume of rinse is large or the microbial load is low, it is advisable to filter a known volume of sample through an appropriate membrane, lay the membrane onto the surface of a pre-poured plate of nutrient agar, and then incubate; any micro-organisms trapped on the membrane should grow into visible colonies over 48–72 hours; the direct epifluorescent filter technique (DEFT) system (see Chapter 10) could also be adapted to examine rinse water.

Finally, two areas which must not be overlooked are the air and the general state of building (e.g. walls, drains, etc.) (refer to Chapter 3). Exposing agar plates to the atmosphere can prove helpful, especially during the summer months when the aerial mould spore level is high and an upward trend in counts can act as a warning of an increased risk of contamination of the yoghurt; the risks of infection by aerial spores

can be reduced by preventing draughts in the processing area. In addition, the general condition of the walls and floors in the processing and packaging areas provides an insight into the overall standard of hygiene.

Effluent treatment

4.11 Background

Water is used in the dairy industry for processing (e.g. heating, cooling, recombining powders) and cleaning purposes (e.g. equipment and dairy premises), and it is safe to assume that any waste water from processing will not contain as high a percentage of polluting materials as the water used for cleaning. This latter waste water or effluent has to be treated before it is discharged into the public sewer or into a river or water way and, from a yoghurt plant, the effluent will consist of milk base, dilute yoghurt and/or bulk starter culture, dilute fruit, dilute stabilising compounds and detergent and/or sterilising agents.

The volume of effluent arising in a dairy plant is dependent on two main factors, the type of dairy product being processed and the degree of water management being exercised and thus the amount of water being conserved. For example, cheese, milk powder and evaporating plants generate larger volumes of effluent than a dairy pasteurising milk, and ratios have been worked out in the dairy industry indicating the volume of water required to process a certain volume of milk. Unfortunately data concerning yoghurt production are not widely available, although the IDF (1981) did report the following water to milk ratios for the production of yoghurt in France: food grade water was $0.5–1.0 l^{-1}$ of milk, boiler water was $0.2–0.35 l^{-1}$ of milk and cooling water was $2.0–4.0 l^{-1}$ of milk. In addition, Hiddink (1995) reported on water consumption for liquid milk or desserts processing in some European countries and the water-to-milk ratios ranged between $0.5–12.9 l kg^{-1}$ of milk. In view of the increased cost of water and effluent treatment, any reduction in water consumption is essential. This can be achieved by proper management (i.e. minimise water leaks from rubber hoses or recovery of the final rinse of the CIP cycle) reducing the demand of the cooling systems by, for example, using cooling towers and air coolers, and making extensive use of heat/cold regeneration in the processing equipment.

In view of the limited technical data available on yoghurt effluent, it is recommended that the reader consults the following International Dairy Federation documents (IDF, 1974, 1978, 1983, 1990, 1997d), Radick (1992), Strydom *et al.* (1993), Barnett *et al.* (1994), Tiwari (1994), Thakare *et al.* (1996), Samkutty *et al.* (1996), Viraraghavan and Varadarajan (1996), Kadam and Saxena (1996), Russell (1996) and Ordolff (1998) for general information regarding dairy effluent treatment.

4.12 Nature of pollution

A yoghurt effluent can contain organic and inorganic matter which is then subject to biological decomposition by micro-organisms. Oxygen is required for this biological process and if highly polluted water is discharged directly into rivers or other water ways, the dissolved oxygen in the water will be utilised. The result is that, in

extreme cases, life in the water reaches a standstill (i.e. stagnant water). The amount of oxygen required to decompose the total solids in an effluent is used, therefore, by major water authorities all over the world to assess whether waste water should be treated before discharge. The parameters used to assess the level of pollution in dairy effluents are as follows:

- Biological oxygen demand (BOD) is the amount or quantity of oxygen required by aerobic micro-organisms to decompose/stabilise the organic matter in effluent held at 20°C for 5–7 days. The sample is presedimented or filtered before conducting the test.
- Chemical oxygen demand (COD) is the amount or quantity of oxygen required for chemical oxidation. The effluent sample is filtered and/or sedimented, boiled in the presence of acid dichromate with silver sulphate as a catalyst and finally titrated. The organic matter reduces part of the dichromate and the balance is determined by titration. Hence, COD is a measure of the amount of oxygen absorbed by the dichromate.
- Permanganate value (PV) is a quick test to determine the chemically oxidisable organic matter in a sample. The effluent sample (sedimented and/or filtered) is boiled in acid or alkaline permanganate and the balance of unoxidised permanganate is determined by iodine titration. The presence of ferrous ions or nitrite in the sample can interfere with the accuracy of the PV test and hence this test is normally carried out before the BOD test as a preliminary indication of the magnitude of the oxygen demand.
- Total organic carbon (TOC) test involves the complete oxidation of all organic carbon constituents in the effluent sample to carbon dioxide.
- Total organic solids (TOS) content of the effluent sample is the difference between the total solids and the ash. The former is determined by drying at >100°C, and ashing takes place on heating the sample to >550°C.
- Miscellaneous tests may comprise the determination of fat, lactose and protein in a dairy effluent, and the level of surface-active agents (from detergent compounds). In the latter test, the sample is treated with methylene blue and, owing to the presence of anionic surfactants, insoluble blue salts are formed. The salts are extracted with chloroform and measured photometrically.

Other tests, which could be of some value in assessing the inorganic pollution likely from a yoghurt effluent, are pH, ammonia nitrogen, nitrate and nitrite, and phosphorus.

4.13   Methods of effluent treatment

A dairy effluent can be treated mechanically, chemically, biologically or by a combination of these methods. The mechanical treatment simply removes the insoluble matter from the effluent with the aid of filters, screens or sedimentation. Another mechanical system is flotation, in which air bubbles are passed through the effluent and, as they rise to the surface, small particles of solid matter become attached; the resultant scum can then be scraped off.

  The use of certain chemical compounds (e.g. iron sulphate or chloride, or aluminium sulphate) can precipitate the dissolved constituents in the effluent, and the

precipitated matter is then removed by mechanical separation. However, chemical treatments cannot remove the lactose or other dissolved sugars.

Biological treatment of dairy effluents is widely practised and purification of any waste water is accomplished either by decomposition of the organic substance(s) by the aerobic activity of micro-organisms, or as the result of anaerobic fermentation. In the oxidative approach, the oxygen is supplied artificially by means of special aeration inlets, but a septic tank is required for the anaerobic process.

The treatment of any type of dairy effluent, including that from a yoghurt plant, is usually carried out using the combined processes of mechanical separation and biological purification and the overall process is divided into three main treatments:

- primary (effluent roughing)
- secondary
- tertiary (effluent polishing).

Figure 4.14 illustrates the different types of process employed for the treatment of effluents from yoghurt plants.

Data regarding the treatment of dairy effluents are not widely published, but a study carried out by Gaster (1972) on a plant producing fruit yoghurt is summarised in Table 4.12. Two biofilters were used and the effluent plant was capable of handling 550000 l day^{-1}. Settlement of the effluent was carried out prior to the roughing stage and because of the nature of yoghurt (low pH) large volumes of sludge were removed. The reduction in BOD was about 75% and the filter beds were relatively small, compared with other creameries, since the greater part of the effluent load was being removed by the high rate biofilters.

Nevertheless, environmental issues and legislation have placed increased pressure on the dairy industry over the last few decades and Stevens (1986, 1993, 1995) has reviewed the legal aspects of dairy effluent treatment and control and developments in wastage control. However, according to Stafford (1992), anaerobic fermentation of the effluent prior to further treatment and discharge into the environment has two main advantages: first, the energy can be recovered and utilised, and second, the reduction in BOD/COD pollution consumes no oxygen; some

Table 4.12 Some data regarding the treatment of effluent from a yoghurt factory

Treatment and/or process	Capacity
Daily throughput	546000 l
Balancing capacity	273000 l
Roughing treatment	Two-stage flocor tower
	590000 l
Polishing treatment	Two filter beds, e.g. alternating double filtration (ADF)
	271000 l
	BOD load
Raw effluent	1000–1500 ppm (2400 ppm highest level)
To the plant	654 kg BOD day^{-1}
To biofilter	1.39 kg BOD m^{-1} day^{-1}
BOD reduction in roughing state	75–85%
To percolating ADF	0.28 kg BOD m^{-1} day^{-1}
Final effluent	15–25 ppm

Adapted from Gaster (1972).

PRIMARY TREATMENT

Crude Effluent

| Screening | Settlement | Disintegration | Separation of Grease and Oil | Balancing | pH Adjustment | Cooling | Nutrient Addition |

SECONDARY TREATMENT   To Sewer

Biological Filtration

1. High Rate
2. Single
3. Double
4. Alternating Double   Filtration

Activated Sludge

| Conventional or High Rate | Extended Aeration | Contact Stabilisation | Oxidation Ditch | Aeration Lagoons |

Balance Tank

Flow Gauges

One or Two Filters

Sludge ← Primary Humus Tank

Sludge ← Secondary Humus Tank

Aeration chamber

Settling

Sludge ←

Contact Zone

Settling

Re-aeration chamber

Sludge

Ditch with Surface Aerator

Settling

Sludge ←

1st part of Lagoon

2nd part of Lagoon

Decanting Wier

TERTIARY TREATMENT

To River ←   Final Effluent

| Micro Strainers | Sand Filters | Grass Plots | Lagoons | Chemical Treatments |

To River

Fig. 4.14   Possible treatments of effluent from a yoghurt plant

Data compiled from Cooper (1974), Zabierzewski and Thom (1978), Synnott *et al.* (1978) and Odlum and McCarthy (1978).

aerobic polishing of the effluent will be required to meet the standards for river quality discharge. Under certain conditions, the aerobic biological treatment of dairy waste water results in poor sludge settling (i.e. bulking) due to the presence of highly soluble inorganic components and the high COD:N:P ratios (Donkin, 1997). The problem is minimised by extending the aeration time, and the incorporation of an anaerobic or anaxion zone to facilitate the degradation of the readily metabolised lactose in the effluent. Furthermore, sludge bulking has been associated with residues containing filamentous bacteria (Donkin, 1997; see also Nyhuis, 1994; Viraraghavan and Wise, 1994; Anderson *et al.*, 1994; Monroy *et al.*, 1995; Malaspina *et al.*, 1995, 1996).

Modification of an effluent treatment plant for a cheese factory in Sweden (i.e. the aeration basin was converted to an equalisation tank, trickling filters were replaced by moving-bed biofilm reactors and a new settling tank was added) achieved average removal efficiences of 98% of both total BOD and P (Rusten *et al.*, 1996). However, removal of fat, oils and grease from waste water can improve biological treatments and the target level should be <10 mg 100 ml^{-1}; the various methods that can be used have been reported (IDF, 1997d) (see also Cordoba *et al.*, 1995; Cordoba and Sineriz, 1997).

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5

Traditional and recent developments in yoghurt production and related products

5.1   Introduction

The accepted homeland of yoghurt is the Balkan peninsula and the Middle East region. To the communities living in those parts of the world, this type of fermented milk product is identified and known as natural/plain unsweetened yoghurt. The per capita annual consumption is high and in Bulgaria, in particular, is $31.5\,\text{kg}\,\text{head}^{-1}$ year^{-1} (IDF, 1977). It is evident, therefore, that yoghurt plays an important role in the diets of these communities. Furthermore, it is customary for yoghurt to be consumed not only as a refreshing drink, but also as a main ingredient during the preparation of a wide variety of dishes including salads and soups; such food habits and their ensuing consumer attitudes may well be a contributory factor to the high annual consumption. Incidentally, recipes for yoghurt dishes are increasingly being included in cookery books, for example, Norris (1972), Hunter (1973), Nilson (1973), Orga (1975), Black (1977), Kay (1978), Newman (1978), Lanigan (1978), Stuart (1979), Hinfey (1980), Poole and Partington (1980), Butross (1982), der Haroutunian (1983), Hoffman and Hoffman (1990), Choate (1993), Fuller (1994), Banerjee (1995), White (1996) and Saleh (1996).

Prior to 1950, the acceptability of yoghurt by communities in other parts of the world (i.e. Western Europe and North America) was limited to very small minorities and to some ethnic groups descended from the Balkans or the Middle East. The reason for this lack of popularity has been attributed to the fact that:

- natural yoghurt has a distinctive acidic, sharp flavour which can limit consumer acceptability;
- yoghurt does not play an important role in the diets of such communities;
- the type of food prepared does not require yoghurt as a raw material;
- the preference for other fermented dairy products, e.g. cheese;
- limited diversity of yoghurt and related products available on the markets;
- lack of consumer knowledge about the health properties of yoghurt and bio-yoghurts.

Despite the proximity of Europe to the Middle East, the popularity of yoghurt did not spread and it was not until the 1950s in Switzerland that a major development in the yoghurt industry took place, namely the introduction of fruit flavoured and sweetened yoghurt. Since that time the popularity of yoghurt had spread to other parts of the world, and consumption has increased significantly (see Table 1.2). It could be argued that the increased acceptability of yoghurt is the result of the fact that:

- Good marketing and advertising campaigns have been used to improve the image of the product and hence increase sales to the consumer.
- The production of low fat yoghurts has been used to encourage the diet conscious consumer to include it as part of his/her slimming programme.
- Communities in western Europe and North America have a preference for sweet products and hence the sweetened yoghurt was readily accepted.
- Yoghurt is consumed as an off-the-shelf dessert and not for the preparation of yoghurt dishes.
- Some of the yoghurt advertisements have been geared towards the younger generation and their response to the message has been enthusiastic.
- Continuous research and development is taking place in order to innovate yoghurt-based products which may lead to wider acceptability by the consumer.

Research and development is of great importance in the present context, for although many recent developments have their origin in the traditional processes, pressures from industry have elicited some interesting products. Some of these yoghurt-based products have been developed by industrial organisations and the available technical data are, as a consequence, somewhat limited. It was decided, therefore, to present the processing techniques in the form of schematic flow diagrams, for in this way the outlines of the process are more easily discerned; relevant scientific publications are referred to where possible. However, Mann (1987a, b, 1990a, b, 1992, 1995, 1996a–c) has published a "digest" of international dairy publications updating the technological and scientific aspects of yoghurt and related products and the reader is referred to some reviews for more information regarding indigenous fermented milk products in different countries (De, 1980; Beuchat, 1983; El-Gendy, 1983; Abou Donia, 1984; Jandal, 1988; FAO, 1990; Punjrath, 1991; Mathur, 1991; Gupta, 1992; Dirar, 1993; Kroger *et al*., 1992; Kurmann *et al*., 1992; Akin and Rice, 1994; Surono and Hosono, 1995; Steinkraus, 1996, 1997).

5.2   Standard commercial yoghurt

Commercial yoghurts are divided into three main categories, plain/natural, fruit and flavoured and these different types of yoghurt are manufactured in either the set or stirred/drinking form (see Fig. 1.3). The latter type is more popular and details of the different stages of the pre-paration of the milk base up until the addition of starter culture are given in Chapter 2. In brief the preliminary treatment of milk includes (a) the standardisation of fat content to $0.5–3.0\,g\,100\,g^{-1}$, (b) fortification of the milk solids-not-fat (SNF) to $12–14\,g\,100\,g^{-1}$, and (c) the addition of sugar and/or stabilisers (optional). The milk base is pre-warmed to about 60°C, homogenised at 17 MPa pressure, heated to 90–95°C for 3–5 min, cooled to 30–45°C and inoculated

with starter culture. Thus, the remaining manufacturing stages are illustrated in Fig. 5.1.

The current trend in commercial fruit yoghurt is towards a low calorie product. This can be achieved in many ways, for example by reducing the fat content in the milk base, by replacing the sugar with low calorie synthetic sweeteners, by replacing the milk fat with fat substitute (see Section 5.11), by the addition of dietary fibre preparations (Fernandez-Garcia and McGregor, 1997) and/or by reducing the milk solids-not-fat in the milk base and adding bulking agents like stabilisers. The latter aspect has been discussed in detail in Chapter 2 (see also Sato *et al*., 1983; Bassett, 1983; Baker, 1983, 1985; Baker and Hulet, 1988, 1989; Marin and Zee, 1992; Ramaswamy and Basak, 1992; Nielsen *et al*., 1993; Walther, 1995; McGlinchey, 1995; Cunin, 1997; Hunt and Maynes, 1997), whilst reference could be made to the following patent applications for a more complete discussion (Streiff *et al*., 1990; Singer *et al*., 1993; Shazer *et al*., 1993; Mehnert, 1996).

In an attempt to improve yoghurt consumption in different markets of the world, the product has been mixed with a wide range of food ingredients in order to provide the consumer with flavours other than fruit types. Some examples may include the use of dried fruit and vegetable powders as additives which contain natural sources of pectin and vitamin C, and such yoghurts may have therapeutic effects for patients with digestive tract disorders (Arkhipova and Krasnikova, 1995). Alternatively, carrot pulp and natural extracts obtained from raw vegetables have been used to flavour the yoghurt (Ryckeboer and Louis, 1992; Vesely *et al*., 1995), whilst Spillman and Farr (1983) evaluated consumer acceptability of a range of veg-
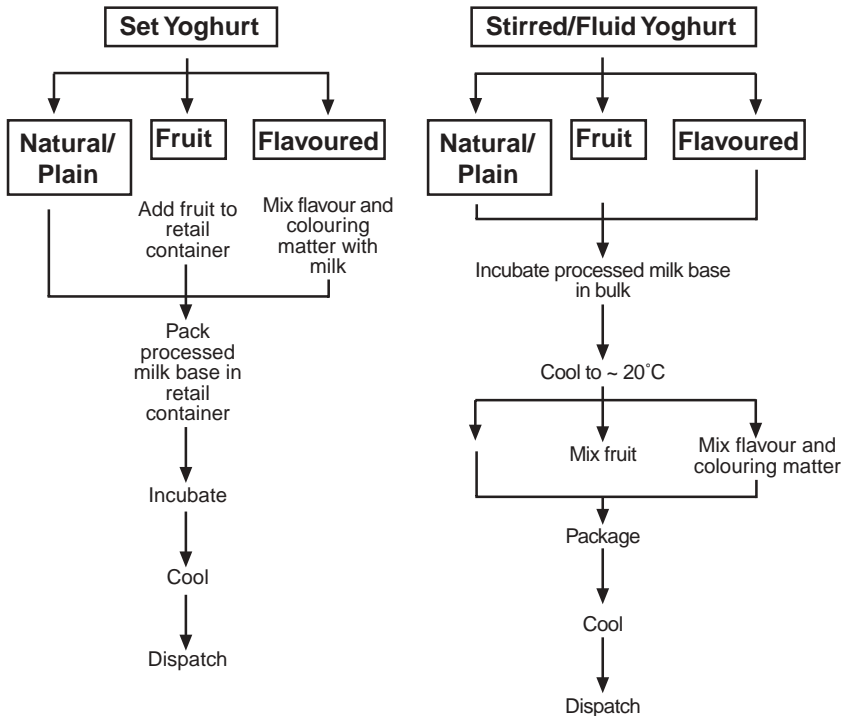
Fig. 5.1 Some of the manufacturing stages of flavoured yoghurts

etable flavoured yoghurts (cucumber, cauliflower, bean sprouts, groundnuts, celery, coconut and spices). Other proposed ideas are: (a) fruited yoghurts with added fibres from soya, oat and gum arabic (Hoyda *et al*., 1990) or cocoa pulp (Pina *et al*., 1998), (b) yoghurt for salad dressing containing salt, spices, dried onions, garlic and parsley (Steinberg, 1983a) or yoghurt dip with added onion, clam, Cheddar and blue cheeses (Steinberg, 1983b), (c) the use of puffed cereal grains that are specially treated (i.e. water-in-oil emulsion) so that the crisp texture of the cereal is maintained when mixed with yoghurt (Kaufman *et al*., 1990), (d) yoghurt fortified with calcium, which is a suitable vehicle to increase the calcium content of the product (Pirkul *et al*., 1997), and (e) special sweet toppings called "Sprinkl'ins" for a yoghurt dessert especially developed for children (Thøgersen, 1996). However, the swelling of cartons of yoghurt flavoured with cereals was attributed to the presence of *Mucor hiemalis* which appeared after 20 or 40 day storage at 12 or 5°C, respectively (Foschino and Ottogalli, 1989).

5.3 Yoghurt made from different mammalian milks

Sheep's, goat's and buffalo's milks are used for the manufacture of yoghurt and these milks are very popular in countries around the Mediterranean, Middle Eastern countries, southern Russia and the Indian subcontinent. Camel's milk may have been utilised by the nomads in the desert, but little published data are available. Although these milks are processed in a similar manner to cow's milk, the casein fractions differ, basically due to numerous breeds of goat and sheep compared with only a few among cows. According to reviews by Kalantzopoulos (1993), Bottazzi (1996) and Tamime and Marshall (1997), the reported quantities of casein components in these milks are:

- minor caseins: cow > sheep > buffalo > goat
- κ-casein: buffalo > goat > cow > sheep
- β-casein: goat > sheep > cow > buffalo
- α_s-casein: sheep > buffalo > cow > goat.

The extent of whey protein denaturation during heating is also different (Law, 1995) and, as a consequence, can affect the rheological properties of yoghurt. Specific studies on goat's, sheep's and buffalo's yoghurt has been reported by many researchers and, for this reason, the technological aspects of such products merit a separate review.

The fermentation of goat's, sheep's and buffalo's milks, including some aspects of the husbandry of these mammals, has been reported by IDF (1981, 1983, 1986, 1996), Epstein (1985), Kehagias (1987), Hansen (1989a), Boylan (1989), Anifantakis (1990), Abrahamsen and Rysstad (1991), Lokeshwar (1992), Mathur (1994), Kalantzopoulos (1994) and Gigli *et al*. (1996). However, some comparative studies using cow's, goat's or sheep's milk for the production of yoghurt give rise to the following suggestions: (a) For the production of drinking or natural set yoghurt, each type of milk should be concentrated to 18–35 g total solids (TS) 100 g⁻¹, diluted with equal volume of boiling water, spontaneously cooled to the incubation temperature and the milk fermented; this method of processing the milk produces good quality yoghurt but, with low fat milk, homogenisation is recommended (Renard, 1983),

(b) After growth of a mixed culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in milk, the expressed whey inhibited the growth of a wide range of undesirable and pathogenic micro-organisms; the inhibitory activity was buffalo > cow > goat (Singh and Kaul, 1982; Singh, 1983), (c) In Iraq, sheep's yoghurt was highly rated by a taste panel and was the firmest, whilst goat's and goat's + cow (50:50) yoghurts had the lowest scores; the starter (*Lactococcus lactis* subsp. *lactis* and *L. delbrueckii* subsp. *bulgaricus*) was recommended for making yoghurt similar to commercial products available in the market (Al-Dahhan *et al*., 1984) and (d) Kehagias *et al*. (1988) evaluated the quality of cow's, goat's and sheep's yoghurt using different commercially available starter cultures.

5.3.1 Goat's milk yoghurt

The gross chemical composition of goat's milk can vary considerably and the total solids (TS) may range between 11.3 and $15.9\,g\,100\,g^{-1}$ (Robinson and Vlahopoulou, 1988); the main causes of this variation are breed, stage of lactation, geographical location and diet. Such a view was confirmed by Kehagias *et al*. (1989) who reported that the best quality set-type goat's yoghurt was made from milk of indigenous breeds because it contained the highest TS. Whilst in India, Singh *et al*. (1991, 1996) reported that the growth of starter cultures in pasteurised goat's milk was faster than in boiled milk, that significant variation ($P < 0.01$) was observed in the growth of three mesophilic and four thermophilic starter cultures in milks obtained from four breeds of goat and that the lowest sensory scores were awarded to yoghurts made with *Lactobacillus acidophilus* and *L. delbrueckii* subsp. *bulgaricus*, and the highest to products made with single strains of *Lactococcus* species. The use of mixed strain starters improved the firmness of dahi (an Indian fermented milk) made from cow's, buffalo's or goat's milk (Katara and Lavania, 1991). However, the rate of acid development of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in sterilised milk was in the following order: goat > goat + cow > cow (Bozanic and Tratnik, 1997; Bozanic *et al*., 1998).

Thus, milk high in TS (*c*. protein $3.8\,g\,100\,g^{-1}$) should be used for yoghurt making and, as with cow's milk, different methods of fortification and processing of the milk can be used (Table 5.1, see also Park, 1994). However, the selection of starter cultures can greatly influence the organoleptic characteristics of goat's yoghurt (Castagnetti and Turtura, 1994). Whilst Ibrahim *et al*. (1990) observed enhanced growth, acid development and peptidase activity of *L. delbrueckii* subsp. *bulgaricus* in goat's milk, the observed inhibition of the yoghurt starter cultures in goat's milk could be associated with either milk containing strong "goaty" flavours or a higher concentration of free fatty acids than in cow's milk (Abrahamsen and Rysstad, 1991). Litopoulou-Tzanetaki *et al*. (1993) achieved a higher than usual concentration of acetaldehyde, diacetyl and acetoin in fermented goat's milk by using a mixture of a commercial yoghurt starter culture plus *Lactococcus lactis* biovar *diacetylactis*. In general, the citrate content in goat's milk is rather low when compared with cow's milk and, as a consequence, such milk may not be suitable for diacetyl production by mesophilic lactococci alone (Abrahamsen and Rysstad, 1991). However, low levels of acetaldehyde in goat's yoghurts have been attributed to the relatively high concentration of glycine in the milk; glycine can inhibit the enzyme involved in the conversion of threonine to acetaldehyde and glycine (Abrahamsen and Rysstad, 1991). The addition of threonine to goat's milk stimulated

Table 5.1 Some suggested processes employed during the manufacture of goat's milk yoghurt

Processes	References
Fortify the milk with 4% cow's skimmed milk powder (SMP), standardise the fat content to $2 g 100 g^{-1}$, homogenise at 19.6 MPa and heat to 80°C for 15 min.	Duitschaever (1978)
Ultrafiltration (UF) and homogenisation of the milk improved the flavour and viscosity of the product.	Abrahamsen and Holmen (1981)
Procedures for making yoghurt and cheese from goat's milk on small farms have been detailed.	Flanagan and Holsinger (1985)
Addition of cow's SMP to goats milk helped to mask the goaty flavour.	Manjunath and Abraham (1986)
Improved coagula characteristics by addition of goat's milk powder or UF of the milk; reverse osmosis (RO) process did not provide a useful method of fortification.	Marshall and El-Bagoury (1986)
Flavouring of goat's yoghurt with guava or plum syrup ($18–20 g 100 g^{-1}$) was not rated significantly different from cow's yoghurt.	Araujo *et al.* (1988)
Yoghurt made from goat's milk heated to 85°C for 20 min and incubated for 42°C for 3 hour was similar to a product made from a mixture of buffalo's and cow's milk.	El-Samragy (1988)
Homogenisation of the goat's milk and possibly the use of EPS starter cultures were identified as the most significant factors in improving the quality of stirred yoghurt.	Alexiou *et al.* (1990)
A selection of production methods have been illustrated in a patent.	Gabriel (1990)
UF of the milk to $16–18 g TS 100 g^{-1}$ followed by heating to 90–92°C for 20 min helped to produce a typical Bulgarian yoghurt.	Baltadzhieva *et al.* (1991)
Fortification of goat's milk with 10% SMP improved the quality of zabadi (an Egyptian fermented milk).	Ahmed (1992)
Vacuum evaporation of milk, homogenisation and heating at 85°C for 15 min produced a thick yoghurt with improved flavour; the addition of stabilisers improved the physical and appearance properties of the product.	Abou-Dawood *et al.* (1993)

acetaldehyde production (Marshall and El-Bagoury, 1986; Rystaad *et al.*, 1990) and some relevant information regarding the behaviour and proteolytic activities of the yoghurt starter cultures in goat's milk have been reported by Telles (1988) and Abd-Rabo *et al.* (1992).

Inoculation rates (≤1.5%) of the yoghurt starter culture have been recommended by Vlahopoulou *et al.* (1994) to produce firmer gels, but other researchers have used ≥2% (Marshall and El-Bagoury, 1986; El-Samragy, 1988; Araujo *et al.*, 1988; Alexiou *et al.*, 1990; Baltadzhieva *et al.*, 1991). However, the viscoelastic properties of goat's yoghurt when using exopolysaccharide (EPS) cultures were lower (storage modulus G' and loss modulus G'' module) than those made from non-ropy starter cultures (Vlahopoulou and Bell, 1993) and similar observations were also reported for cow's milk yoghurt (see Chapter 2).

Nevertheless, EPS starter cultures produce thicker yoghurt and the product can be diluted with water (ratio 1:0.3 or 1:0.4) and $7 g$ sugar $100 g^{-1}$ added for the production of drinking yoghurt (van Dender *et al.*, 1991), whilst Hashimoto

and Antunes (1997) recommended the heat treatment of goat's milk at 90°C for >5 min during the production of yoghurt using EPS cultures. Alternatively, UF goat's milk retentate has been used to improve the characteristics and composition of a cultured-type beverage (Miocinovic *et al.*, 1990), whilst in Poland consumer acceptability of goat's fermented milk products were in the following order: drinking yoghurt > cultured acidophilus milk > kefir (Pieczonka and Pasionek, 1995).

5.3.2 Sheep's milk yoghurt

The technology of both traditional and industrial sheep's yoghurt have been reported by Irvine (1989) and Anifantakis (1990). The main differences in the manufacturing stages are first, in the traditional process the milk is boiled, filled into containers at 95°C, allowed to cool to 45°C, inoculated with starter culture and fermented to the desired pH, and finally transferred to the cold store; such a method produces a set-type yoghurt with a crusty layer. Second, the industrial process may include standardisation of the fat content, homogenisation and heating the milk to 95°C only. The addition of aroma (e.g. fruit or flavouring substances) is optional because the majority of sheep's yoghurt is sold unflavoured. The use of two-stage homogenisation at 13.8 MPa and 3.5 MPa, respectively, has been reported by Smith (1989), whilst Muir and Tamime (1993) have examined the effect of homogenisation of the milk on the extent of serum separation and firmness of set- and stirred-type sheep's yoghurt (see Fig. 5.2). Furthermore, using milk from a commercial flock of milking sheep in Scotland, details of the effect of seasonal variation on the gross chemical composition, changes in indices of stability, microbiological quality and organoleptic properties of yoghurt have been given by Muir *et al.* (1993a–c) and Tamime *et al.* (1993) (see also Bonczar *et al.*, 1998).

Inherently, sheep's milk contains high levels of protein (*c.* 5.8 g 100 g^{-1}), and does not require fortification of the milk SNF during the production of yoghurt (Muir *et al.*, 1993a). As mentioned elsewhere, homogenisation of the milk can improve the firmness (see Fig. 5.2) and reduce syneresis of sheep's yoghurt (Muir and Tamime, 1993), whilst Kisza *et al.* (1993) recommended heat treatment of the milk at 91°C for 30 s to reduce the fermentation time compared with cow's milk. The same
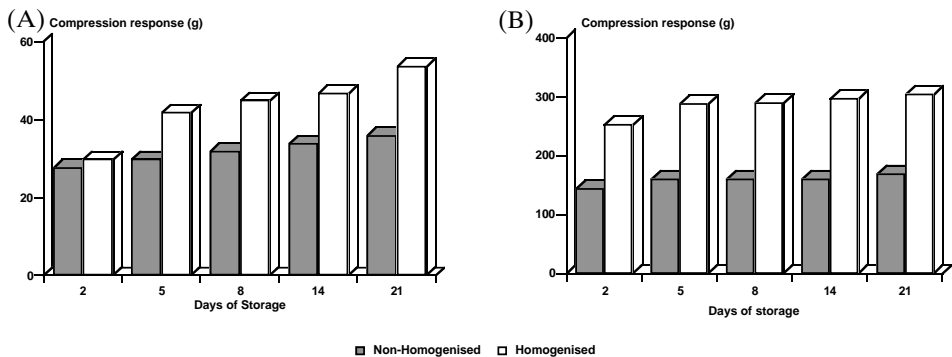
Fig. 5.2   Firmness of sheep's yoghurt (non-homogenised and homogenised) during storage for 21 days at 5°C

Data compiled from Muir and Tamime (1993).

Note: (A) Stirred yoghurt and (B) set yoghurt; to convert g force to Newtons (N), multiply 9.81 × 10^{-3}.

authors used a mixed starter culture consisting of *S. thermophilus* and *L. acidophilus* which resulted in a superior product when compared with a yoghurt starter culture (see also Creed, 1996).

Since the lactation period of sheep is about 6 months, the availability of milk for processing in dairies all the year around is limited. Hence a problem is encountered in maintaining a steady output and availability of sheep's yoghurt on the market. Some attempts have been made to preserve sheep's milk by freezing (Young, 1986, 1987; Giangiacomo and Messina, 1991). The stability of the milk during storage is governed by the temperature of freezing and the size of the block being frozen. Anifantakis *et al.* (1980) recommended the addition of $2\,g\,100\,g^{-1}$ Na-citrate and $0.1\,g$ $100\,g^{-1}$ ascorbic acid before freezing in order to improve the stability during storage (i.e. up to 11 months) and after thawing when it is heated for yoghurt making. Oxidation of the fat was more pronounced in a 7 cm thick block of frozen milk stored at −20°C, in the presence of ascorbic acid, and when compared with a 2 cm block stored at −30°C; although the free fatty acid content increased during storage, the yoghurt made from the thawed milk was acceptable by the taste panel. However, in a recent study, Voutsinas *et al.* (1996a, b) concentrated sheep's milk by RO (whole and skimmed – the latter was mixed with the cream after concentration) before freezing, and they reported: (a) no significant differences in lipolysis during storage at −20°C for up to 8 months, (b) although the initial total viable and coliforms counts were high, the number decreased during storage, and (c) the thawed and reconstituted concentrates were stable for the production of yoghurt especially for whole milk, but the product had a slight grainy texture and the extent of syneresis was higher when compared with yoghurt made from fresh sheep's milk. These results may suggest, in part, some degree of storage stability of frozen sheep's milk, but more research is required to overcome some of the faults observed during the manufacture of yoghurt.

Isolates of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* from traditional Greek yoghurt have been characterised for flavour and proteolytic activity (Kalantzopoulos *et al.*, 1990a, b; Georgala *et al.*, 1995), and combinations of these organisms have been recommended for the industrial production of sheep's milk yoghurt. In an earlier study Kehagias and Dalles (1984) noted that the β-galactosidase activity of starter cultures in sheep's milk was double that observed in a similar product made from cow's milk. However, the screening and selection of lactic acid bacteria from gioddu (a Sardinian fermented milk made with an "artisanal" starter culture plus enzymic extracts of aromatising yeasts) resulted in a sheep's product with good keeping quality, improved flavour and appearance, and a firmer product with low syneresis (Deiana *et al.*, 1992).

5.3.3 Buffalo's milk yoghurt

In Egypt, small producers manufacture zabadi by boiling buffalo's milk for 30 min, cooling it to 40–42°C, inoculating with a starter (i.e. previous day zabadi) and incubating in the retail container. By contrast, the industrial process is similar to yoghurt making since the fat content is standardised to about $3\,g\,100\,g^{-1}$, the milk is then heated (e.g. 85–90°C for 5–10 min) and finally the milk is fermented in the retail container; the addition of flavour(s) is optional (Shalaby *et al.*, 1992; Mahran, 1996; Iniguez *et al.*, 1997; see also Garg, 1988). It is of interest to point out that homogenisation is not used during the preparation of buffalo's milk yoghurt, perhaps because the milk contains $(g\,100\,g^{-1})$ protein 4.3 and fat 8.6 (Spanghero and Susmel, 1996),

which is suitable for the production of a set-type yoghurt with a creamy layer. Furthermore, a similar processing approach (i.e. non-homogenisation of the milk) is found in countries where buffalo's milk is used for the production of other fermented milk products; Singh (1979) homogenised buffalo's milk, but the pressure(s) was not reported.

As with cow's milk, different fortification and/or fat standardisation methods have been used for buffalo's milk yoghurt. Table 5.2 illustrates some examples and the processing parameters. The use of buffalo's milk powder for fortification of the

Table 5.2 Some examples of processing buffalo's milk during yoghurt making

Comments	References
As with cow's milk, strain selection and combination is important to produce good quality buffalo yoghurt.	Lal *et al*. (1978); Khana and Singh (1979)
Use of a 5% inoculation rate and incubation at 43°C for 3 hours was recommended for skimmed buffalo yoghurt.	Patel *et al*. (1983)
Reduction of dissolved O_2 to $2.9\mu g\,g^{-1}$ in milk prior to heating at 90°C for 10 min increased the rate of acid development of the starter culture and the thiol content.	Shekar and Bhat (1983)
Lactose hydrolysis of the milk (30–40%) increased the acetaldehyde content in the product and gave the highest sensory score.	Abdou *et al*. (1984)
Milk preserved with lactoperoxidase required $1\frac{1}{2}$ hours more to reach the desired acidity in buffalo yoghurt.	Kumar and Mathur (1986)
Milk is concentrated to 1/2 or 2/3 its volume to produce yoghurt, but wheying was evident when the product was stored at 33–38°C.	Reddy *et al*. (1987)
Best misti dahi was produced from partially concentrated milk (about $18\,g\,TS\,100\,g^{-1}$) + sucrose $14\,g\,100\,g^{-1}$, using mixed strains of mesophilic starter cultures.	Gosh and Rajorhia (1990b)
Use of stored UHT milk ($g\,100\,g^{-1}$) (fat 4.5 and SNF 8.5) gave bitter sensory scores when compared with dahi made by heating the milk to 90°C for 5 min.	Sharma and Prasad (1990)
Addition of stabilisers to milk or reducing the fat content to $1.5\,g\,100\,g^{-1}$ decreased the diacetyl and volatile fatty acids levels in the product.	Shukla *et al*. (1986); Shukla and Jain (1991)
Addition of 10–$12\,g\,sucrose\,100\,g^{-1}$ to skimmed milk inhibited the growth of *L. delbrueckii* subsp. *bulgaricus* during dahi production.	Amin *et al*. (1992)
Heat treatment of skimmed milk at 85°C for 5 min was recommended for yoghurt making in Spain.	Iniguez *et al*. (1992)
Standardisation of fat to $3\,g\,100\,g^{-1}$ and SNF to $10\,g\,100\,g^{-1}$ produced the best quality dahi.	Chawla and Balachandran (1993, 1994)
Milk heated at 80–82°C for 20 min, cooled to about 31°C, inoculated with single strain *S. thermophilus* and incubated for 10–12 hours produced an acceptable product	Tawfik *et al*. (1993)
Milk (fat $6.3\,g\,100\,g^{-1}$ and protein $4.7\,g\,100\,g^{-1}$) heated to 75°C for 5 min produced the most acceptable and firmest yoghurt.	Cardoso Castaneda *et al*. (1994)
Good bio-yogur was produced from mixed buffalo's (70%) and cow's (30%) milks that had been heated to 90°C for 5 min and fermented with *S. thermophilus* and *L. acidophilus*.	Iniguez *et al*. (1995)

milk is not widespread because it is not readily available, but recent studies of such a powder made from skimmed UF retentate have been reported by Patel and Mistry (1997). The gross composition $(g\,100\,g^{-1})$ of skimmed buffalo's milk powder is protein 67.5, fat 1.6, ash 8.6 and lactose 18.7.

Miscellaneous additives such as whey proteins (Ahmed and Ismail, 1978a, b), groundnut protein (Venkateshaiah et al., 1982), defatted soyabean flour (El-Deeb and Hassan, 1987; Magdoub et al., 1992), cooked wheat grain (Hamzawi and Kamaly, 1992) and cow's SMP (El-Shibiny et al., 1977) have been used to fortify milk to produce an acceptable buffalo yoghurt. The use of membrane filtration is somewhat limited for the industrial production of buffalo yoghurt, but studies in this area have suggested: (a) a two-fold concentration by UF and standardisation of the fat content to $5.5\,g\,100\,g^{-1}$ was recommended by Haggag and Fayed (1988), (b) UF could be used to manipulate buffalo's milk, for example $10\,g\,SNF\,100\,g^{-1}$ or $11\,g\,SNF\,100\,g^{-1}$ plus $3\,g\,fat\,100\,g^{-1}$ for the production of zabadi (Khorshid et al., 1992), and (c) RO of buffalo's milk >1.5-fold produced dahi that was very thick, lumpy, lacking flavour and had low acidity (Kumar and Pal, 1994).

Milk obtained from buffalos given a yeast culture in their feed affected the growth and biochemical behaviour of two mesophilic and three thermophilic single strains of lactic acid bacteria (Ibrahim, 1991). As the starter cultures employed for the production of dahi are not well defined, the general consensus is that yoghurt microfloras have been used, even though the preference in India may be to use mixed mesophilic strains including Lac. lactis biovar diacetylactis (Gosh and Rajorhia, 1990a). However, the antibacterial activity of S. thermophilus MD-2, MD-8 and D-3 strains in buffalo's milk dahi (i.e. $4.5\,g\,fat\,100\,g^{-1}$ and $10.5\,g\,SNF\,100\,g^{-1}$) against pathogenic micro-organisms was greater in the cell free extracts which may suggest that inhibitor substance(s) other than lactic acid may be present (Gupta and Tiwari, 1990; Dave et al., 1992; see also Dzurec et al., 1992). β-galactosidase activity of the same starter culture strains in dahi made up to $21\,g\,100\,g^{-1}$ TS was reported by Dave et al. (1993), whilst the incorporation of nisin into dahi and its effect on the yoghurt starter culture was studied by Gupta and Prasad (1988, 1989).

The microstructure of buffalo dahi is influenced by the level of heating applied to the milk. According to Tomar and Prasad (1989) milk heated to 70°C resulted in a product which was soft, had an open structure and the casein was near spherical in shape (i.e. a size of about 300 nm), whilst milk heated at 90°C for 30 min gave a firm curd and the micelle size was about 235 nm and elongated in shape; the protein matrix consisted of a long micellar chain (see also Turambekar and Kulkarni, 1991).

Thermisation of misti dahi at 65°C for 30 min decreased the starter cultures count (i.e. consisting of Lac. lactis biovar diacetylactis and subsp. cremoris) by about $3\log_{10}$ colony forming units (cfu) ml^{-1} and a further $1\log_{10}$ cfu ml^{-1} after storage at 30°C for 30 days (Chander et al., 1989, 1992). A similar observation was reported by Sarkar et al. (1992a, b) when the product was heated at 60°C for 10 min (see also Mann and Joshi, 1997).

It was suggested that the nutritive quality of zabadi could be improved by the addition of electrolytic iron or ferric chloride up to $8\,mg\,100\,g^{-1}$ with no effect on the quality of the product (Mahran et al., 1996). However, buffalo's milk fortified with groundnut or soya milk enhanced the growth of Bifidobacterium bifidum, whilst the addition of 3 mM glycine produced the firmest curd with a starter count $>1 \times 10^8$ cfu ml^{-1} at pH 3.89 (Murad et al., 1997).

5.3.4 Camel's milk yoghurt

Camel's milk is popular in countries that have arid regions and tropical temperatures. It is generally opaque-white in colour. The gross chemical composition can vary considerably and the main causes of variation are breed, stage of lactation, type of fodder and availability of drinking water. Some data are available on the composition of camel's milk, and the range of the various components ($g\,100\,g^{-1}$) reported in a recent review are as follows: TS 9.8–14.4, fat 3.2–5.5, lactose 3.4–5.5, protein 2.7–4.5 and ash 0.6–0.9 (Hassan *et al.*, 1987; Hagrass *et al.*, 1987; Farah, 1993; see also Mohamed, 1990; Hafez and Hamzawi, 1991; Gorban and Izzeldin, 1997).

Farah *et al.* (1990) heated camel's milk to 85°C for 30 min, cooled it to 27°C and fermented it with mesophilic lactic cultures (homo- or hetero-fermentative) for 24 hour. The products were evaluated organoleptically by 13 Somali nomads, nine Somalis (i.e. city dwellers) and three Canadians, and at the same time compared with susa (a traditionally fermented milk from Somalia). The products were highly acceptable and similar to susa, and the authors recommended the controlled fermentation of camel's milk in rural areas in order to improve the quality of susa and utilise wasted surplus milk during the rainy season.

However, Gran *et al.* (1990) and Abu-Tarboush (1996) observed that the growth of mixed or single strains of *S. thermophilus* (four) and *L. delbrueckii* subsp. *bulgaricus* (three) was higher in cow's than in camel's milk, but proteolysis was higher in camel's milk. Nevertheless, in mixed cultures, the yoghurt starters released the same amount of free amino groups except for the *L. delbrueckii* subsp. *bulgaricus* strain LB12 (Abu-Tarboush, 1996). A similar behaviour was also reported for *L. acidophilus* and four species of bifidobacteria grown in camel's milk (Abu-Tarboush, 1994; Abu-Tarboush *et al.*, 1998).

5.4 Pasteurised/UHT/long life/heat shock yoghurt

Depending on the standard of hygiene observed during the manufacture of yoghurt and the microbiological quality of the ingredients and packaging materials, the shelf life of yoghurt is around 3–4 weeks under refrigerated conditions. Various techniques have been used in order to improve the keeping quality of yoghurt, such as:

- freezing and drying
- gas flushing
- addition of preservatives
- use of aseptic equipment
- application of multiple frequency microwaves
- sterilisation by heat

(discussed under separate headings elsewhere)

and each of these approaches has its adherents.

A post-production heat treatment helps to prolong the shelf life of the product, since the application of heat inactivates the starter culture bacteria and their enzymes, as well as other contaminants, for example yeasts and moulds. Traditionally, yoghurt was heated for a few hours over low fires of a special type of wood. The end product was referred to as smoked yoghurt (see Fig. 1.2) and it was preserved over the winter months by placing in jars and covering with either olive oil or tallow. However, in a mechanised plant, the time–temperature relationships

which are used to achieve the desired effect of pasteurisation are similar to those used for liquid milk processing, although in general a lower energy input is required for yoghurt since the level of acidity is much higher than in milk (Gavin, 1966; Puhan, 1979; Driessen, 1984).

5.4.1 Technology of manufacture

Two main problems have been associated with the manufacture of pasteurised yoghurt. First, a reduction in viscosity and whey syneresis may occur and second there may be loss of flavour (this is only significant in plain/natural yoghurt). Table 5.3 illustrates the heat treatments that can be applied to produce yoghurt with longer keeping quality. To overcome some of these problems, especially when yoghurt is heated to temperatures above 70°C, the following precautionary measures are recommended:

- Cooling the yoghurt first to 20°C, and then proceeding with the heat treatment; in some instances, the heating is in two stages (i.e. 60–68°C for 5–20 min followed

Table 5.3 Reported processing conditions for the manufacture of stirred-type pasteurised/UHT yoghurt

Time	Temperatures (°C)	Improvement of shelf life	References
30 min	50–55	3 weeks at 15°C	Rakshy (1966)
15–40 s	57–70	Reducing unwanted microbial counts	Sebela (1979)
20–30 min	58–60	Inactivation of β-galactosidase	Scolari *et al.* (1983)
5 min	58	Inactivation of yeasts	Waes (1987)
	60–65	40 or 10 days at 6–8°C and 15–20°C, respectively	Karabasevic *et al.* (1983)
	60–65	6–8 weeks at 12°C	Neirinckx (1972)
30 min	60	30 days at 20°C	Goh (1985)
1 min	60 or 70		Prekoppova and Slottava (1979)
5–20 min	60–68 then to 77	Aseptic yoghurt	Barua and Hampton (1986)
5 min	64	3 weeks at 20°C	Vanderpoorten and Martens (1976)
30 s	65		van der Loo (1980, 1981)
Flash	65–70	Hot filling	von Klupsch (1977a); Mulcahy (1972)
20 min	65	7 days at 27°C	Luck and Mostert (1971)
5 min	70	21 days at ~5°C	Mohammed *et al.* (1985)
30–40 s	70	Hot filling	Dellaglio (1977, 1979)
15–30 min	70	30 or 60 days at 20°C and 4°C, respectively	Guldas and Atamer (1996)
Few s	75	4–6 weeks at 20°C	von Schulz (1969); Bake (1979)
~2 min	85	No refrigeration required	Keefer and Murray (1988)
20 s	85	3 months at 37°C	McKenna (1987)
10–15 min	85–88	1 year at 20°C	Anon. (1979a)
27 s	85	>4 weeks at 20°C	von Holdt (1978)
	88	Few weeks	Hermann (1980)

by heating to 77°C) in order to stabilise the protein without gelatinising the added starch (Barua and Hampton, 1986).

- Homogenisation of the heated yoghurt before packaging is recommended, for example, cool the heated yoghurt to about 65°C, homogenise at 5 MPa, cool to 7°C add flavour and package (Hermann, 1980).
- Hot filling of yoghurt after pasteurisation is widely practised and final cooling takes place in the retail container (see Table 5.3 for illustrated examples).
- Addition of special stabilisers is sometimes recommended, but on average, <1 g 100 g^{-1} is added depending on the type used; the following are some examples: (a) carrageenan and starch plus citrate (Barua and Hampton, 1986), (b) xanthan and guar gum mixture at a ratio of 2:1 plus disodium phosphate (Hermann, 1980), (c) the use of Gelodan which is a mixture of starch, pectin, gelatin and milk proteins (Berg and Møller, 1994; Guldas and Atamer, 1996), and (d) agar, carrageenan or pectin plus citric acid (Keefer and Murray, 1988). However, Petersen (1989) reported that carrageenan is added as a texturiser and to rebuild the rheological properties of the product after heating.
- Recommended processing equipment should be used including plate, tubular or scraped surface heat exchangers and plant to package the heated yoghurt aseptically.

Set-type yoghurt can be heat treated in the retail container and some examples are 75°C for 5–10 min (Bake, 1979), 58°C for 5 min (van der Loo, 1980), 85°C for 35 min (Pavey and Mone, 1976), 65–85°C for 30–120 min (i.e. depending on the size of the pot in order to sterilise the centre of the product) (Deschamps, 1985), 60–85°C in an autoclave for up to 50 min and pressures up to 0.2 MPa (Egli and Egli, 1976a, b, 1977, 1980) and 72°C in a water bath for 30 min (Aziz, 1985).

It is evident, therefore, that it is technically feasible to prolong the shelf life of yoghurt by the application of heat, although some controversy may exist regarding its definition as yoghurt; most existing standards stipulate that yoghurt must contain an abundant and viable population of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (Glaser, 1992). Tamime and Deeth (1980) suggested that it would be reasonable to reserve the term yoghurt for the traditional product and to designate the heat-treated product as pasteurised, UHT or long life yoghurt. Such an approach could help to ease the existing controversy, for essentially the only difference between pasteurised yoghurt and a traditional yoghurt is the low viable count of starter organisms in the former; this difference may, however, be relevant in relation to the nutritional and therapeutic aspects of the product (see Deeth and Tamime, 1981; Marshall and Tamime, 1997a, b; Buttriss, 1997, and Chapter 9). Nevertheless, von Klupsch (1977b) has recommended that the stability of heated cultured milk products should be tested during storage for 3 day at 30–37°C, 15 day at ambient temperature and 60 day at about 5°C, and the product should not show any sign of gas production or syneresis during these storage periods.

5.4.2 Processing effects on properties of product

The other constituents of yoghurt that may be most affected by heat are the vitamins and the enzymes. de Felip *et al*. (1979), comparing heated yoghurt (HY) and unheated yoghurt (UY), reported the following observations: (a) The thiamin content in both types of yoghurt was not affected by heat or cold storage, (b)

Vitamin B_6 losses appeared to be greater during the storage of HY than with UY, that is, 85% compared with 50%, (c) Folic acid decreased to a trace concentrations in HY after 15 days, but in UY a similar reduction took 30 days, (d) Pantothenic acid was initially reduced by 70% in HY, and (e) Heat treatment reduced the activities of the enzymes protease, cellulase, amylase and β-galactosidase by 60%, 25%, 50% and 100%, respectively. However, identification of the starter microflora in thermally treated, set-type, plain yoghurt using gene probes and polymerase chain reactions were dependent on the heat treatment applied and the results differed for the streptococci or lactobacilli (Lick *et al.*, 1996).

The inactivation of β-galactosidase has been reported by many researchers (Speck, 1977; Speck and Geoffrion, 1980; Lusiani and Bianchi-Salvadori, 1978; Kolars *et al.*, 1984; Gilliland and Kim, 1984; Savaiano *et al.*, 1984; Savaiano and Levitt, 1987; McDonough *et al.*, 1987; Schaafsma *et al.*, 1988; Dewit *et al.*, 1988; Lerebours *et al.*, 1989; Pochart *et al.*, 1989; Marteau *et al.*, 1990) and the reviews by Rao *et al.* (1985), Bourlioux and Pochart (1988), Fernandes and Shahani (1989), Abrahamsen (1991) and Savaiano (1994) are recommended for further reading. The presence of this enzyme in yoghurt is highly desirable, particularly for consumers deficient in lactase. Gallagher *et al.* (1974) showed that yoghurt does not have the same adverse effects as milk on lactose intolerant patients and this benefit is due to the presence of active β-galactosidase; a test on lactose-intolerant humans fed heated yoghurt confirmed the effect by measuring hydrogen in the breath. However, Hottinger *et al.* (1992) patented a process for preparing a long-life yoghurt in which each microbial flora of the starter culture has a level of 10^6–10^{10} cfu ml^{-1} after heating; a mutant strain of *L. delbrueckii* subsp. *bulgaricus* is used which lacks a fragment of the DNA containing part of the β-galactosidase gene, to ensure the survival of the micro-organisms.

An alternative method, which can be used to pasteurise yoghurt, is the application of the multiple frequency or microwave technique, known as the Bach system. The principle of this method is well documented by Bach (1977, 1978) and, in brief, it consists of a two-stage, rapid dielectric heating of yoghurt in plastic cups. The first stage is applied horizontally (low frequency microwaves with high penetration), while the second stage is applied vertically (high frequency microwaves with low penetration). The actual pasteurisation is at a lower temperature than required for a conventional process and the treatment takes place during the passage of the yoghurt cups through a water bath. The two stages are complementary to each other and are needed to achieve adequate pasteurisation. According to Bach (1977), this system results in the destruction of yeasts and moulds, but has no adverse effect on the milk proteins or the starter bacteria; the keeping quality of yoghurt is extended to 4–6 weeks at room temperature. In addition, the use of this technique does not require the addition of special stabilisers to the yoghurt. According to Reuter (1978), the additional processing cost is marginal when set against the improved shelf life of the yoghurt.

5.5 Drinking yoghurt

5.5.1 Background
Drinking yoghurt is categorised as stirred yoghurt of low viscosity and this product is consumed as a refreshing drink. The traditional Turkish yoghurt drink is known

as ayran, and Akin and Rice (1994) have detailed the stages of manufacture. Ayran can be produced from full-fat milk, and after fermentation, the yoghurt is mixed with about 35% water and $1 \, g \, salt \, 100 \, g^{-1}$, churned to remove the butter granules, packaged and stored at 5°C. However, if the fat is standardised to $1.5 \, g \, 100 \, g^{-1}$ and the SNF in the milk is not fortified, the stirred yoghurt (i.e. ayran) is mixed with salt ($1 \, g \, 100 \, g^{-1}$), packaged and stored in the refrigerator. The Turkish standard of ayran is as follows ($g \, 100 \, g^{-1}$): water 90.5, TS 9.5, SNF 8, lactic acid 1.6, salt 1 (optional) and free from pathogenic micro-organisms (Akin and Rice, 1994). In the Lebanon, a similar product to ayran is made from low fat milk and flavoured with mint extract.

The European and North American types of drinking yoghurt are made from a milk base low in fat and milk solids and the manufacture of such products is possible in most types of yoghurt plant. Under normal production practice the yoghurt coagulum is handled very carefully, but when drinking yoghurt is manufactured, the positive pumps are replaced with centrifugal pumps to transfer the yoghurt from the incubation tanks to the coolers. Alternatively, higher speeds of agitation are used to break the coagulum after fermentation, or sometimes the cold yoghurt is passed through a homogeniser without the application of pressure.

Up to the 1980s, relevant published data on drinking yoghurt were reported by many researchers (Pedersen and Poulsen, 1971; Grozdova, 1971; Rousseau, 1974; Morley, 1978, 1979a, b; Rhodes, 1978; Anon., 1979a, 1980a, 1981, 1986d; Lang, 1979, 1980; Ross, 1980; Hendricus and Evers, 1980; Yaygin, 1980; von Klupsch, 1981; Lavrenova *et al.*, 1981), whilst Mann (1983a, b, 1985a, b, 1988a, b) has published an update of the technological and scientific aspects of drinking yoghurt (see also von Klupsch, 1984; Charalambous, 1986; Driessen and Loones, 1992).

5.5.2 Processing aspects

According to Bylund (1995), commercial processes for the manufacture of drinking yoghurt could be classified into the following types:

- Homogenise stirred yoghurt, cool and package; shelf life 2–3 weeks at 5°C.
- Homogenise stirred yoghurt, pasteurise (i.e. low temperature) and aseptically package; shelf life 1–2 months at 5°C.
- Homogenise stirred yoghurt, UHT and aseptically package; shelf life several months at ambient temperature (see Fig. 5.3).

In general, milk alone is normally used for the production of drinking yoghurt but in some instances other food additives may be added to the milk. Some examples may include the addition of malt extract (Zobkova *et al.*, 1985), whey concentrate or soyabean flour (Rossi and Clementi, 1984; Kolesnikova *et al.*, 1986), whey: buttermilk mixture (60:40) (Srivastava *et al.*, 1985), processed tomato and SMP (Yokota *et al.*, 1989), sweet cream buttermilk (Choprea and Gandhi, 1989, 1990; Gritsenko *et al.*, 1993), enzyme-hydrolysed lupin seed milk (Han *et al.*, 1985), red ginseng extract (Song *et al.*, 1992) and yoghurt cultures and edible acid (Hidalgo and Dalan, 1984). It could be argued, however, that some of these products should be known as beverages rather than drinking yoghurts.

The milk base and any miscellaneous additives are normally fermented with a yoghurt starter culture, but a wide range of mixed cultures have been used. Some examples are shown in Table 5.4. Slow acidification of milk by *L. delbrueckii* subsp.
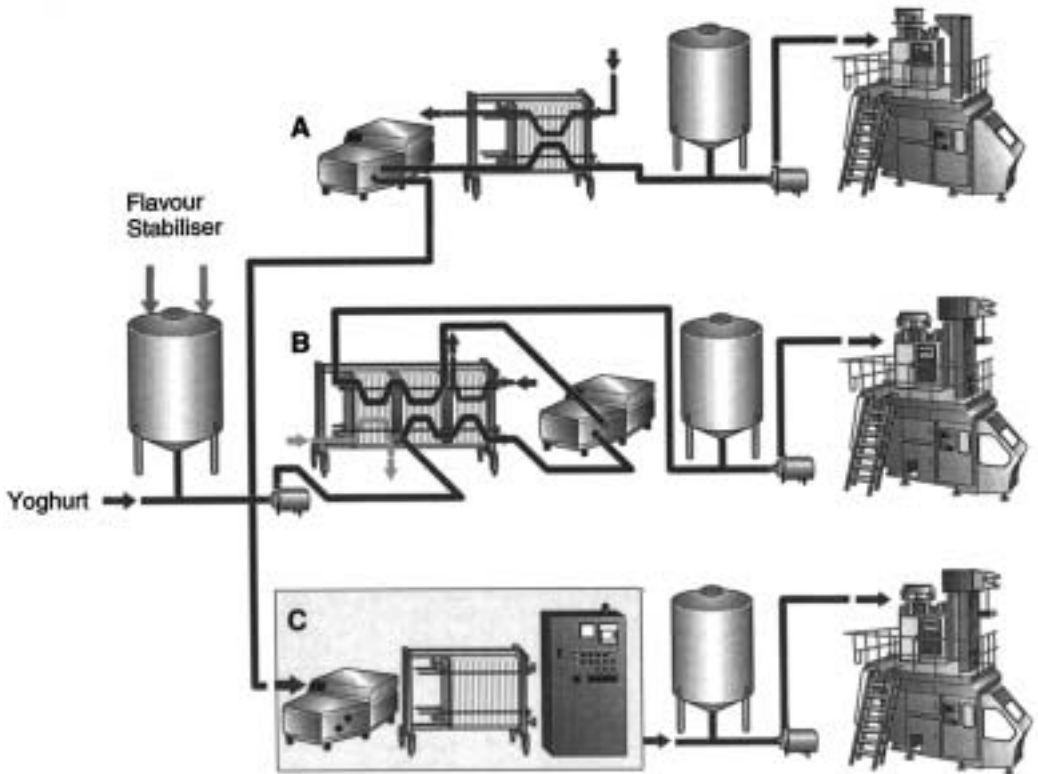
Fig. 5.3 Illustrations of some processing plants that could be used for the manufacture of drinking yoghurt with the anticipated shelf life indicated: A, homogenised and cooled, shelf life 2–3 weeks, refrigerated; B, homogenised, pasteurised and aseptically packaged, shelf life 1–2 months, refrigerated; C, homogenised, UHT treated and aseptically packaged; shelf life several months at room temperature

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Table 5.4 Some examples of starter cultures used for the manufacture of drinking yoghurt or beverages

Micro-organisms	References
S. thermophilus and *Lac. lactis* subsp. *lactis*	Koroleva *et al.* (1985)
L. delbrueckii subsp. *bulgaricus, L. paracasei* subsp. *paracasei*, *Lac. lactis* subsp. *cremoris*, *Lac. lactis* subsp. *lactis* and *Acetobacter aceti*	Kolesnikova *et al.* (1986)
Yoghurt culture, *L. acidophilus* and *B. bifidum*	Rossi and Clementi (1984)
L. delbrueckii subsp. *bulgaricus*	Siscar *et al.* (1985)
L. paracasei subsp. *paracasei*	So (1986)
S. thermophilus (single strain) or with *L. acidophilus*	Srivastava *et al.* (1985), Han *et al.* (1985) and Yukalo *et al.* (1991)
L. delbrueckii subsp. *bulgaricus* and/or *Lactobacillus helveticus* with or without *S. thermophilus*	Yokota *et al.* (1989)
L. delbrueckii subsp. *bulgaricus* and *L. acidophilus*	Choprea and Gandhi (1989, 1990)

bulgaricus and *Lactobacillus paracasei* subsp. *paracasei* for >48 and 140 hours, respectively, helped to minimise the precipitation of protein in the product (Kang and Lee, 1985; So, 1986). However, whey separation may be a problem during the manufacture of drinking yoghurt and it is necessary to incorporate a stabiliser into the milk base (Towler, 1984; Foley and Mulcahy, 1989; Tuohy, 1990). Syneresis was mimimised in a cultured beverage made from sweet buttermilk by the addition of gelatin or carboxylmethyl cellulose (Choprea and Gandhi, 1990), apple pectin paste (Yukalo *et al.*, 1991) or about $0.4\,g\,100\,g^{-1}$ Mexpectin RS450 (Anon., 1983a, 1984). van Hooydonk *et al.* (1984a, b) reported that variations in the sequences of processing of drinking yoghurt (e.g. homogenisation following instead of preceding pasteurisation or with homogenisation both before and after pasteurisation) did not affect the stability of the product; they recommended that single homogenisation at $\geq 15\,MPa$ was sufficient in the presence of added pectin (about $0.4\,g\,100\,g^{-1}$). A similar view regarding the effect of upstream homogenisation (i.e. prepasteurisation) or with downstream homogenisation (i.e. after pasteurisation) on the stability of laban (a Middle Eastern natural yoghurt) was put forward by McKenna (1987). However, storage studies at different temperatures on the shelf life of liquid yoghurt were reported by Lee *et al.* (1993) and the product was stable for 16 days at 5°C and 10°C, 12 days at 15°C and 6 days at 20°C; the viable cell counts of the yoghurt organisms were selected as an index of quality that could be related to the sensory taste of the product during storage.

Drinking yoghurt is normally flavoured with fruit purees or juices and consumers studied in the U.S.A. preferred strawberry and raspberry (White *et al.*, 1984; Ryan *et al.*, 1984), while in Germany, sensory tests with children aged between 8–14 ($n = 222$) have identified the optimum sugar content as $8.3\,g\,100\,g^{-1}$ (Endres, 1992). However, consumer attitudes to natural fruit juice versus added flavours and colorants in drinking yoghurt were in favour of the former product (Cramwinckel and Herstel, 1988a, b). Other fruit flavours which have been used in drinking yoghurt are carrot and apple concentrate (Kolesnikova *et al.*, 1986), pineapple (Srivastava *et al.*, 1985), lemon or orange concentrates (Arsov, 1983) and fruit juices, concentrates or essences (Evers, 1983).

The processing of drinking yoghurt at the Dan-Maelk factory in Denmark has been given in detail (Anon., 1986a–c). The gross chemical composition of the product ($g\,100\,g^{-1}$) is: fat 3.5, protein 3.8 and sugar 8; sterile fruit (i.e. free from stabilisers and preservatives) is added at a rate of $15\,g\,100\,g^{-1}$. The product is packaged aseptically in a screw cap gable carton using a Cherry-Burrell QL-9 machine fitted with a Posi-Fill® rotary-type valve that can handle fruit pieces up to 1.3 cm. Illustrations of other types of containers (cartons, glass bottles or non-translucent plastic bottles) that are used to package drinking yoghurt have been reported (Anon., 1987a, 1989, 1997; Kimbrell and Willman, 1993; Reiter, 1994). However, the ability of plastic bottles to absorb flavour compounds from drinking yoghurt has been studied by Linssen *et al.* (1992) (see also Chapter 2 and Section 2.13.5 and Tagliaferri, 1989).

The chemical composition of drinking yoghurt may vary from one country to another to meet consumer demand and a typical formulation ($g\,100\,g^{-1}$) might be as follows: fat up to 1.5, milk SNF about 9, sugar up to 8, stabiliser(s) about 0.5, fruit syrups or puree 5–15. As mentioned elsewhere, the product is sometimes heat treated (pasteurised or UHT) in order to prolong its keeping quality. Nevertheless, no appropriate data are available on the overall sales of drinking yoghurt in dif-

ferent markets but in the U.S.A. the sales of such products in 1992 were estimated to be about U.S.\$ 13 million (Pontikis, 1992). Also as mentioned in Chapter 9, ayran was used successfully for oral administration of rehydration salts and was preferred by children to water for the treatment of gastroenteritis (Caglayan *et al*., 1989).

5.5.3   Other beverage products

Soft drinks are extremely popular worldwide and, according to Duitschaever and Ketcheson (1974), a yoghurt beverage (flavoured with natural orange, lemon, cherry or apple) has the effect of improving the thirst quenching quality and refreshing taste of ordinary yoghurt and causing a pleasant tingling sensation on the tongue. However, the fermentation of milk by lactic acid bacteria and yeasts is widely used in east Europe and Russia for the manufacture of kefir and koumiss, and this type of fermentation releases lactic acid, alcohol, carbon dioxide and aromatic flavouring compounds into the product. A process has been developed for the Japanese market in which a yeast (genus *Kloeckera*) is precultured in the milk before the production of yoghurt. The milk is then sterilised, cooled to incubation temperature and finally inoculated with a mixed culture of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. Details of the process have been reported by Kuwabara (1970). The yoghurt beverage has the following characteristics: it contains aromatic flavouring compounds produced by the yeast, but no alcohol or gas: it contains a higher viable cell count of the starter cultures than conventional yoghurt, since the yeast metabolites enhance the activity of the starter culture; and the beverage does not suffer from whey separation.

A rather different Bulgarian beverage, which is specially formulated for the market in Russia, consists of 35–54% yoghurt, 20–40% natural fruit or vegetable puree, 28–30% syrup plus apple pectin and 0.1–0.2% citric acid. The mixture is homogenised, sterilised at 120–130°C for 50–70 s, cooled and packaged (Arolski *et al*., 1979), but the popularity of the product, particularly against a wider market, has not been tested. Kondratenko (1994) reported a high protein product made from high protein powders (casein and blood hydrolysate or casein and whey protein) and cultured with *L. delbrueckii* subsp. *bulgaricus* ($\leq 2.5 \times 10^9$ cfu g^{-1}) which is suitable for dietetic or sports purposes; this product could be consumed as a beverage rehydrated in milk, water or juice. Alternatively, yoghurt-like beverages could be made with vegetable flours (soyabean, peas, lupin and horse bean) fermented with a yoghurt starter culture; however, reduced lactic acid production was observed when compared with a milk-based beverages and *L. delbrueckii* subsp. *bulgaricus* exhibited no significant growth (Rossi, 1982).

5.5.4   Carbonated yoghurt

Carbonated yoghurt can be manufactured in either a liquid or a dry form. The former type is, in effect, a carbonated, flavoured drinking yoghurt, while the dry mix gradually releases carbon dioxide (CO_2) when the powder is reconstituted with water. Liquid carbonated yoghurt can be made using one of the following techniques. (a) A soya protein whipping agent is used with stabilisers (carboxymethylcellulose and xanthan gum) in the yoghurt/milk mixture; the liquid product, on shaking, develops frothiness which is maintained during consumption (Igoe and

Taylor, 1983). (b) The processed milk base is carbonated with CO_2, followed by fermentation with the starter culture (Castberg and Rystaad, 1990; see also Meyer and Mizandjian, 1991). (c) Carbonation of a yoghurt beverage was achieved by homogenising the product (i.e. yoghurt containing sugar and type 428 yoghurt stabiliser) at 4.8 Pa and 4°C (Choi and Kosikowski, 1985; Driessen and Loones, 1992).

The dry carbonated yoghurt has been explained in detail by Schenk (1980). He has reported the following advantages when using certain carbonates: (a) The presence of metal carbonates in the mix tends to neutralise the acid in the yoghurt, so that carbonated yoghurt is less acidic and has a pH around 7, (b) Although different types of metal carbonates could be used, the addition of calcium carbonate rather than sodium carbonate is advantageous; the former compound tends to dissolve at a slower rate in water, and so gradually releases the CO_2 into the reconstituted product, otherwise the carbonated yoghurt tends to go flat within a very short period of time, and (c) The addition of various types of calcium compound to the dried mix improves the opacity of the carbonated yoghurt, since the calcium reacts with various acids to form insoluble salts (see also Anon., 1998). However, the beverage concentrate, details of which have been given by Kolesnikova *et al.* (1986), could be diluted with carbonated water to produce a fizzy beverage (see Section 5.5.2).

5.6   Lactose hydrolysed yoghurt (LHY)

During the manufacture of yoghurt, only part of the available lactose is utilised by the starter culture bacteria as an energy source with the production of lactic acid. The excess lactose could be utilised to sweeten the yoghurt without increasing its calorific value. This effect could be achieved by hydrolysing the lactose using β-galactosidase (in powder or liquid form), which splits the lactose into glucose and galactose; the relative sweetness of lactose and these monosaccharides is, compared to a degree of sweetness for sucrose equal to 1, as follows: lactose 0.4, galactose 0.6 and glucose 0.7. Commercial preparations of β-galactosidase are mainly produced from yeasts, fungi and, to a lesser degree, bacteria (Broome *et al.*, 1983, Gunther, 1984). However, Engel (1973) observed that only 50% hydrolysis of the lactose was necessary to produce an acceptable yoghurt in terms of sweetness. Up until the late 1970s, relevant data on the manufacture of LHY were reported by Tamime (1977a, b, 1978a) and reviewed by Driessen and Loones (1992), IDF (1993) and Khedkar *et al.* (1994). The process of lactose hydrolysis in milk could be carried out using one of the following methods:

• Process A – low temperature hydrolysis at <10°C during overnight storage;
• Process B – high temperature hydrolysis at 30–35°C for $\frac{1}{2}$ hour;
• Process C – high temperature hydrolysis at 30°C where the enzyme is added to the processed milk base along with the starter culture.

In processes A and B it is essential to agitate the milk and to adjust the pH to about 6.6; proceed to manufacture the yoghurt as illustrated in Fig. 5.1. Inactivation of the β-galactosidase is achieved by the heat treatment. In process C, the slow rate of acid development by the starter culture gradually reduces the β-galactosidase activity and total activation may occur below pH 5.0 (see also Lelieveld, 1984).

Hydrolysis is only desirable, of course, during the manufacture of fruit/flavoured yoghurt, since plain/natural yoghurts are not sweetened at all. Nevertheless, although a reduction in the level of lactose in natural yoghurt does improve its therapeutic value (Gallagher *et al.,* 1974), current clinical studies confirm that β-galactosidase originating from the starter culture is sufficient for lactose maldigestors, and there is no need to hydrolyse the lactose in the milk base (Rosado *et al.*, 1992; Rosado, 1998; see also Chapter 9). However, work in this field has associated the enhanced activity of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in lactose hydrolysed milk with the availability of glucose and/or galactose in the milk. Hemme *et al.* (1978, 1979) and Marschke and Dulley (1978) have detected some proteolytic activity in commercial samples of β-galactosidase (possibly due to contamination during its preparation) and the improved activity of the yoghurt starter culture may be associated with the liberation of essential amino acids (Lee *et al.*, 1990a) rather than with the presence of glucose and/or galactose. Nevertheless, despite these contradictory views, many researchers have reported shorter coagulation times for the lactose hydrolysed milks (Ismail and El-Nimer, 1980; Dariani *et al.*, 1982; Effat *et al.*, 1983; Shchelokova *et al.*, 1985; Kreuder, 1988; Arsov, 1990). However, Arsov and Godic (1993) and Arsov and Torkar (1995) concluded that the causes of increased activity of starter cultures in lactose hydrolysed milk could be determined more clearly only using a pure culture of *S. thermophilus*. In a separate study, Arsov (1990) observed no enhanced activity by one of two commercial yoghurt starter cultures, while Sharma and Dutta (1986) suggested that stimulation of acid production by either of the yoghurt organisms in hydrolysed milk was strain dependent.

The quality of LHY may be influenced by a multitude of factors. First, lactose-hydrolysed milk can have an inhibitory effect on the growth of some strains of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in mixed culture (Abd El-Hady *et al.*, 1985). Second, some β-galactosidase preparations may cause off-flavours in the product when hydrolysis levels exceed 60%, while others are suitable at 80% hydrolysis (Dariani *et al.*, 1982; Broome *et al.*, 1983; Toba *et al.*, 1986a). Consequently, yoghurt treated with β-galactosidase during fermentation received slightly lower scores for flavour, texture and consistency than the control (Ismail *et al.*, 1983).

On some occasions it may be desirable to use the β-galactosidase of *Aspergillus oryzae* to obtain a higher oligosaccharide content (4–19 times) then that obtained with the control yoghurt (Toba *et al.*, 1986b). However, the use of hydrolysed whey concentrate or hydrolysed reconstituted SMP and dried whey may cause gelation of the milk base or affect curd stability of the yoghurt, and the recommended processing methods for LHY have been reported by Shah and Jelen (1987), Shah *et al.* (1993) and Atamer *et al.* (1995). Also, if the use of lactulose in the manufacture of LHY is desired, it should be added after the lactose hydrolysis in order to reduce the loss of lactulose due to β-galactosidase activity (Olano *et al.*, 1986). Furthermore, an alcoholic LHY beverage can be made from either whey or skimmed milk using β-galactosidase from *A. oryzae* and fermentation with *Zymomonas mobilis* and *L. delbrueckii* subsp. *bulgaricus* for ethanol and lactic acid production, respectively (Miyamoto *et al.*, 1987).

Further processing methods to produce low calorie and low lactose yoghurt may include: (a) combined UF and β-galactosidase hydrolysis of milk which produces yoghurt with a lactose level <0.1 g 100 g^{-1} (Streiff *et al.*, 1990; Khorshid *et al.*, 1993; Abbas *et al.*, 1996a–c; see also Shady and Abdel-Razik, 1997), (b) production of a

good quality yoghurt with *L. delbrueckii* subsp. *bulgaricus* alone in hydrolysed milk fortified with glucose oxidase and hydrogen peroxide (Tahajod and Rand, 1993), and (c) the use of a β-galactosidase preparation from lactic acid bacteria rather than yeasts (Sinha and Dutta, 1985; Kobayashi *et al*., 1989; Toba *et al*., 1990; Yang *et al*., 1993; Somkuti and Steinberg, 1995).

It is clear, therefore, that yoghurt can be produced from lactose hydrolysed milk, but the incentive for commercial production is limited because the process is still not economic in comparison with the addition of normal sweetening agents. However, Smith and Bradley (1984) have reported a net saving of U.S.$0.0061 *per* 227 g cup of sundae-style LHY and a similar view was confirmed by Botha *et al*. (1987). Alternatively, the production cost of LHY could be reduced by replacing SMP with a whey/caseinate blend in the milk base before hydrolysis (Whalen *et al*., 1988). It could be argued of course, that the use of immobilised enzymes might offer an attractive solution, but the economics of the process will be the decisive factor.

5.7   Concentrated/strained yoghurt

5.7.1   Introduction and nomenclature

Traditionally, the containers used by the nomads in the Middle East for the production of yoghurt were made from animal skin and the yoghurt was left in these skins until it was consumed. While the yoghurt was hanging in the animal skin some of the liquid phase would have been absorbed into the skin, while some of the whey that had seeped through the skin would have been lost by evaporation. In this way concentration of the product took place and the new product was referred to as concentrated/strained yoghurt. This latter product would have had a better keeping quality than normal yoghurt, mainly as a result of the higher concentration of lactic acid.

Evidence of the production of strained yoghurt can be found in many countries such as the Balkans, eastern Mediterranean, Turkestan and the Indian subcontinent. Table 5.5 shows the variety of names by which this product is known in different countries. For hygienic reasons, the use of cloth bags rather than animal skins is now widely practised. In some countries an attempt has been made to introduce standards, for example Lebanon (Anon., 1965), Jordan (Anon., 1980b; Ibrahim *et al*., 1996) and Saudi Arabia (Salji *et al*., 1983, 1987a, b), where it is stipulated that labneh (see Table 5.5) shall have a specific chemical composition based on fat, total solids and salt. The latter compound is basically added as a flavouring agent, as a preservative or, possibly, to neutralise the acidic taste of the product.

Labneh is normally consumed with bread as part of a main meal, but the possibility of promoting this product in Europe and North America has not seriously been considered. For example, a dairy spread in which labneh is mixed with chives or, alternatively, a dairy dessert made by mixing fruit/flavours with the concentrated product, could prove popular. A rather similar traditional Indian dish is called shrikhand, made from chakka and sugar. Nutmeg and saffron extract are used as flavouring agents (Ganguly, 1972). However, such products, including ymer (a Danish fermented milk product), are similar in composition to labneh (Table 5.6) and the only evident differences are that chakka is made from buffalo's milk (Atreja and

Table 5.5 Synonyms for concentrated/strained yoghurt in different countries

Traditional names	Countries
Labneh, labaneh, lebneh, labna	Eastern Mediterranean
Tan, than	Armenia
Laban zeer	Egypt, Sudan
Stragisto, sakoulas, tzatziki	Greece
Torba, suzme	Turkey
Syuzma	Russia
Mastou, mast	Iraq, Iran
Basa, zimne, kiselo, mleko-slano	Yugoslavia, Bulgaria
Ititu	Ethiopia
Greek-style	United Kingdom
Chakka, Shrikhand[a]	India
Ymer[a]	Denmark
Skyr[a]	Iceland

Data compiled from Azimov (1982), Tamime and Crawford (1984), Tamime and Robinson (1988), FAO (1990), Kassaye *et al.* (1991), Kurmann *et al.* (1992), Akin and Rice (1994) and Doeff (1994).

[a] Refer to text.

Deodhar, 1987) and both the Danish and Indian products are made with mixed strains of mesophilic lactic acid bacteria (see Section 5.3.3). The microflora of skyr, which is an Icelandic fermented and concentrated product, consists of a yoghurt starter culture, *L. helveticus* and lactose-fermenting yeasts (Tamime and Robinson, 1988).

5.7.2 Processing methods
The traditional method of production (i.e. home, rural and/or small scale) consists of straining cold and unsweetened natural/plain yoghurt using a cloth bag, animal skin or earthenware vessel (Yonez, 1965; Zmarlicki *et al.*, 1974a, b; Tamime and Robinson, 1978; Robinson, 1977). In some parts of the world, the large-scale manufacture of labneh is also possible using large cloth bags (about 25 kg capacity) which are piled on top of each other to assist in removal of whey. The cloth bag method, in comparison with large- or factory-scale operations, is slow, labour intensive, unhygienic, cumbersome and gives low yields due to residues left in the bag (Tamime, 1993; El-Samragy, 1997). The different methods available to manufacture strained yoghurt in large volumes are as follows:

- cloth bag or the "Berge" system
- mechanical separators
- ultrafiltration
- product formulation

5.7.2.1   Cloth bag or Berge system
The cold full-fat stirred yoghurt (natural/plain) is emptied into cloth bags, about 25 kg, and stacked on top of each other in a vertical press which is located in a refrigerated room. Pressure is applied in order to assist whey drainage for a duration

Table 5.6 Chemical composition (g 100 g^{-1}) of concentrated/strained yoghurt and related products

Country/produce	Total solids	Fat	Protein	Lactose[a]	Ash	References
Lebanon/labneh						
commercial ($n = 3$)	22.1	9.0	ND	ND	ND	Tamime and Robinson (1978)
standard	26.0	10.0	–	–	–	Anon. (1965)
Saudi Arabia/labneh						
commercial ($n = 18$)	22.9	7.6	9.6	3.8	1.2	Salji *et al.* (1983, 1987a, b)
standard	22.0	7.0	–	–	–	and Salji (1991)
Jordan/labneh						
standard	23.0	9.0	–	–	–	Ibrahim *et al.* (1996)
USA/labneh						
experimental ($n = 3$)	23.2	8.9	7.4	5.0	1.5	El-Samragy and Zall (1988)
UK/Greek style						
commercial ($n = 4$)	24.1	10.2	6.2	6.6	0.9	Tamime (1993)
experimental ($n = 4$)[b]	24.7	11.5	8.7	3.8	0.7	Tamime *et al.* (1989b, 1991b)
experimental ($n = 12$)[c]	23.0	10.8	8.0	3.5	0.7	
Egypt/labneh						
experimental	26.6	9.8	11.0	4.0	1.5	Hofi (1988)
experimental	26.1	10.0	10.3	3.6	1.1	
Greece/stragisto						
commercial	22.4	10.7	8.2	ND	1.7	Veinoglou *et al.* (1978)
India/chakka[d]	23.0	Tr	14.0	3.3	2.2	
Iceland/skyr	20.9	0.4	15.8	3.6	1.0	Tamime and Robinson (1988)
UK/labneh anbaris	31.2	4.8	18.6	7.0	ND	
Israel/labneh anbaris	46.5	20.0	17.7	4.0	3.4	
Ethiopia/ititu	20.9	9.1	7.2	ND	0.7	Kassaye *et al.* (1991)

[a] In some instances the lactose content was calculated by difference. [b] Product made by traditional (cloth bag) method. [c] Product made by UF of warm yoghurt.
[d] Shrikhand is made from chakka sweetened with sugar and fortified with cream (Patel and Abd El-Salam, 1986; Boghra and Mathur, 1992).

ND, not determined; –, not specified; Tr, trace; n, number of samples tested.

of 12–18 hours. The pressing time can be reduced if the pressure is increased to $2\,kg\,kg^{-1}$ of yoghurt and labneh will be ready for packaging after pressing for 6 hours (Abou-Donia *et al.*, 1992b). Alternatively, a long and horizontal cloth filter can be used; the long sides are supported on poles and may be gently oscillated up and down, while slight lateral pressure is applied. This method of concentrating the yoghurt is known as the modified Berge system and was developed in France in the 1960s for the production of fresh curd cheese (Berge, 1964; Maggs, 1964; see also Töral *et al.*, 1987).

Preliminary studies on the effect of using various strains of yoghurt starter culture on the rate of whey drainage were first reported by Tamime (1977b, 1978b) and Tamime and Robinson (1978). They concluded that strains producing exopolysaccharides (EPS) are not suitable because of the longer time required for the removal of whey and that the best labneh was produced from $16\,g\,TS\,100\,g^{-1}$ of yoghurt (see also Gilles and Lawrence, 1981; Jensen and Nielsen, 1982; Hamad and Al-Sheikh, 1989). Al-Kanhal (1993) observed that traditional labneh made from fresh milk had the best organoleptic scores when compared with a similar product made from recombined milk or cultured buttermilk concentrated using a quarg or nozzle separator. Fat losses in the whey were minimised during the manufacture of chakka or shrikhand by homogenisation of the milk base before the fermentation stage (Desai *et al.*, 1985; Patel and Chakraborty, 1988) or using less than 3l of fermented milk for concentration (Rao *et al.*, 1987). Alternatively, shrikhand could be produced from skimmed chakka, together with the addition of cream and sugar in order to reduce the fat losses (Rao *et al.*, 1987b) or fortified with iron (Boghra and Mathur, 1992; Boghra *et al.*, 1997).

Different methods that can be used for the manufacture of labneh-type products may include: (a) the use of a specially designed packaging container where the whey is drained from the yoghurt and collected at the bottom of the plastic cup (Varan, 1994; Grusin, 1994); an illustration of this system is shown in Fig. 5.4, (b) the use of high solids low fat yoghurt which can be mixed with cream and mashed fruit (Cavaliere *et al.*, 1994a), and (c) the use of vacuum filtration to concentrate the yoghurt (Akin *et al.*, 1995).

5.7.2.2 Mechanical separator

Dagher and Ali (1985) produced labneh from heated yoghurt by centrifugation for 5 min at different speeds between 4000 and 11 700 g, and organoleptically all these labnehs were similar to the control (cloth bag) samples (see also El-Kenany, 1995). Factory-scale production of labneh using the quarg or nozzle separator in Saudi Arabia has been reported by Salji *et al.* (1983, 1987a, b). Skimmed milk should be used for the manufacture of yoghurt and the fermented milk is stirred vigorously, thermised at about 60°C, filtered to remove any large clots, cooled to about 40°C and concentrated to $18\,g\,100\,g^{-1}$ solids, cooled to about 15°C, blended with cream or fruit (optional) and finally packaged. Further accounts of this process have been reported by Rasic (1987), Hansen (1989b), Lehmann *et al.* (1991), Mortensen (1995) and Bylund (1995) and a typical example is shown in Fig. 5.5. If whole milk is used instead, the nozzles of the separator will clog. Recent developments in the design of such separators have made it feasible to use fermented whole milk for the production of concentrated yoghurt (Lehmann *et al.*, 1991). After acidification, the fermented milk is processed as described above, but before the separation stage, it is de-aerated for 15–20 min to assist the separation of the whey in the separator. A

Fig. 5.4   Illustration of a specially designed packaging container in which the product is concentrated during storage, distribution and retailing

typical chemical composition (g 100 g⁻¹) for concentrated yoghurt is total solids 24 and fat 9.6, whilst the composition of the whey is 6.1 g 100 g⁻¹ total solids, consisting mainly of lactose and minerals, but about 0.5 g fat 100 g⁻¹.

Recently, Kehagias *et al*. (1994) have reported that compositional differences between strained yoghurt and quarg can be attributed to the structural changes brought about during the fermentation of milk, that is, fast and slow acidification using thermophilic and mesophilic starter cultures, respectively. Also, the same authors (Kehagias *et al*., 1992) reported differences in the yield and recovery of milk solids when using goat's or cow's milk (see Rao *et al*., 1987b). In another process for the production of labneh, yoghurt is blended with 25–100% of its volume with brine (3–12 g salt 100 g⁻¹) and the mixture is concentrated using a centrifugal separator (Kharrazi, 1984).

Whey from milk coagulated in a smoked wooden vessel (gorfa) is removed gradually using a wooden pipette for the production of ititu in Ethopia (Kassaye *et al*., 1991; Beyene and Abrahamsen, 1997). As the whey is removed, the gorfa is filled with fresh milk to provide an on-going fermentation and the concentrated product has a shelf life of two months without refrigeration. The precise role(s) of the smoking process are not known, but a similar effect was described by Kimonye and Robinson (1991) with respect to iria ri matii (a Kenyan fermented milk in smoked gourds). Similarly, the yoghurt can be heated gently and the whey allowed to drain to give concentrated yoghurt (Rasic, 1987); such a method of manufacture resembles the traditional process of ymer making.

5.7.2.3   Ultrafiltration (UF)

Two different systems of UF have been used for the production of labneh, the fermentation of UF retenate that has the solids content desired in the final product
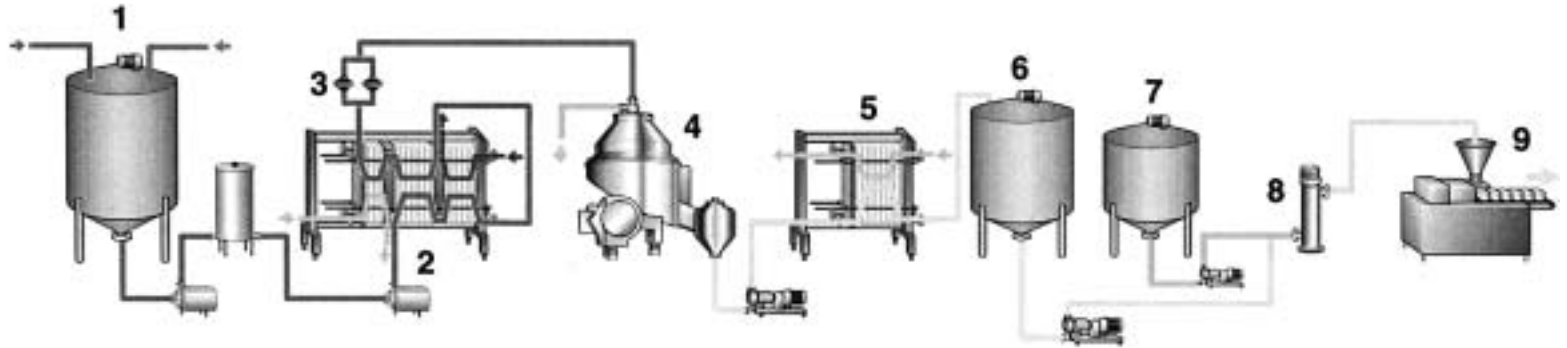
Fig. 5.5   Flow chart for the manufacture of strained yoghurt by mechanical separation
1, Ripening tank; 2, plate heat exchanger for thermisation; 3, filter system; 4, quarg separator; 5, plate cooler; 6, intermediate tank; 7, cream tank; 8, dynamic mixer; 9, packaging machine

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and UF of yoghurt at 40°C to produce a concentrate at about $24\,g\,TS\,100\,g^{-1}$. In the former system of production (Veinoglou *et al.*, 1978; Ibrahim, 1979; Abd El-Salam and El-Alamy, 1982; El-Samragy and Zall, 1988; Hofi, 1988, 1990; El-Samragy *et al.*, 1997) the UF retentate may be fermented in the retail container – as with the manufacture of natural set yoghurt – and the firmness of the product is much greater when compared with a similar product made using a traditional (cloth bag) method or by UF of warm yoghurt (Tamime *et al.*, 1989b). Also chakka and shrikhand have been produced by the UF technique where the yield has increased by 23% compared with the traditional method of manufacture and the UF product was highly rated (Patel and Chakraborty, 1985b, Sharma and Reuter, 1989, 1992).

According to Vesely *et al.* (1989), Robinson and Tamime (1993) and Tamime (1993), a wide range of UF plants are available on the market for the production of strained yoghurt on a large scale. A typical example is illustrated in Fig. 5.6 and according to the supplier, the manufacturing process is as follows. Standardised milk (e.g. $12.5\,g\,100\,g^{-1}$ total solids and $3.5\,g\,100\,g^{-1}$ fat) is preheated to 60°C, homogenised at 14.7 MPa, heated in a plate heat exchanger (PHE) to 95°C and held for 5 min in a holding tank before cooling to 40–45°C in the regeneration section of the PHE. After the fermentation period, the warm yoghurt is heated at 58–60°C for 3 min in the PHE, cooled to 40°C, concentrated in a two- to four-stage UF plant, cooled in a plate cooler to about 20°C and finally packaged. The degree of concentration using a four-stage UF plant, for example, could be adjusted to give 14, 16, 19 and $22\,g\,100\,g^{-1}$ total solids, respectively. However, the highest flux rate during UF was observed at a temperature $\geq 50°C$, but the total viable counts of the yoghurt starter organisms were lower than with labneh ultrafiltered at $\leq 45°C$ (Tamime *et al.*, 1991b). Attia *et al.* (1991a, b) reported that UF carried out at elevated temperatures $>45°C$ increases the fouling rate of the UF membranes, which may affect the processing
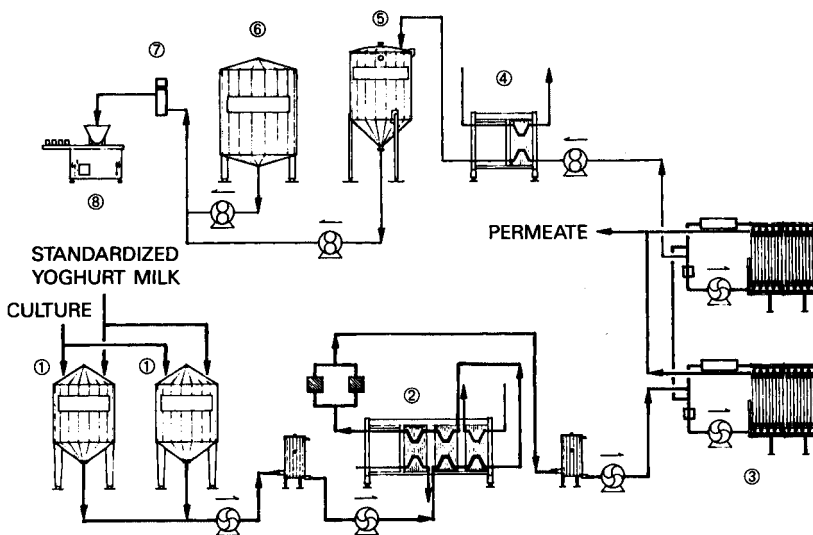
Fig. 5.6 Flow chart for the manufacture of strained yoghurt by UF
1, Fermentation tanks; 2, plate heat exchanger; 3, two- to four-stage UF plant; 4, plate cooler; 5, buffer tank; 6, fruit tank (optional); 7, in-line mixer; 8, packaging machine

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conditions in large-scale operations where the equipment needs to be washed more frequently. It is possible to recommend that UF of yoghurt should be at 45–50°C, since at this high temperature, labneh can be produced within the shortest time and the firmness of the product is similar to traditional labneh (Tamime *et al.*, 1991b).

The ultrafiltration of heated (about 50°C) fermented and coagulated skimmed milk with different UF modules have been extensively studied by Sachdeva *et al.* (1992a, b) and Sharma *et al.* (1992a, b) for the production of good quality quarg. However, the concentration of L(+)- and D(−)-lactic acid in the product is governed by many factors such as the type of starter culture, the type of milk and the method of concentration, that is, UF or traditional method (Akin, 1997).

5.7.2.4 Product formulation

It is feasible to manufacture strained yoghurt from recombined dairy ingredients (Tamime, 1993). The process involves reconstitution of powder(s) in water and blending it with anhydrous milk fat, stabilizer (e.g. Cremodan Mousse 31, Danisco Ingredients (U.K.) Ltd.) and salt (optional). The recombined milk is handled and processed in a similar way to the production of yoghurt. After the fermentation stage, the product is precooled to about 20°C, packaged and the final cooling to 5°C takes place in the cold store. Typical compositions (g $100 \, g^{-1}$) of strained yoghurts are full-fat: fat 10, SNF 14.8, salt 0.5, stabiliser 0.8 and total solids 26.1, and low-fat: fat 4.2, SNF 17.4, salt 0.5, stabiliser 0.9, total solids 23.0. However, as mentioned later, the rheological properties of recombined labneh will be different from those of labneh made by the traditional method or from UF retentate.

5.7.3 Miscellaneous properties

A wide range of aspects, besides the processing methods used for the manufacture of strained yoghurt, can affect the quality of the products.

The firmness of labneh (UF or traditional method) made from goat's or sheep's milk was lower than that of the cow's milk product (Mahdi, 1990; Mahdi *et al.*, 1990). However, the highest yield of strained yoghurt was for sheep > goat > cow (Giannoukou *et al.*, 1992), whilst in India, the yield of chakka was greatest with buffalo's milk (26.2%) and lowest with cow's milk (24.0%) (Subramonian *et al.*, 1995).

Standardisation of the milk base (cow's or buffalo's milk) is highly recommended to produce chakka with a specified compositional standard (Kulkarni *et al.*, 1995), a view which is applicable to labneh-type products as well. The utilisation of buttermilk or whey protein concentrates (WPC) has been successful for the production of labneh or chakka (El-Samragy *et al.*, 1988b; Mahfouz *et al.*, 1992; Al-Kanhal, 1993; Karthikeyan *et al.*, 1996). Gelatin (but not sodium alginate) can be used as an additive to improve the consistency of chakka (Desai *et al.*, 1987; Agnihotri and Pal, 1996, 1997), as does the use of Gelodan SB 253, Nisin and/or an EPS starter of *Leuconostoc* species (Sarkar *et al.*, 1996a, b).

Although the starter culture employed to ferment the milk during labneh making should consist of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, mesophilic lactic acid bacteria are widely used in India for the production of chakka. However, Patel and Chakraborty (1985a) recommended the use of a yoghurt starter culture instead as the fermentation time was reduced by 4–6 hours; addition of $10 \, \mu g \, g^{-1}$ of diacetyl improved the flavour of the product (see also Khanna *et al.*, 1982; Patel

et al., 1993; Kadu *et al.*, 1994). A similar observation (i.e. reduced fermentation time) was also reported by Suryawanshi *et al.* (1993) and Subramonian *et al.* (1995, 1997) when using a combined starter culture of *S. thermophilus* and *L. acidophilus*. Rao *et al.* (1986, 1987b) reported that the highest yield and best organoleptic properties were observed in chakka made from milk fermented with *Lac. lactis* subsp. *cremoris*; labneh made with *B. bifidum* was not accepted by a taste panel due to the high level of acetic acid in the product (Mahdi, 1990; Mahdi *et al.*, 1990). The use of different starter culture combinations for making labneh were reported by Abou-Donia *et al.* (1992a) and Amer *et al.* (1997), whilst El-Samragy *et al.* (1988a) produced an acceptable labneh using *L. delbrueckii* subsp. *bulgaricus* in combination with *Enterococcus faecalis*.

The heat treatment of shrikhand at 70°C for 5 min extended the shelf life of the product to 15 days at 36°C or >70 days at <10°C, and it retained its overall acceptability (Prajapati *et al.*, 1991, 1992, 1993). Alternatively, Indian labneh packed in containers and covered with a layer of soyabean oil was still acceptable after 30 days storage at room temperature (Hassan *et al.*, 1986); a similar method is used in the Middle East to preserve labneh anbaris (see Section 5.7.5). Other modifications in the production methods of strained yoghurt may include lactose hydrolysis of the milk base (Tamime, 1978a, b; Tamime and Robinson, 1978), replacement of the butterfat with vegetable oils (Hefnawy *et al.*, 1992; Taha *et al.*, 1997a), addition of fruits (Bardale *et al.*, 1986), direct acidification of the milk (Ibrahim *et al.*, 1994) and carbonation of the milk for production of a gel rather than acidification or enzymatic coagulation (Caron *et al.*, 1992). The production of acetic and propionic acids is a method suggested by Haddadin *et al.* (1996, 1997) for utilisation of the whey from labneh (see also Atamer *et al.*, 1993).

The therapeutic and nutritional properties of strained yoghurt could be similar or slightly better than yoghurt. Thus, antibacterial properties of Indian fermented milk products against a wide range of pathogenic micro-organisms have been reported by Balasubramanyam and Varadaraj (1995) and Sarkar *et al.* (1996a), while a market survey in Egypt found that labneh (*n* = 28) contained different quantities of the vitamin B complex ($\mu g \, 100 \, g^{-1}$): niacin (93.2–184), biotin (1.3–2.6), vitamin B$_6$ (23.5–36.1), vitamin B$_{12}$ (0.21–0.29) and folic acid (3.7–5.2). The addition of propionibacteria to the yoghurt starter culture increased the vitamin B$_{12}$ and folic acid contents in labneh by 210% and 25%, respectively, whilst storage of labneh at 6°C for 10 day did not markedly affect their level (Khattab, 1991; see also El-Samragy *et al.*, 1997).

Ultimately, the microbiological properties of any type of strained fermented milk reflect the standards of hygiene during manufacture and the method of production. Thus, Lalas and Mantes (1984, 1987) reported that the low counts of lactic acid bacteria in strained yoghurt suggested that the yoghurt had been subjected to heat treatment before concentration, whilst the yeast and mould and total colony counts were $<25 \, cfu \, g^{-1}$ and up to $6.8 \times 10^5 \, cfu \, g^{-1}$, respectively. Yamani and Abu-Jaber (1994) found out that commercial Jordanian traditional labneh obtained from 18 dairy factories had mean psychrotropic and mesophilic counts of 2.6×10^6 and $4.4 \times 10^6 \, cfu \, g^{-1}$, respectively, and these figures increased after 14 day storage at 7°C to 1.1×10^7 and $1.4 \times 10^7 \, cfu \, g^{-1}$, respectively (see also Mihyar *et al.*, 1997). However, Upadhyay *et al.* (1984, 1985) found a positive correlation between the chemical changes and microbial counts of shrikhand and a sensory evaluation of fresh and stored samples.

In some instances, milk can be contaminated by undesirable components during the manufacture of strained yoghurt. For example, the radioactive material ^{131}I in milk (amounting to 6–12 kBq kg^{-1} which was equivalent to the dosages that Greece received during the Chernobyl accident) reduced the count of lactic acid bacteria in strained yoghurt by 45% (Vosniakos *et al.*, 1991). Hassanin (1994) reported that 70% of aflatoxin M_1 present in milk was recovered in labneh because this potential heptocarcinogen tends to be associated with the casein fraction of the product.

5.7.4 Microstructure

The microstructure of labneh (Fig. 5.7) made from cow's milk using the traditional (cloth bag) method, fermentation of UF retentate and UF of warm yoghurt, and the effect of smoothing these products by passage through a lactic curd structuriser was first reported by Tamime *et al.* (1989a). They found that: (a) SEM (scanning electron microscopy) at low magnification showed that there was no noticeable effect of the processing on the microstructure of labneh but, in some unsmoothed samples, small lumps of fluffy protein aggregates were found that were hollow and disappeared after smoothing (Fig. 5.7 a and b), (b) the microstructure of all the labneh samples at high SEM magnification were composed of casein particle chains and clusters and only subtle differences were observed; however, the smoothed samples
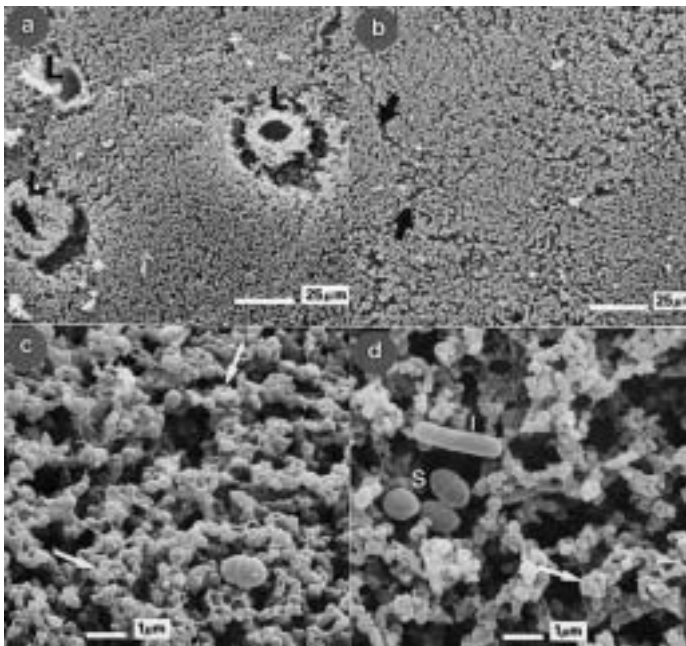
Fig. 5.7 Microstructure (SEM) at low magnification of UF labneh before (a) and after (b) passage through the lactic curd structuriser. L, small hollow protein lumps; black arrows in (b) show fluffy areas after the smoothing stage. Traditional (cloth bag) labneh at higher magnification before (c) and after (d) passage through the structuriser. Separation of fluffy areas (white arrows) is clearly noticeable; I, lactobacilli and S, streptococci

After Tamime *et al.* (1989a). Reproduced by courtesy of *Scanning Microscopy International*.

had slightly less compact and more open matrices, possibly due to the formation of larger pores as a result of the mechanical action of the structuriser (Fig. 5.7 c and d), and (c) a TEM (transmission electron microscope) examination of all the labnehs showed chains of agglomerated casein particles and fat globules; the chains were shorter after passage through the structuriser and there was some evidence of casein micelle fusion. However, in a separate study, Tamime *et al.* (1991a) found that the processing temperature (35–55°C) of UF resulted in an increase in the dimensions of the casein particles forming the protein matrix of the labneh (Fig. 5.8). Concentrating the yoghurt at 55°C resulted in the formation of complex micellar chains compared with the more simple structure of UF labneh concentrated at 35°C or the traditional product. Also the smoothed products appeared whiter and brighter, possibly due to the formation of appendages at the surface of the casein particles (Mottar *et al.*, 1987, 1989). Labneh made from goat's or sheep's milk was similar and less uniform than a similar product made from cow's milk (Tamime *et al.*, 1991c) (see Fig. 5.9).

As mentioned elsewhere, different methods for the manufacture of labneh are available. Ozer *et al.* (1997, 1998) have evaluated the rheological properties of prod-
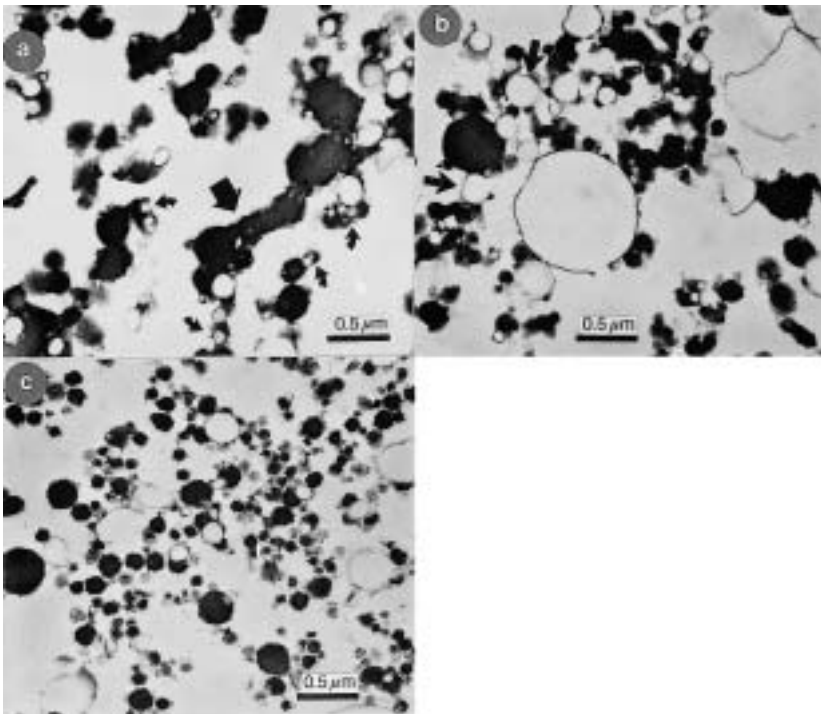
Fig. 5.8 Microstructure (TEM) of the protein matrix of unsmoothed UF labneh (a) concentrated at 55°C (protein matrix (large arrow) and minute fat globules (small arrows) embedded in the casein micelles); (b) unsmoothed UF labneh concentrated at 35°C (arrows illustrate association of fat globules with casein particle chain); (c) unsmoothed traditional labneh

After Tamime *et al.* (1991a). Reproduced by courtesy of *Scanning Microscopy International*.
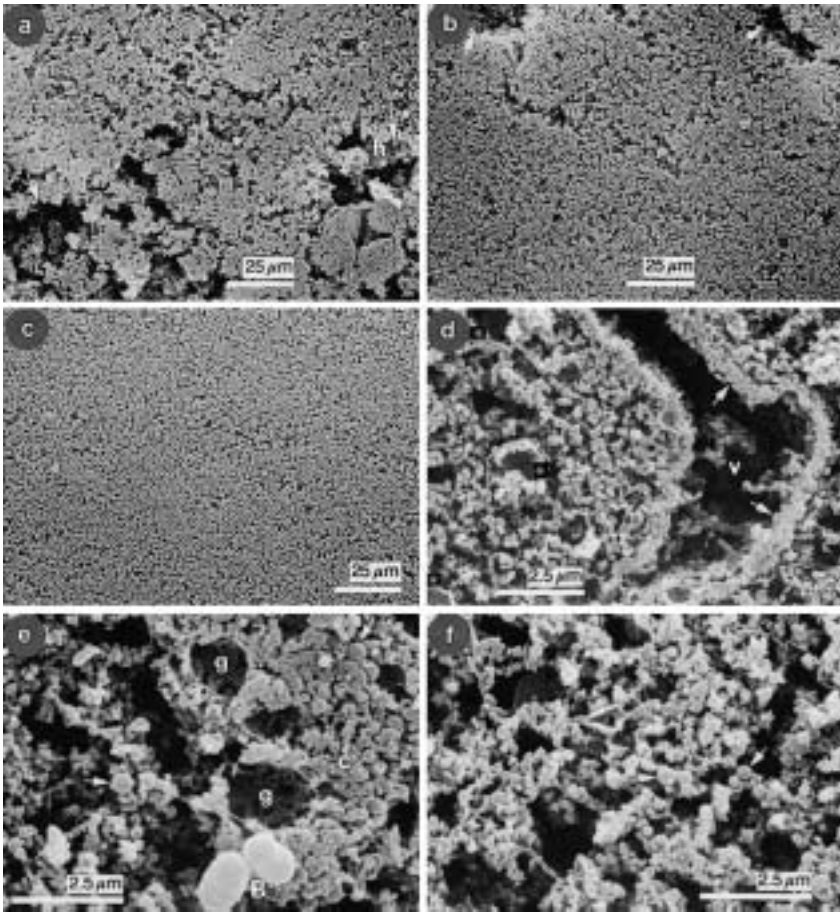
Fig. 5.9 Microstructure (SEM) of unsmoothed UF labneh made from goat's milk (a) contained many void spaces (arrows). Sheep's milk (b) was more uniform and cow's milk (c) had a uniform structure. (d) Sheep's labneh showing residues of fat globules membrane (asterisks); compact protein particles (arrows) formed the walls of a small void space (v) in the matrix. (e) Smoothed goat's labneh made by the traditional method and UF procedure (f); g, fat globule; B, bacteria; c, compact casein clusters and arrows illustrate large casein micelles which have smooth surfaces

After Tamime *et al.* (1991b). Reproduced by courtesy of *Scanning Microscopy International*.

ucts made by: (a) the traditional method of draining some of the whey from normal full fat yoghurt through a cloth bag, (b) concentrating full fat milk by UF or RO to $23 \, g \, 100 \, g^{-1} \, TS$ prior to fermentation, (c) concentrating full fat yoghurt (14–16 g $100 \, g^{-1} \, SNF$) by UF or RO to $23 \, g \, 100 \, g^{-1} \, TS$, (d) reconstituting full fat milk powder to give a milk base for fermentation of $23 \, g \, 100 \, g^{-1} \, TS$, and (e) a combination treatment that might involve, for example, concentrating skimmed milk yoghurt by UF and adding cream to provide the desired fat content.

The precise choice of system will affect both the chemical composition and the physical properties of the end product and some typical figures are shown in Table

Table 5.7 Chemical composition (g 100 g^{-1}) of some samples of labneh made by different methods[a]

Product	Total solids	Protein	Lactose	Fat	Ash
Traditional method	23.3	8.0	5.2	9.2	0.8
UF (before fermentation)	22.4	8.3	5.2	8.2	0.8
UF (of yoghurt)	22.6	8.1	5.5	8.5	0.9
RO (before fermentation)	23.2	6.8	9.0	6.3	1.1
RO (of yoghurt)	22.2	6.4	8.8	6.6	1.0
Direct reconstitution	22.5	6.4	8.7	6.1	1.3

[a] For details refer to text.

After Ozer *et al.* (1997).
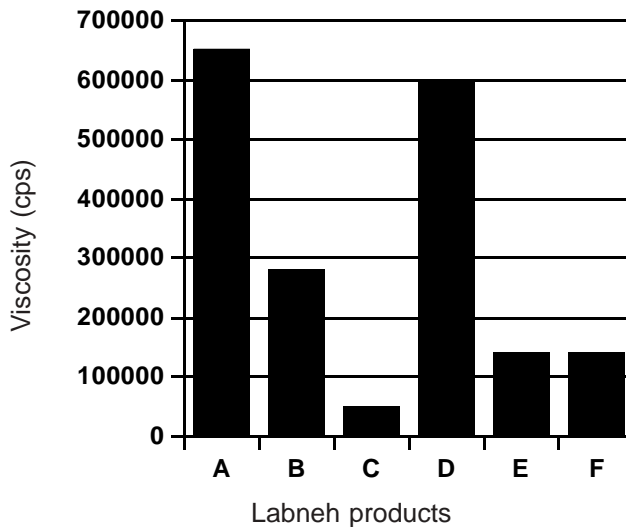
Fig. 5.10 Viscosity values of the test samples of labneh after overnight storage at 4°C. Results are average of three replicates

A, traditional; B, UF of yoghurt; C, RO of yoghurt; D, UF before fermentation; E, RO before fermentation; F, direct reconstitution

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5.7. The contrasting values for protein are of especial note with respect to the viscosity of the products and the effect of the higher protein levels is evident in Fig. 5.10. However, the precise level of protein is not the only factor to influence the physical properties, for it is clear that, while the traditional labneh has a lower protein content than the product made by UF of yoghurt, its viscosity is double that of labneh made from UF milk. It has been suggested by Ozer *et al.* (1997, 1998) that these variations are a reflection of structural differences between the gels, a point that is borne out to some extent by some dynamic rheological studies that were carried out using a stress-controlled rheometer. Thus, as shown in Fig. 5.11 and 5.12, the storage and loss moduli of labneh made by the various methods (see above) showed the same pattern as the results shown in Fig. 5.10. The differences are
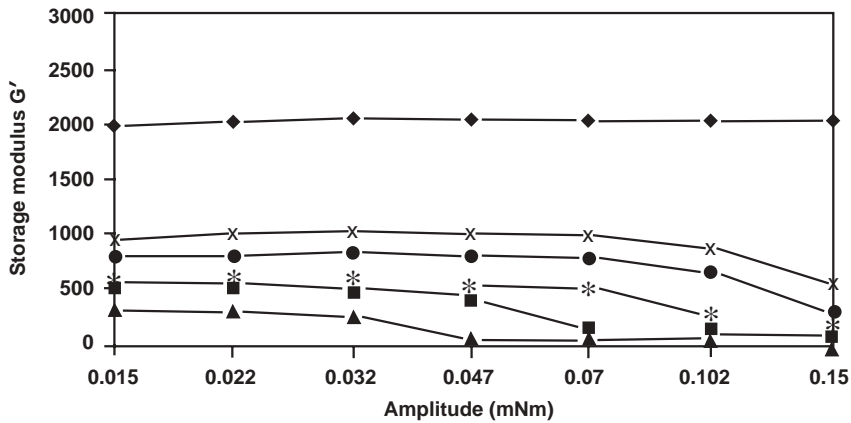
Fig. 5.11 Typical storage modulus patterns of different types of labneh after overnight storage at 4°C; test conditions are: amplitude range 0.015–0.15 m Nm, frequency 0.25 Hz, parallel plates (10 mm radius and 1 mm gap setting) at 25°C measuring temperature. Results are average of three replicates

−◆− Traditional; −●− UF of yoghurt: −▲− RO of yoghurt; –x– UF before fermentation; −∗− RO before fermentation; −■− direct reconstitution

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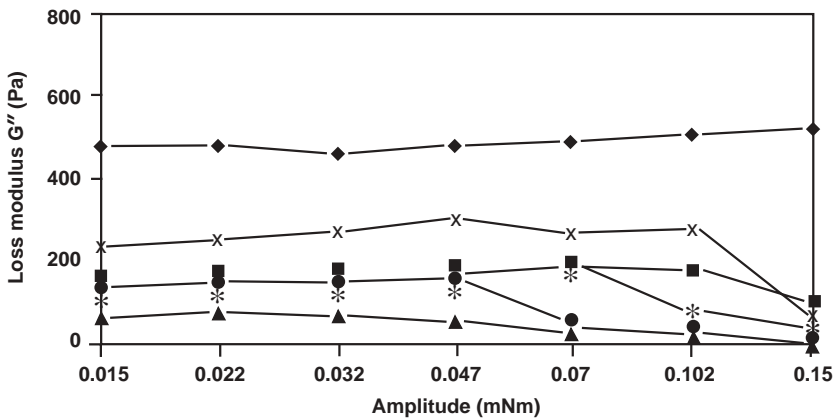
Fig. 5.12 Typical loss modulus patterns of different types of labneh after overnight storage at 4°C; test conditions are similar to those shown in Fig. 5.11. Results are average of three replicates

−◆− Traditional; −●− UF of yoghurt: −▲− RO of yoghurt; –x– UF before fermentation; −∗− RO before fermentation; −■− direct reconstitution

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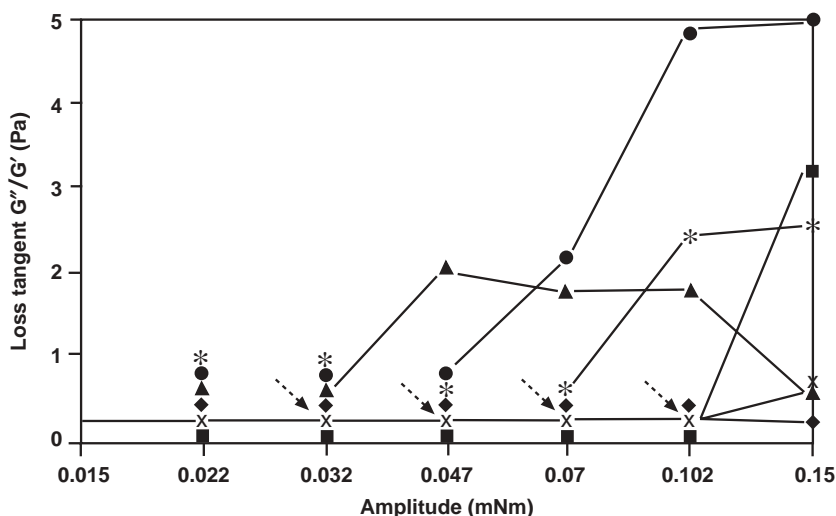
Fig. 5.13 Variation in loss tangent values of labneh samples as a function of amplitude; arrows indicate the breaking point of the structure in each sample. Results are average of three replicates

−◆− Traditional; −●− UF of yoghurt: −▲− RO of yoghurt; −x− UF before fermentation; −∗− RO before fermentation; −■− direct reconstitution

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illustrated even more vividly by the calculations of the loss tangent values (Fig. 5.13) (G''/G') in that, while the structure of the traditional labneh did not break down at all under the experimental stresses applied, all the other samples showed some degree of instability. The labneh made from by UF (before fermentation) came structurally closest to the traditional product. This result confirms the proposal of Tamime *et al*. (1989a, b) that UF offers an excellent alternative to the cloth-bag method for making labneh. Nevertheless, the apparent superiority of the traditional labneh as revealed in Fig. 5.11 and 5.12 should be noted, for if the concentrated yoghurt is used as a base for a speciality like tzatziki, then the traditional product tends to give a better quality retail item.

5.7.5 Related products
Concentrated/strained yoghurt (i.e. labneh) is sometimes used as a raw material for the manufacture of some traditional dairy products popular in the Middle East. The process mainly involves extraction of more whey from the concentrated yoghurt and, in some extreme cases, the final product is dried (see Section 5.9). These traditional foods are produced from surplus milk during the spring and the summer months of the year and are used during the winter. Examples of such products are labneh anbaris and shankleesh or shankalish.

5.7.5.1 Labneh anbaris
This type of concentrated yoghurt has a total solids content between 30 and 40 g 100 g⁻¹ (Tamime and Crawford, 1984) and in some instances even higher (Rosenthal

Fig. 5.14 Small-scale vertical press for the production of labneh anbaris or yoghurt cheese

et al., 1980) (see also Table 5.6). The traditional process starts with labneh (24 g TS $100 g^{-1}$) and the end product is shaped into balls and partially sundried. Alternatively, the labneh is pressed for a longer duration to remove more whey (see Fig. 5.14; Hessabi, 1995) and then it is shaped into balls; however, by using this method to produce high solids strained yoghurt, aerial contamination with micro-organisms could be minimised. The balls are then placed in earthenware vessels or glass jars and further preserved in olive oil (see Fig. 5.15). In areas where goat's and/or sheep's milk is used to replace cow's milk, the end product is much stronger in flavour.

As long as the product is kept submerged in olive oil, the shelf life of the product is about 12–18 months at ambient temperature. Tamime and Crawford (1984) preserved labneh anbaris with K-sorbate (0.1 g $100 g^{-1}$) or by heating the product in oil at 65°C for 55 min. After one year of storage at 20°C, the microbial counts in the control (without any treatment), K-sorbate or heated products were: (a) total viable count (non-lactic acid bacteria); 3.0×10^4, 4.5×10^3 and 2.0×10^2 cfu g^{-1}, respectively, (b) yeast and mould counts; $>1.0 \times 10^3$ cfu g^{-1} in the control sample and no growth at 10^{-1} dilution in the experimental products, and (c) coliforms were not recovered in any of the samples.

The consistency of this product resembles "lactic curd" or "pates fraiches" cheese, and Davis (1971) reported on a similar product called "yoghurt cheese". The typical

Fig. 5.15 Illustration of labneh anbaris preserved in olive oil

manufacturing process for "yoghurt cheese" is as follows: heat milk (whole or skimmed) to 70°C, cool to 46°C, add yoghurt starter culture and gently stir. Allow the milk to cool to 30°C without agitation, add the following ingredients (annatto, rennet and starter culture consisting of *Lac. lactis* subsp. *lactis* and subsp. *cremoris*), stir for 2–3 min, and after 2–3 hours cut the coagulum coarsely (2–3 cm in size). Run the curd and whey (by gravity) into a coarse cloth bag, drain the whey for 24 hours at 20–25°C and transfer the curd into a clean coth bag. Re-suspend for further draining for 24 hours at 5–10°C. Mix the curd with sorbate and salt (optional), pack and store under refrigeration.

A typical analysis $(g\,100\,g^{-1})$ of yoghurt cheese would be:

Product	Total solids	Protein	Fat	Lactose	pH
Experimental	40	15.0	17.2	5.5	3.8
Market in Qatar	Nd	20.0	22.0	7.6	3.95

Nd: not determined
After Keceli (personal communication).

Given that the water activity (A_w) of the test sample was 0.85, the salt content was $1.0\,g\,100\,g^{-1}$ and the pH was 3.8, it is not surprising that the product was microbiologically stable. In fact, the only real problem could be fungal growth on the surface of such products, a risk that, in practice, is eliminated by the anaerobic conditions imposed by the covering of olive oil.

In Poland, pre-concentrated milk (e.g. $30–40\,g\,100\,g^{-1}$ TS), is used to manufacture a product called super yoghurt, and this approach could help to overcome the hygienic problems associated with the use of the cloth bags. However, a novel cultured milk product called YoCheese has been developed in the U.S.A. and has the combined attributes of cottage cheese and yoghurt (Willrett *et al*., 1990). Similarly in Japan, yoghurt made with *S. thermophilus, L. acidophilus* and *Bifidobacterium* sp.

was added to a soft-type cheese (i.e. similar to cream cheese or quarg in appearance) for the production of yoghurt cheese (Ariga *et al.*, 1989).

5.7.5.2 *Shankleesh or shankalish*
The procedure for the manufacture of shankleesh is somewhat similar to that of labneh anbaris differing only in the following aspects: (a) it is made from either low fat yoghurt or the fermented buttermilk which is the by-product of ghee making, (b) herbs and/or spices such as thyme (*Thymus vulgaris*) are added and (c) during the ripening or maturation period in earthenware jars, indigenous moulds grow on the surface of the product and participate in the biochemical changes that occur. Thus, according to Toufeili *et al.* (1995), this fermented milk product could be classified as a surface mould ripened cheese variety, the only indigenous type native to the Middle East.

Shankleesh, in the Lebanon, is normally made from sheep's milk, but local dairy factories also produce it from goat's and cow's milks. In some instances, the product is not mixed with herbs but is sold as white shankleesh. According to Dagher (1991), Robinson (1995a) and Toufeili *et al.* (1995), the manufacturing stages for shankleesh are as follows: dilute cold yoghurt with iced water, churn to remove the butter granules, heat the buttermilk at 90°C for 15–20 min to maximise the floculation of the proteins, cool and strain in a cloth bag for 48 hours at 6°C. Traditionally, the concentrate is mixed with salt, spices and herbs, shaped into large balls, partially dried in the sun and placed in earthenware jars to ripen at ambient temperature for one month; however, during this period moulds grow on the surface and before dispatch, the balls are washed with water, and covered with powdered thyme (Dagher, 1991). Alternatively, the shankleesh could be partially dried in an oven at 60°C until the moisture is about 64 g 100 g^{-1}, prior to the curd being mixed with salt (2.5 g 100 g^{-1}), kneaded manually and shaped into balls (i.e. 100 g each). The balls are then placed in earthenware jars and matured at 6°C and 85% relative humidity (Toufeili *et al.*, 1995).

The compositional quality of shankleesh may vary from one country to another due to inherent differences in the traditional methods used to manufacture this product. Table 5.8 illustrates the proximate gross composition of traditionally and laboratory-made shankleesh. This product and labneh anbaris are normally con-

Table 5.8 Proximate chemical composition (g 100 g^{-1}) of traditionally and laboratory-made shankleesh

Product type	Moisture	Protein	Fat	Lactose	Ash	References
Traditionally made						
NR	30.0	–	12.3[a]	–	–	FAO (1990)
NR	44.0	35.0	5.6	3.0	12.2	Dagher (1991)
Laboratory-made						
Cow	59.8	33.0	2.0	2.3	2.0	Toufeili *et al.* (1995)
Goat	58.9	31.4	4.0	2.6	3.1	
Sheep	56.0	32.2	6.1	2.8	3.0	

NR, not reported.

[a] The fat content was calculated from the reported fat-in-dry matter content.

sumed with bread and olive oil as appetisers, and the possibility of developing such products for markets in Europe and North America, perhaps as basic ingredients for the preparation of cocktail dips, clearly exists.

5.8 Frozen yoghurt

5.8.1 Background, standards and marketing

Frozen yoghurt is classified into three main categories, soft, hard or mousse (Fig. 5.16). These products resemble ice cream in their physical state and they are characterised simply as having the sharp, acidic taste of yoghurt combined with the coldness of ice cream. In addition these products contain high levels of sugar and stabilisers/emulsifiers compared with yoghurt, since these compounds are required during the freezing process to maintain the air-bubble structure.

The historical background of, and technical data on, frozen yoghurt has been discussed in detail by Kosikowski (1977), and Mann (1977, 1979) has compiled several international digests on frozen yoghurt; Lang (1979) and Rothwell (1993) have also reviewed developments in this field. In most countries, frozen yoghurt does not have national standards of identity in terms of chemical composition, minimum yoghurt content, heat treatment of the yoghurt/ice cream mix before freezing and the count of the starter microflora at the time of consumption (Mitten, 1989; Kimbrell *et al.*, 1990; Rothwell, 1993; Childs, 1994; Anon., 1995a, 1996; Westerbeek, 1995a, b, 1996). However, Westerbeek (1996) has pointed out that, in the Netherlands, the standards for frozen yoghurt stipulate that it should contain a minimum yoghurt content ≥70% and have a pH <5, but in the U.S.A., consumers favour frozen yoghurt higher in pH (Brown *et al.*, 1991a). Little data are available on the production figures and market of frozen yoghurt in different countries but, in the U.S.A. (Knuston, 1978; Dryer, 1994; Keehner, 1996) in 1993, the market volume was about 550 million litres.

PRODUCT NATURAL STIRRED YOGHURT
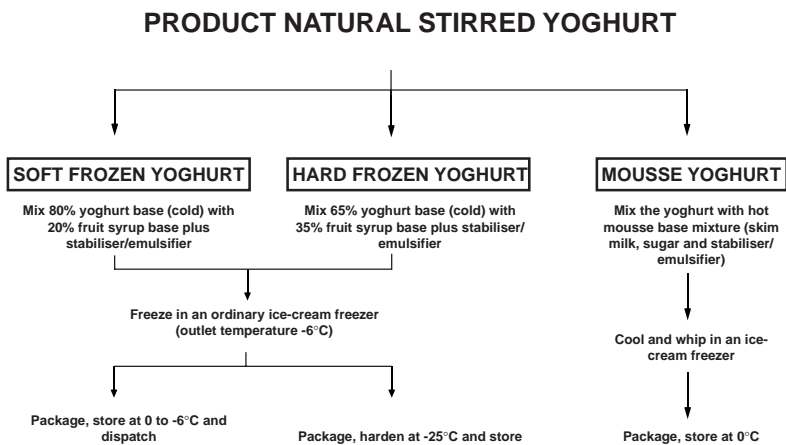
Fig. 5.16 Frozen yoghurt

For further information refer to Kurmann (1969), Crisp and John (1969), Ziemba (1971), Anon. (1977b, 1978a, 1979b), Igoe (1979a), Redfern and Rizk (1979), Gautneb *et al.* (1979), Hekmati and Bradley (1979), Kankari and Antilar (1980), Hulsbusch (1980), Kosikowski (1981), Bradley and Hekmati (1981), Miles and Leeder (1981) and Bray (1981).

5.8.2 Technology of manufacture

In general terms, the various stages involved in the manufacture of the different types of frozen yoghurt are similar (see Fig. 5.16) and some recipes for frozen yoghurt prior to the 1980s have been reported by Bradley and Winder (1977), Collins (1977), Chandan (1977), Mitten (1977), Grosser (1978), Morris (1979) and Speck and Hansen (1983). Basically, the process consists of mixing cold, natural stirred yoghurt with the cold fruit syrup base, stabilisers/emulsifiers and sugar (the latter ingredients are added hot for the manufacture of mousse yoghurt (see Fig. 5.16), then freezing the mix in a conventional ice cream freezer. The chemical composition of the yoghurt/fruit mix and the temperature during storage can ultimately affect the physical characteristics of these frozen yoghurt products, and Table 5.9 illustrates some suggested formulae for their manufacture; the recommended percentages of yoghurt and fruit range from 65–80 to 20–35%, respectively.

More recently, McGill (1995) has patented a container for tempering and dispensing frozen products including frozen yoghurt, while other processes for the manufacture of frozen yoghurt may include:

- no fermentation of the milk base
- direct or indirect fermentation of the milk base (Olsen, 1990a, b; Anon., 1993a).

Thus, these products may be made from yoghurt or a blend of ice cream mix containing sugar and yoghurt at a ratio of 50:50 to make frozen yoghurt with 89–90% overrun (Olsen, 1990a). Also, in some instances the processed milk base or ice cream mix could be inoculated with concentrated starter culture before freezing (Olsen, 1990b). Figure 5.17 shows a flow chart of the equipment required for the production of frozen yoghurt (see also Bylund, 1995), and the following patents provide some additional information (Carvel, 1990; Curry and Beach, 1991; Bee *et al.*, 1994; Heinrich, 1995).

Although the procedures for manufacture are well established, the following recommendations may help to eliminate defects in frozen yoghurt. (a) Ensure that the fruit syrup base is pasteurised and, except in the case of mousse yoghurt, cold prior to its addition to the yoghurt. (b) Gently mix the yoghurt and fruit syrup base, since vigorous agitation can lead to loss of the refreshing taste in the frozen yoghurt. (c) Replace the air at the whipping/freezing stage by nitrogen to achieve a longer shelf life for frozen yoghurt (Jochumsen, 1978). (d) Replace the normal sweetening agent

Table 5.9 Suggested chemical composition (g 100 g^{-1}) of frozen yoghurt mixes

Ingredients	Frozen yoghurt		
	Soft	Hard	Mousse
Fat	2–6	2–6	3
Milk SNF	5–10	5–14	12
Sugar	8–20	8–16	8
Stabilisers/emulsifier	0.2–1.0	0.2–1.0	2.4
% Overrun	50–60	70–80	90

Adapted from Anon. (1977a, c), Mitten (1977), Collins (1977) and Bradley and Winder (1977); for comparison refer to Olsen (1990a, b), Williams (1990/91), Rothwell (1993) and Anon. (1996).
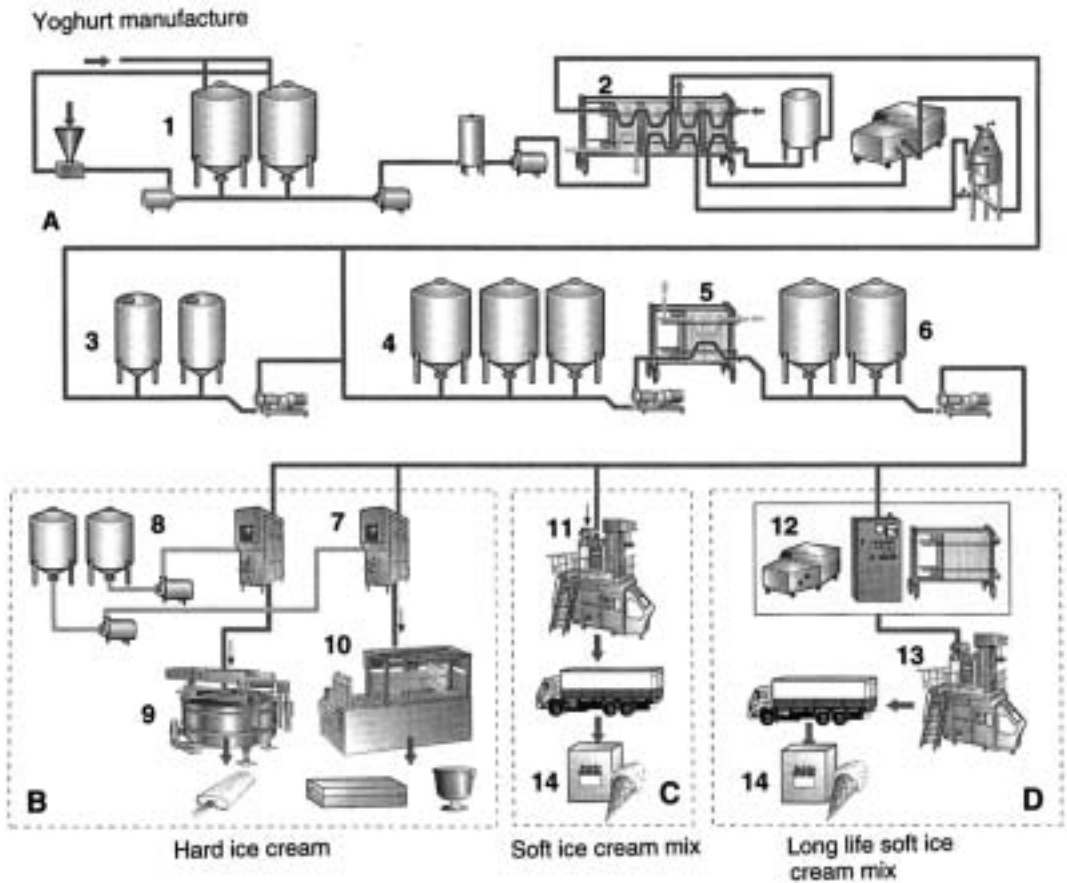
Fig. 5.17 Simplified flowchart for the production of frozen yoghurt: 1, mixing tanks; 2, pasteuriser; 3, bulk starter tanks; 4, incubation tanks; 5, cooler; 6, buffer tanks; 7, ice cream freezer; 8, aroma tanks; 9, bar freezer; 10, cup/cone filler; 11, packaging; 12, UHT treatment; 13, aseptic packaging; 14, soft-ice machine at the retailer

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(e.g. sugar and/or corn syrup) of the fruit base by lactose-hydrolysed whey (Aries, 1977, 1978) (e) Mousse yoghurt without sugar cannot be stored at $<0°C$, since whey syneresis can occur upon thawing and a partial collapse of the foam occurs.

The chemical compositions of some commercial frozen yoghurts in the U.S. market are shown in Table 5.10. The data illustrate a wide variation in the milk components used. Meyer (1989) provided a comprehensive and detailed ingredient comparison of frozen yoghurts marketed in the U.S.A. The fat content in the mix can affect the quality of frozen yoghurt. Venkateshaiah *et al*. (1994, 1996) reported that a fat level of up to $5\,g\,100\,g^{-1}$ produced the most acceptable yoghurt, while in Egypt, $10\,g$ fat $100\,g^{-1}$ was recommended (Gooda *et al*., 1993; Salem *et al*., 1994a, b); the overrun is increased by increasing the fat content (Chen *et al*., 1984). Thus, during the preparation of the mix base, a number of ingredients will be used besides the yoghurt and it is essential that the fat and SNF contents are calculated properly to achieve a balanced mix. The algebraic method for calculation is recommended, espe-

Table 5.10 Range of composition (g 100 g^{-1}) of commercial frozen yoghurts sold in the U.S. market

Flavour	Fat	Protein	Ash	Total solids	pH
Vanilla	1.8–5.9	3.5–3.8	0.7–1.0	28.8–34.2	6.37–7.10
Chocolate	3.2–5.7	2.9–4.2	0.9–1.1	31.1–37.6	6.36–7.10
Strawberry	1.7–5.3	1.6–3.2	0.8–1.1	31.2–37.6	4.37–5.70

Data compiled from Tieszen and Baer (1989).

cially when considering the economics of the operation and the quality of the end product. Hypothetical examples have been reported by Hyde and Rothwell (1973) and Marshall and Arbuckle (1996) for the preparation of ice cream mixes and these examples could also be applicable for frozen yoghurt (see also Appendix IX).

As mentioned elsewhere, American consumers prefer frozen yoghurts with a high pH (Speck and Hansen, 1983; Guinard *et al.*, 1994), whilst Gooda *et al.* (1993) concluded that, while low pH mixes improve the overrun of frozen yoghurt, the products gain slightly lower organoleptic scores after 60 day storage than similar products frozen at pH 5.

The milk SNF of the milk base can be adjusted using different ingredients, such as a 50:50 slurry prepared from soyabean and skimmed milk or buttermilk (Rajasekaran and Rajor, 1989), UF of milk and addition of hydrolysed WPC (Maric *et al.*, 1990; Opdahl, 1990; Opdahl and Baer, 1991), skimmed milk, SMP, yoghurt, cream or vegetable oils and sucrose or maltodextrin (Fuisz, 1993; Malone and Sage, 1993, 1994) and condensed cottage cheese whey (Baig and Prasad, 1996a, b).

The combination of fat (10 g 100 g^{-1}) and starter culture (3%) was highly recommended by Salem *et al.* (1994a, b) for the production of frozen yoghurt. However, the survival of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in frozen yoghurt is of great importance in order to maintain the therapeutic image of the product. Bielecka *et al.* (1982, 1988) reported no inactivation of the starter organisms in frozen yoghurt after 10 months storage at −25°C, and Stenby (1993) reiterated the importance of using special cultures for frozen yoghurt. The viability of the starter culture in frozen yoghurt has been studied by many researchers (Miles and Leeder, 1981; Mashayekh and Brown, 1992; Brown *et al.*, 1991b; Whithead *et al.*, 1993; Childs, 1994; Frison and Agostini, 1994; Thompson and Mistry, 1994; Hong *et al.*, 1996; Andreini, 1997), and observed differences in the counts could be attributed to: (a) the base mix not being properly fermented, (b) the base mix having been heat treated after fermentation and before freezing, and (c) the sensitivity of the starter culture to freezing. Nevertheless, Mashayekh and Brown (1992) and Thompson and Mistry (1994) have reported some reduction in β-galactosidase acitivity (i.e. to about 70%) in frozen yoghurt and, in extreme cases, very low activity makes promoting the efficacy of frozen yoghurt for lactose maldigestors very difficult (Savaiano, 1994). However, improving the survival of the yoghurt bacteria in the frozen product has been achieved using a microentrapment method (Sheu *et al.*, 1993).

Halambeck *et al.* (1984) reported that the use of pure EPS-producing starter cultures was not suitable for the production of frozen yoghurt, because the polysaccharide material interfered with the aggregation of fat and casein. The defect can be minimised by using a blend of non-EPS and EPS starter organisms (Stenby, 1993; Hong *et al.*, 1996).

Consumer acceptability of flavoured frozen yoghurt varies with country (see Chen *et al.*, 1984; van Beckevoort, 1991; Venkateshaiah *et al.*, 1994), as do the types of container used to package frozen yoghurt; some examples have been reported (Anon., 1990a, 1991c, d, 1992b; Friedman, 1991a; Gorski, 1996).

5.8.3 Related products

As with yoghurt, frozen yoghurt has been made successfully from sheep's milk (Smith, 1989; Martinou-Voulasiki and Zerfiridis, 1990) and from buffalo's milk (Mahran *et al.*, 1996; Taha *et al.*, 1997b). Alternatively, low fat Greek-style or strained yoghurt with added pieces of fruit have been used for the production of frozen yoghurt, but no detailed formulations have been reported (Anon., 1990a).

Low calorie frozen yoghurt can be produced from milk low in total solids or with the use of fat substitutes and artificial sweetners. In the former approach, a $70\,kcal$ $100\,g^{-1}$ frozen yoghurt was made by reconstituting SMP to $16\,g\,TS\,100\,g^{-1}$ and, after fermentation, adding carboxymethylcellulose $(0.05\,g\,100\,g^{-1})$ and gelatin $(0.2\,g$ $100\,g^{-1})$, homogenising and then freezing (Therrien *et al.*, 1982). Elsewhere, frozen yoghurt has been prepared from skimmed milk, a blend of artificial sweetners with aspartame, non-metabolisable bulking agents, β-galactosidase to hydrolyse the lactose, sucrose polyesters to replace the milk or dietary fat and yoghurt starter cultures to ferment the mix before freezing (Wolkstein, 1986); however, the sucrose polyester could be replaced by starch-based fat substitutes (Steinsholt and Bjørke, 1995; see also Anon., 1995b).

Developments in soft-serve formulations of frozen yoghurt and products "spoonable" at domestic freezer temperatures have been reported by Morley (1984) and Andreasen (1990). Collier and Cardwell (1988) made a similar product by blending yoghurt with ice milk mix (e.g. at a ratio of 40 : 60) and 8% grape puree (*Vitis rotundifolia*) before freezing; sensory evaluation studies indicated consumer acceptability of these types of fruit flavoured frozen yoghurt. Powder preparations for the manufacture of soft-serve frozen yoghurt have been reported in different countries (Devshony, 1987; Huber and Rowley, 1988; Anon., 1991a, 1992a; Spano, 1995).

Frozen yoghurt has also been proven to be an acceptable vehicle for incorporation of bifibobacteria and *L. acidophilus* into the human diet (McBean, 1990; Morel, 1990). *B. bifidum* and *L. acidophilus* survived well in high pH frozen yoghurt with an average count of each of $3.6 \times 10^6\,cfu\,ml^{-1}$ after 8 weeks' storage at $-29°C$ (Laroia and Martin; 1991; see also Otero *et al.*, 1997), and a similar observation was reported by Modler and Villa-Garcia (1993) for *Bifidobacterium longum*. The same microflora was used in ice cream making and, after storage for 16 weeks at $-20°C$, the count of each organism was about $1.0 \times 10^7\,cfu\,ml^{-1}$ (Christiansen *et al.*, 1996). However, a slight drop in the count was anticipated before and after freezing due to the incorporation of air at the whipping stage and freezing. Other workers observed no survival of *B. bifidum* in low pH 3.9–4.6 ice cream mixes (Tamime *et al.*, 1995a). Recently, zabady was made by replacing 33% and 50% of the yoghurt starter culture with *B. bifidum* DI or BB_{12}, respectively, during the manufacture of the base from which frozen zabady was made (Kebary, 1996); the numbers of bifidobacteria that survived after 5 weeks' storage averaged $10^7\,cfu\,ml^{-1}$. Arany *et al.* (1995) have reported that, using a roll-tube repair-detection procedure, recovery of cells of bifidobacteria from frozen yoghurt was significantly ($P < 0.01$) better than with the

pour plate method. However, it could be argued that damaged cells are unlikely to survive passage through the digestive tract and hence pour plate counts could provide a more realistic picture.

Hong *et al.* (1996) evaluated three different commercially available bio-cultures (ABT, ABY-2 and AC-180) for their effect on the texture and flavour of frozen yoghurt and they reported that the highest readings for hardness, cohesiveness and elasticity were for the product made with the ABT culture, and that the sensory evaluation scores did not differ significantly between the frozen yoghurts made with the three cultures. An improved nutritional value for "Bellevue" frozen yoghurt is suggested by the use of vegetable oils rather than milk fat along with bifidobacteria and *Lactobacillus* species (Kawano, 1985; see also Taha *et al.*, 1997b), whilst a soft-serve frozen yoghurt that is fat-free and cholesterol-free and has no added sugar has been described (Anon., 1991b).

5.9   Dried yoghurt

5.9.1   Introduction
The primary objective of manufacturing yoghurt in powder form is to store the product in a stable and readily utilisable state. Traditionally, natural/plain yoghurt, which is low in fat, is concentrated, shaped into flat rolls and sun dried (see Kurmann *et al.*, 1992). The dried yoghurt is normally utilised by the desert dwellers in the preparation of food dishes, soups or even consumed like biscuits with tea. However, the first commercial attempts to produce dried yoghurt were aimed at the do-it-yourself consumer market and the reconstituted yoghurt lacked a high viable cell count of starter culture organisms, as well as the pleasant taste, firm body/texture and the attractive appearance of ordinary yoghurt. However, there has been a considerable effort made to improve the quality of dried yoghurt and in general the powder forms are now divided into two different types. In the first type, the reconstituted yoghurt is incubated for a few hours to allow the coagulation process to take place, while in the second type the gel is formed within a very short period of time – so-called instant yoghurt. Neither of these products has gained consumer acceptability because the reconstituted product does not resemble fresh yoghurt. Nevertheless, yoghurt powder can be easily used to prepare a beverage drink. A wide range of patents have been filed in many countries (Ferguson, 1963; Chamay, 1967; Simon and Devallerie, 1968; Anon., 1973a, b; Bohren, 1974; Schur, 1978; Trop, 1980, 1986; Duffy, 1981; Cajicas, 1981a, b, 1990; Rudin, 1984; Tokumaru *et al.*, 1987, 1989; Costanzo and Calcavecchia, 1989; Usacheva *et al.*, 1991, 1992; Kunizhev *et al.*, 1992; Beutler *et al.*, 1993). It is evident from the method of processing that many additives are used to give the powder a yoghurt-like appearance and taste upon rehydration. Some examples of these additives are sucrose, dextrose, stabilisers (i.e. xanthan gums, starch, locust bean gum, Na-alginate), sequestering agents, calcium coprecipiate, organic acids and acidogen (see also Mazaleva and Gugin, 1966; Vitez, 1968; Charon, 1968; Gavin, 1969; Radaeva *et al.*, 1970; Vitanov *et al.*, 1973; Schober, 1973; Schober and Landwehr, 1973; Blanchaud, 1973a, b). The milk may be fermented with a combination of cultures such as *S. thermophilus* and *L. helveticus* or a yoghurt culture and *L. acidophilus* (Rudin, 1984; Beutler *et al.*, 1993).

5.9.2 Processing methods

Traditional products such madeer, oggtt and plain kishk (see Section 5.9.3) are produced by Bedouins in some Middle Eastern countries. Milk from different species of mammals has been used for the production of these products. Normally, skimmed or buttermilk from churned fermented milk is concentrated, shaped into flat rolls and dried in the sun (Al-Mashhadi *et al.*, 1987; Al-Ruqaie *et al.*, 1987; Al-Mohizea *et al.*, 1988). However, Al-Raquaie and El-Nakhal (1987) have produced successfully an acceptable tamar oggtt from cultured skimmed milk and chopped dates (i.e. Tamar in Arabic). Evidence of the production of dried yoghurt can be found from western Asia to Turkestan where the product is called churpi or zurpi, in Nepal chura, in Turkey kurut, in Tibet tschurra, in the former U.S.S.R. katyk and in Algeria klila (Tamime and O'Connor, 1995).

Basically there are two methods of drying that could be employed commercially for the manufacture of dried yoghurt (spray-drying or freeze-drying) and although the latter method of drying would seem the more attractive – the temperature of drying (20–35°C) is much lower than with spray drying (55–60°C) so ultimately causing the least damage to the milk constituents, and/or loss of flavour – it is far too expensive to be considered on a commercial scale. However, another drying method known as air-diffusion (dispersion) drying has been used to dehydrate dahi, and the dried product had properties similar to a freeze-dried one, but with improved reconstitutability (Baisya and Bose, 1974). In a separate study, they reported that the reconstitution properties of dried dahi were improved in the presence of lecithin and corn starch (Baisya and Bose, 1975; Baisya *et al.*, 1978). Rathi *et al.* (1990) freeze dried dahi at −20°C for 12 hour, and the reconstituted product received slightly lower sensory scores, but much lower curd tension and viscosity measurements than a fresh product. The poor rheological properties of the reconstituted dahi were due to the destruction of the gel structure during the drying process. However, a process for the manufacture of dried yoghurt with a predetermined geometrical shape was reported by Costanzo and Calcavecchia (1989) who recommended freeze drying at −30 to −40°C. However, Sharma *et al.* (1992a) and Sharma and Arora (1993) observed that increasing the milk solids in yoghurt to $18.8\,g\,100\,g^{-1}$ TS resulted in an improved yield of freeze-dried yoghurt from 0.22 to $0.31\,kg\,m^{-2}\,h^{-1}$, and a reduction in the drying time per unit output of 25.8%; a further increase in the milk solids imparted a chalky taste to the dried product.

At present, powdered yoghurt is produced commercially using spray drying, but some precautionary measures should be considered. First, the concentration of yoghurt, before drying, should be carried out at 50–60°C and second, the drying conditions should be moderate to ensure a high viable cell count of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in the dried product. In addition, concentrating the yoghurt at higher temperatures increases the scorching onto the surfaces of the evaporator and causes discoloration of the final powder. Masters (1991) and Caric (1994) have provided some specifications for spray – drying buttermilk. The acidified milk was concentrated to $36\,g\,100\,g^{-1}$ TS at 58°C in an evaporator with a degassing stage and spray dried at 43°C with an integrated fluid bed as a cooler. A scraped surface evaporator might also be used to concentrate the yoghurt before drying.

De and Patel (1989, 1990) produced chakka and shrikhand powders using a spray drier by maintaining the inlet and outlet temperatures at 190 and 95°C, respectively;

the speed of the atomizer was controlled at 25 000 revolutions min^{-1} (RPM). However, for shirkhand powder making, the chakka was mixed with sugar and water and the blend was homogenised before drying.

Both APV and Niro companies are leading drier manufacturers and yoghurt can be dried in a three-stage drying plant. An illustration is shown in Fig. 5.18, and on average, the yoghurt is concentrated to 35 g 100 g^{-1} TS, preheated and atomised into the drying chamber with inlet and outlet air temperatures at 160 and 65°C, respectively. The semi-dried yoghurt particles fall down to the bottom of the drying chamber onto an integrated fluid bed drier; such particles form a fluidised layer which is further dried. Later, the powder is transferred to an external fluid bed drier for final drying and cooling. During drying, the product temperature is about 55°C and the powder outlet is at 25°C. Incidentally, the spent drying air from both the drying chamber and external fluid bed drier is drawn through a series of cyclones to recover the fine powder particles (fines) from the air. The fines are fed back to the external bed drier where they are mixed with the bulk of the powder to maximise the yield. Such dried yogurt contains 2 g 100 g^{-1} moisture and has a tapped bulk density of 0.5 g cm^{-3} (see also Gendrel *et al.*, 1990).

Thus, there are different types of dried yoghurt products (traditional or industrial) available to consumers in different markets and Table 5.11 illustrates some examples. Some of these products contain low quantities of fat because either skimmed milk or the buttermilk from churned fermented milk was used to make the yoghurt. The microbial counts (cfu g^{-1}) in a commercial dried yoghurt of non-lactic acid bacteria, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* were as follows: $<1 \times 10^4$, 1×10^3 and 1×10^4, respectively (Anon., 1983b), whilst Pan *et al.* (1995) reported that the count of lactobacilli in dried yoghurt was 7×10^5 cfu g^{-1} (see also Rybka and Kailasapthy, 1995; Kim *et al.*, 1997). In the U.K., high and low acid
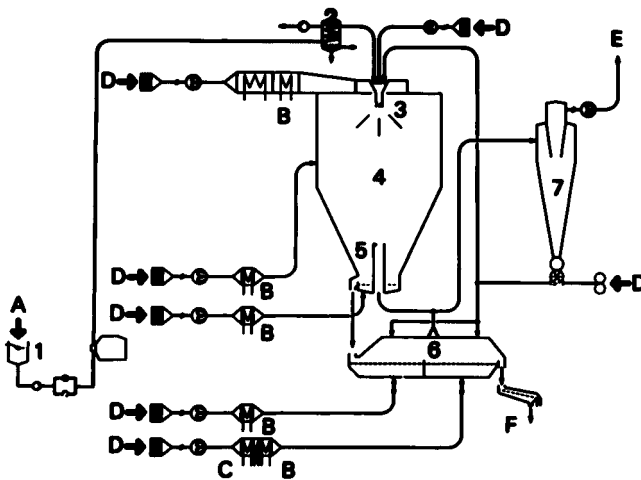
Fig. 5.18 Schematic illustration of a three-stage drying plant for the manufacture of dried yoghurt: A, product inlet; B, steam; C, cooling water; D, air inlet; E, air outlet; F, product outlet; 1, feed tank; 2, preheater; 3, atomizer; 4, spray drying chamber; 5, integrated fluid bed; 6, external fluid bed; 7, cyclone

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Table 5.11 Chemical composition (g 100 g^{-1}) of different types of dried yoghurt products

Product		Total solids	Protein	Fat	Lactose	Ash
Oggtt						
Sheep	(WM)	95.6	31.7	39.3	19.3	5.3
	(BM)	91.9	37.3	14.5	32.5	7.6
Cow	(WM)	96.3	26.2	25.4	38.7	6.0
	(BM)	93.7	31.1	11.0	44.4	7.2
Goat	(WM)[a]	92.5	30.4	18.9	37.3	6.5
	(WM)	93.1	26.3	28.9	34.8	5.3
Madeer						
Unknown[a]		91.8	36.4	13.4	34.4	7.6
Goat		96.1	35.5	15.3	37.4	7.9
Yoghurt						
Cow (SM)[a]		96.0	33.0	4.0	52.0	7.0
Cow (SM)[a]		96.0	35.0	1.0	54.4	5.6
Kishk (plain)						
Iranian[a]		95.6	54.4	7.9	29.5	3.8

WM, Whole milk; BM, buttermilk; SM, skimmed milk.

[a] Commercial samples.

Data compiled from von Taleban and Renner (1972), Anon. (1983d), Sawaya *et al.* (1984), Al-Mashhadi *et al.* (1987), Al-Ruqai *et al.* (1987), Al-Mohizea *et al.* (1988) and Holland *et al.* (1991).

dried yoghurts are produced to suit different applications within the food industry and the dried yoghurt is packaged in 25 kg multiwall paper sacks with sealed polyethylene liners (Anon., 1987b).

Kim and Bhowmik (1990) reported that the survival rate of the yoghurt organisms was influenced by the processing conditions of the spray drier and they recommended that the product inlet feed temperature be at 30°C, the air inlet and outlet temperatures be at 160 and 60°C, respectively, the atomising air pressure be at 98 kPa and the hot air flow be 0.23 m^{-3} min^{-1}. The survival rate of *S. thermophilus* was higher than *L. delbrueckii* subsp. *bulgaricus*, but both organisms showed similar survival patterns in freeze-dried yoghurt powder (Kim and Bhowmik, 1990, 1995). However, the fermentation characteristics of the yoghurt microflora and other lactic acid bacteria in an oats-based sour dough and one with enzymatically treated oats influenced the aroma of the products (Marklinder and Lonner, 1992); the intracellular leucine aminopeptidase of *L. delbrueckii* subsp. *bulgaricus* minimised the liberation of bitter peptides (Tchorbanov *et al.*, 1993).

As the formation of the yoghurt gel, after rehydration of some powders, relies entirely on the presence of stabilising agent(s), the yoghurt has a different mouthfeel from the fresh product and this difference could prove to be a limiting factor in terms of acceptability. Alternative outlets for dried yoghurt may include:

- Reconstitution of the powder to 24–26 g 100 g^{-1} TS for the production of labneh (see also Kharrazi, 1990; Maroudas, 1992).
- Hill (1974) reported that when adding yoghurt (in liquid form) to dough in the manufacture of baked goods, it could be advantageous to bakers to use the dried form since they are more familiar with handling dry ingredients (see also Fluckiger, 1973). Also, dried yoghurt can be used in confectionery coatings (Main, 1991; Anon., 1991g; Herbertz, 1997).
- The results of field trials on poultry feeding with dried yoghurt, compared with

skimmed milk powder, favoured the former product, due either to an increased availability of nutrients (i.e. metabolisable energy (ME) and gross protein value (GPV)) or to a reduction in the amount of lactose (Simhaee and Keshavarz, 1974).

- Products, such as yoghurt-flavoured wafers and chocolates with yoghurt flavour inners, have appeared on the market in Europe and North America, and the manufacturers of such products may prefer to use dried yoghurt in their processes.
- Dried yoghurt can also be used for the manufacture of yoghurt-flavoured candy (Peterson, 1979), soup preparations (Rezai, 1985), dips (Main, 1991) and oil emulsion products (Milkova and Stamova, 1992).

5.9.3   Kishk and related products

These products are dry forms of yoghurt–cereal (or other additives) mixtures which are made traditionally throughout the region between the eastern Mediterranean and the Indian subcontinent. According to Kurmann *et al*. (1992) and Tamime and O'Connor (1995), many names are applied to dried fermented milk and, depending on the ingredient and/or additivies used, it is possible to classify them as follows:

- Products containing parboiled cracked wheat or flour found in the Arab countries are called kishk, kushuk, keshkeh, kichk, burghul yoghurt, hugut, zhum or kushik, in Greece and Turkey trahana, in Nepal and Tibet chura and in India kadhi (see also Ghosh and Kulkarni, 1990).
- Products containing vegetables, herbs and/or spices are found in Egypt where the product is called kishk siamy, in Greece and Turkey kapestoes, trahanocirv or zamplaricos.
- Products containing other types of cereals (e.g. oats and barley, see Tamime *et al*., 1997a, b), sorghum (i.e. in Sudan um-kushuk), chick pea, rice or maize (see Dirar, 1993) and pearl millet (Dhankher and Chauhan, 1987a, b).

Milks from different species of mammals (cow, goat, sheep or buffalo) or a mixture of these have been used for the production of kishk. Traditionally, skimmed milk or the buttermilk from churned fermented milk is normally used and whey or milk plus soy-milk has been used in laboratory-made kishk. All these aspects pose problems for the classification of kishk, while the ratio of cereal to fermented milk, which may range between 1:2 and 1:4, affects the quality of the product. Recently, Tamime and O'Connor (1995) have reviewed kishk extensively in terms of its chemical composition (Table 5.12), microbiological quality, nutritional value and methods of manufacture (see also Tzanetakis, 1996).

The main cereal additive (i.e. parboiled cracked wheat) is known by different names such as burghol, bourghoul, burghul or bulgur. The method of preparation could be described as follows. A soft wheat variety is cleaned of stalks, dirt and other cereal grains using a rotary cylindrical machine which is known locally in the Lebanon as ghorbal; it has been illustrated by Tamime and O'Connor (1995). The same machine sizes the wheat kernels into three fractions (i.e. large, small or broken), and the large grains are used to make burghol by steeping the grains in boiling water for 1 hour until soft and then drying in the sun for 24 hours. On the following day, the dried grains are moistened with water (about $20\,g\,100\,g^{-1}$), cracked

Table 5.12 Proximate range of chemical composition (g 100 g^{-1}) of different types of kishk

Product	Additive	Moisture	Protein	Fat	Carbohydrate	Fibre	Ash
1. Fermented milk							
Commercial	WB[a]	5.5–13.0	8.9–23.5	1.6–16.1	31.0–65.3	0.7–2.5	2.0–9.1
	WB	8.4	17.8	6.4	68.8	9.3	7.0
Laboratory	WB	7.5–9.5	14.5–19.7	–	–	~2.4	4.4–8.7
	WF[b]	6.0–12.5	17.6–19.1	–	~56.3	–	3.6–4.6
	MF	–	18.3	–	–	–	2.2
	CF[b]	5.2	25.8	–	–	–	4.9
	RF[b]	12.4	19.3	–	62.5	–	4.7
	MaF[b]	11.8	17.6	–	60.1	–	4.6
	WB[b,c]	8.7	20.3	6.4	66.7	9.0	[d]
	OB[b,c]	8.2	20.5	9.7	63.0	6.7	[d]
	BB[b,c]	8.4	18.8	6.8	67.9	8.4	[d]
2. Fermented milk +/or soy milk							
	WB	9.1–9.2	16.1–17.2	–	–	–	–
	WB, WF, CF[b]	5.2–9.9	18.3–28.2	–	–	–	3.5–5.3
3. Fermented whey							
	WB	9.7	13.3	–	–	–	–

WB: wheat burghol; WF: wheat flour; MF: malted flour; CF: chick pea flour; MaF: maize flour; OB: oats burghol; BB: barley burghol. Dash indicates no results reported.
[a] Average of 25 commercial Lebanese samples of kishk (Tamime, unpublished data). [b] Computed on dry matter basis. [c] After Tamime *et al*. (1997b). [d] For details refer to Table 5.13.
Data compiled from Hassan and Hussein (1987), Abou-Donia *et al*. (1991) and Tamime and O'Connor (1995).

and dehusked. The burghol is separated from the husk by density fractionation using a mechanical winnowing machine (Tamime *et al.*, 1997a). The same machine sizes the burghol into fine or coarse and the latter fraction is used in kishk making. This process can cause the loss of some nutrients from the wheat grain. Tamime *et al.* (1997a) have reported on the losses from burghol made from wheat, oats and barley. In particular, the different parboiled cracked cereals revealed significant differences in the fibre, carbohydrate and mineral content, and these can, in turn, influence the nutritional properties of kishk (see also Oner *et al.* (1993) on the use of soya beans in trahana making).

Details of the many different traditional methods employed for the manufacture of kishk in different countries in the Middle East have been reviewed by Tamime and O'Connor (1995) (see also FAO, 1982, 1990; Farr, 1982; Jandal, 1989, 1994, 1996; Dagher, 1991). Figure 5.19 illustrates the traditional manufacturing stages of kishk
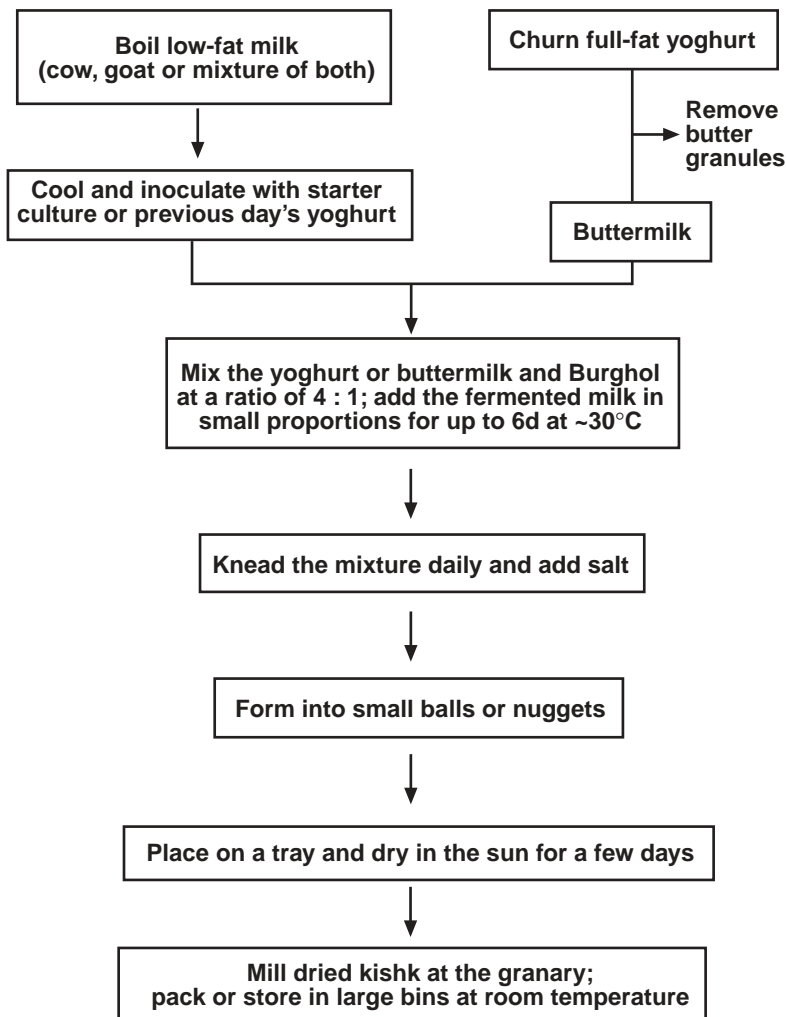
Fig. 5.19 Illustration of the traditional method for the manufacture of Lebanese kishk

in the Lebanon. Ibanoglu *et al.* (1996) used response surface methodology to study the effect of the barrel temperature of a twin screw extruder, the feed rate and the screw speed on starch gelatinisation in trahana making. A regression equation for predicting starch gelatinisation suggested that barrel temperature had the most pronounced effect, followed by feed rate and screw speed (see also Ibanoglu and Ainsworth, 1997).

Garnier (1957), Morcos *et al.* (1973), Robinson (1978), Robinson and Cadena (1978), Cadena and Robinson (1979), Salama *et al.* (1992), Damir *et al.* (1992) and Ibanoglu *et al.* (1995a, b, 1997) have investigated in detail the potential value of kishk for preserving milk protein from spoilage and concluded that the method could prove valuable. The protein content of kishk is high, giving an excellent amino acid content, whose the level is increased due to the metabolic activity of the starter cultures during the fermentation. Kishk contains high concentrations of phenyalanine, threonine, isoleucine, leucine, arginine, valine, tyrosine and lysine, but it has low amounts of tryptophan and sulphur-containing amino acids. The amino acid spectrum of the end product was close to the FAO/WHO (1973, 1985) standard, and only tryptophan and, to a lesser degree, lysine and threonine were at limiting values. The loss of tryptophan could be attributed to the decomposition of the amino acid during the fermentation and sun-drying stages; the tryptophan content of laboratory-made kishk was similar to that suggested by FAO/WHO (1985) (see also Cadena and Robinson, 1979; Sawaya *et al.*, 1984). However, the *in vitro* digestibility of trahana was influenced by the ingredients used including the ratio of fermented milk to cereal used (Ibanoglu *et al.*, 1995a).

Details of the mean concentrations of minerals in kishk have been given by Tamime and O'Connor (1995) and the values are influenced by the type of cereal used. Tamime *et al.* (1997b) profiled the spectrum of the minerals in kishk made with wheat, oats and barley burghol (see Table 5.13). It is evident that the product is a good source of minerals that originate from the milk and cereal, and the wheat burghol including soy milk and chick pea flour are good sources of iron which is deficient in milk.

Kishk is a good source of the B vitamins, but deficient in vitamin C and the fat-

Table 5.13 Mean concentrations (mg 100 g^{-1})[a] of minerals in kishk made from burghol manufactured from wheat, oats and barley

Minerals	Type of kishk		
	Wheat	Oats	Barley
Sodium	1360	1360	1356
Potassium	799	752	753
Phosphorus	552	629	549
Calcium	439	460	425
Magnesium	116	129	107
Copper	0.4	0.3	0.3
Zinc	3.6	3.9	3.2
Iron	21.6	9.3	7.6
Manganese	1.7	3.2	0.9

[a] Data computed on dry matter basis.

After Tamime *et al.* (1997b).

soluble vitamins. The increase in the niacin and riboflavin or provitamin A could be attributed to the activity of the starter culture and addition of tomatoes, respectively (Tamime and O'Connor, 1995). Losses of thiamin (*c.* 30%), but not riboflavin, occurred when the trahana was dried in an oven at 55°C for 48 hour (Ibanoglu *et al.*, 1997).

Kishk (as a dish) is prepared by reconstituting the dried product with water and then simmering the mix gently over a fire. The consistency of this product is rather similar to porridge and it is normally consumed with bread. In some instances, flavouring agents such as chopped onions, tomatoes and/or coriander are added to the gruel mix. Alternatively, kishk is widely used in the Middle East in soup preparations. Although the flavour of kishk or trahana is influenced by the type of lactic acid bacteria used to ferment the milk (Abou-Donia *et al.*, 1991; Lazos *et al.*, 1993), the survival rate may be irrelevant because these products are heated after rehydration.

The unusual flavour and nature of kishk is widely enjoyed among the rural communities in the Middle East, but the introduction of such a mixture to other societies may be rather restricted in terms of appeal and acceptability. However, Cadena and Robinson (1979) conducted an experimental trial in Mexico in which a gruel-type food called atole was replaced by a yoghurt cereal product and the yoghurt-based equivalent was readily accepted by children and mothers, especially when the product was flavoured with strawberry and vanilla extracts. It is safe to assume, therefore, that a flavoured and sweetened kishk could prove to have wide acceptability among communities accustomed to gruel-type foods.

A wide range of basic sensory schemes have been used to evaluate the properties of laboratory-made kishk and related products (see Tamime and O'Connor, 1995), but Muir *et al.* (1995) have developed a sensory vocabulary using a professional panel of eleven assessors to characterise kishk. The descriptors developed were:

- Seven attributes for aroma (overall intensity, creamy/milky, acid/vinegary/sharp, fruity/sweet, cooked, cereal and cardboard).
- Ten attributes for flavour (overall intensity, cream/milky, acid/vinegary/sharp, fruity/sweet, cooked, cereal, cardboard, apple, bitter and salty).
- Five attributes for aftertaste (overall intensity, persistence, acid/vinegary/sharp, cereal and cardboard.
- Five attributes for mouthfeel (viscosity, grainy/floury/chalky texture, sticky/gluey texture, slimy texture and mouth-coating character).

Scottish oatmeal porridges and kishks made from goat's, cow's and mixtures of both milks were evaluated using this sensory scheme and the results could be summarised as follows. First, the oat products were substantially different from the kishk due to the fermented milk component and second, the kishks made from goat's milk were clearly distinguishable from those made with yoghurt of bovine origin.

In another study, Tamime *et al.* (1997b) evaluated kishks made with different cereals and the sensory profiles showed substantial differences between them. Differences in mouthfeel (i.e. grainy, sticky and slimy character) were associated with cereal type. Partial squares regression (PLS2) models derived from the chemical composition of these products were successfully fitted, after cross validation, for grainy, sticky and slimy character. Only the model of grainy character was of predictive value.

The microbiological quality of kishk (commercial and laboratory-made) and related products varied widely which reflects the standards of hygiene during production (see review by Tamime and O'Connor, 1995). Owing to the acidic nature of the product (about 3.8 pH after rehydration), the low moisture content ($<10\,g$ $100\,g^{-1}$) and the presence of salt ($c.\ 3\,g\,100\,g^{-1}$), kishk should exhibit a high degree of microbiological safety. According to Tamime and O'Connor (1995) and Aytac (1996), the microbiological counts (cfu g^{-1}) of these products were: *Enterococcus faecium* 3.4×10^2, range of total counts $<10 \times 10^3$–2.6×10^7, range of lactic acid bacteria 4.5×10^3–2.2×10^7, and range of yeast and mould 9×10^1–1.4×10^4. The majority of organisms making up the total counts were spore formers belonging to the genus *Bacillus*, and these spores will not be killed when the kishk is cooked. Consequently, if a kishk gruel is prepared, boiled and then allowed to stand at ambient temperature for several hours prior to consumption, toxins generated by *Bacillus cereus*, for example, could cause problems. This could be the reason why the death of two people in Iran, who had clinical symptoms of botulism food poisoning, was associated to the consumption of kishk. Haydarynia (1990) confirmed that *Clostridium botulinum* could survive in laboratory-made kishk and then grow and produce toxins in the gruel but, as the genus is anaerobic, long-term survival in dry kishk is unlikely.

5.10 Bio-yoghurt

The overall nutritive value of yoghurt is well established (see Chapter 9), but special types of yoghurt are often manufactured for dietetic and/or therapeutic purposes and are known as bio-yoghurts. The fact that most strains of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* do not survive in the intestinal tract may be a limiting factor if yoghurt is used for antibiotic therapy and/or any other medicinal purposes. The starter cultures employed in the manufacture of bio-fermented milks including yoghurt-related products are shown in Table 5.14 (Marshall and Tamime, 1997a, b). The main organisms belong to the following genera: *Lactobacillus*, *Bifidobacterium*, *Enterococcus* and *Pediococcus*. The latest nomenclature, classification, physiology and biochemistry of the microfloras used in bio-fermented milks have been reviewed by Sneath *et al*. (1986), Bezkorovainy and Miller-Catchpole (1989), Barlows *et al*. (1992), Wood and Holzapfel (1995), Tamime *et al.* (1995a) and Fuller (1997).

However, although the incorporation of *L. acidophilus* and *Bifidobacterium* species into the yoghurt starter culture may contravene some existing definitions of yoghurt, the resultant milk product is reported to be of excellent therapeutic value. Tamime *et al*. (1995a) and Tamime and Marshall (1997) have reviewed a wide range of products (fermented, dried, frozen confectionery, cheese, baby food, unfermented milk) that are available in different markets. Table 5.15 illustrates some examples of fermented milk products that are available in the European market (Tamime, 1997).

It is evident, however, that knowledge of the potential value of bio-yoghurt in medicinal therapy is limited at the present time, and furthermore that the financial rewards to industry will be dependent on the response and back-up of the medical profession. Nevertheless, since there have been some publications regarding the role of lactic acid bacteria in health and disease (Wood, 1992; Salminen and von Wright,

Table 5.14 Starter cultures that are used during the manufacture of biofermented milks and their principal products

Starter organisms		Principal metabolites
L. acidophilus	DL	lactate
paracasei subsp. *paracasei*	L(+)	lactate
paracasei biovar *shirota*	L(+)	lactate
rhamnosus	L(+)	lactate
reuteri	DL	lactate, CO_2
B. bifidum		lactate, acetate
adolescentis	L(+)	lactate, acetate
breve	L(+)	lactate, acetate
infantis		lactate, acetate
longum	L(+)	lactate, acetate
lactis		lactate, acetate
E. faecium	L(+)	lactate
faecalis	L(+)	lactate
P. acidilactici	DL	lactate

Data compiled from Devriese and Pot (1995), Marshall and Tamime (1997a, b), Tamime and Marshall (1997) and Tamime (1997).

1993), and it is evident that only those lactic organism(s) that are of human origin and able to proliferate in the intestinal tract of human beings should be considered to be of likely therapeutic benefit.

5.11 Fat-substitutes yoghurt

One method which can be used to manufacture a low fat, light or low calorie yoghurt involves the use of fat substitutes (i.e. materials with the same functional and organoleptic properties as fats but without the calories) to replace the fat in the milk base. Many different types of fat substitute are available on the market and the technically developed fat substitutes are divided into two main types: modified starches or proteins which have good emulsifying or gel properties along with low calorie values; and modified products which contain bonds resistant to digestion, for example, glycerol ethers and complex carbohydrates or fatty acids esters. Thus, it is possible to propose the following approach to the classification of fat substitutes based on their origin or method of processing:

• Modified starches and hydrocolloids including fibre-based products,
• Modified milk, egg and/or soya proteins known as microparticulated proteins,
• Synthetic compounds containing modified ester bonds.

Technical data on these fat substitutes have been reported by Murray (1988), Hendley and Seymour (1988), Lee (1989), Anderson (1990), Keuning (1990), Friedman (1991b), Iyengar and Gross (1991), Altschul (1993), Akoh and Swanson (1994), Miller (1994) and Artz and Hansen (1996). Some patents provide more technical information on fat substitutes (Neilsen *et al*., 1993; Ohkuma *et al*., 1993; Rhodes, 1995).

Table 5.16 provides some examples of fat substitutes that are used for fat replacement in yoghurt, butter spreads, sour cream, processed and natural cheeses,

Table 5.15 Commercial and developed bio-fermented milk products

Product	\<Microflora\> 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
AB®, Diphilus®, Cultura®, Biomild®, LA7			✓						✓										
Acidophilus-Bifidus yoghurt, Lünebest®	✓	✓	✓						✓+	✓+									
Bioghurt®	✓		✓																
Biokys®			✓						✓								✓		
Olifus®	✓+		✓						✓						✓+				
Progurt®			✓						✓						✓	✓			
BA®, Biobest®, Bifidus yoghurt	✓	✓							✓+	✓+	✓+								
Bifidus milk									✓+	✓+	✓+								
Bifighurt®	✓									✓									
Bifilact®, Bifilakt®								✓					✓						
ABT, Biogarde®	✓		✓						✓										
Bifilus®, Onaka®, Procult 3®, BBA®										✓									
Aktifit®	✓		✓			✓			✓										
BRA® yoghurt			✓	✓							✓								
Pro Viva®, Prima Liv®							✓												
Symbalance®			✓	✓	✓					✓									
Vita®			✓		✓				✓										
Gaio®, Praghurt	✓+	✓																✓	
ABC®, Miru-Miru®			✓		✓							✓+	✓+						
ACT4®	✓		✓		✓				✓										
Yoke®	✓		✓		✓														
Yakult®					✓+	✓+													
Mil-Mil®	✓	✓	✓						✓+		✓+								
Koumiss		✓	✓																✓
Acidophiline			✓											✓					✓
LC1®, Fysig®, Timi Active®			✓																

1, *S. thermophilus*; 2, *L. delbrueckii* subsp. *bulgaricus*; 3, *L. acidophilus*; 4, *L. reuteri*; 5, *L. paracasei* subsp. *paracasei* and biovar *shirota*; 6, *L. rhamnosus*; 7, *Lactobacillus plantarum*; 8, *Lactobacillus* sp.; 9 → 12, *B. bifidum, longum, infantis, breve*, respectively; 13, *Bifidobacterium* sp.; 14 → 16, *L. lactis* subsp. *lactis*, subsp. *cremoris*, biovar *diacetylactis*, respectively; 17, *P. acidilactici*; 18, *Ent. faecium*; 19, kefir yeast.

Data compiled from Tamime and Marshall (1997) and Tamime (1997).

Table 5.16 Classification and some examples of fat substitute products that are used in dairy products

Type/trade name	Technical information		Trade name	Technical information	
	Source	Energy value		Source	Energy value
Modified starches and hydrocolloids					
Gums	Many		Maltrin®	Corn	4 kcal g^{-1}
N-Oil®	Tapioca	3.6 kcal g^{-1}	N-Lite®D	Tapioca	3.8 kcal g^{-1}
Paselli®	Maltodextrin	4 kcal g^{-1}	Litesse™	Polydextrose	1 kcal g^{-1}
Lycadex®	Potato/waxy maize	NR	NatuReal®	Oat	16.8 kcal g^{-1}
Crestar®	Potato	3.8 kcal g^{-1}	Amalean®	Starch	343 cal g^{-1}
Stellar®	Maize	15.4 kcal g^{-1}	Rice® Complete	Rice	16 kJ g^{-1}
Orbitaron®	Maltodextrin	16.7 kJ g^{-1}	Optagrade®	Corn	NR
Tapiocaline®	Tapioca	3.5 kJ g^{-1}	Slendid	Methyl esters of polygalacturonic acid	NR
Modified fibres					
Fibraline®	Inulin	4.2 kJ g^{-1}	Swelite®	Pea	4.2 kJ g^{-1}
Fibrex®	Sugarbeet	2.8 kJ g^{-1}	JustFibre®	Cellulose	0 kJ g^{-1}
Raftaline®	Inulin	4.2 kJ g^{-1}	Oatrim®	Oat	~16 kJ g^{-1}
Sofalite®	Pea	0.5 kcal g^{-1}	Vivacel®	Microcrystalline celluose	0 kJ g^{-1}
Microparticulated protein					
Simplesse®	Milk	16.4 kJ g^{-1}	Trailblazer®		NR
Lita®	Corn	NR	Miprodan®	Milk	NR
Dairy-Lo™	Milk	4 kcal g^{-1}	AMP	Milk	3.9 cal g^{-1}
Danpro®	Soya	11 kJ g^{-1}	Nutrilac®	Milk	~19 kJ g^{-1}
Domovictus®	Milk	15.5 kJ g^{-1}	Globula	Milk	NR
Synthetic compounds					
Olestra®	Sucrose, polyester	NR	Many products	(EPG, TACTA, DUR-Lo and Jojoba oil), but are not widely used in dairy products (see Tamime *et al.*, 1994)	

NR, Not reported.

Note: fat-based products such as Delios® and Tropicana® are made from vegetable oil and coconut milk, respectively and have been used in dairy products.

Conversion 1 kcal g^{-1} to 1 kJ g^{-1}, multiply by 4.18.

Data compiled from Huyghebaert (1990), Anon. (1991f, 1994), Blenford (1993) and Tamime *et al.* (1994).

liquid milk and frozen desserts including ice cream (Tamime *et al.*, 1994; Anon., 1994; Phillips and Barbano, 1997). A wide range of scientific papers have been published on fat substitutes, although it is far beyond the remit of this publication to review this topic in detail. Nonetheless, the role of starches as fat substitutes or fat enhancers in yoghurt formulations has been discussed (Doreau 1993, 1994; Anon., 1995c; McGlinchy, 1995), whilst the role of pectin, inulin, rice-based flour, microparticulated whey proteins and insoluble dietary fibre in food and yoghurt making has been reported (LaBarge, 1988; Harrigan and Breene, 1989; Anon., 1990b, 1991e, 1993b; Singer and Dunn, 1990; Kalab, 1990; Riisom, 1991; Singhal *et al.*, 1991; Kratz, 1993; Paquin, *et al.*, 1993; Lieske and Konrad, 1994; Orthoefer *et al.*, 1995; Franck, 1995; Robinson, 1995b; Buchheim and Hoffmann, 1994; Fernandez-Garcia and McGregor, 1997).

Farooq and Haque (1992) produced successfully a low calorie yoghurt using skimmed milk, SMP, modified starch, Aspartame® and sugar esters. The sugar esters, mainly stearates with a hydrophilic–lipophilic balance in the range of 5 to 9 were derived from edible fats and oils. This substitution produced a yoghurt with body, texture and mouthfeel characteristics similar to an equivalent product without sugar esters. In a separate study, low calorie yoghurts were made from reconstituted SMP (about $14\,g\,100\,g^{-1}$ TS) and seven types of starch-based fat substitutes (Litesse™-improved polydextrose, N-Oil®II, Lycadex® 100 and 200 – maltodextrin, Paselli® SA2, and P-Fibre 150 C and 285 F) added at a rate of $1.5\,g\,100\,g^{-1}$, and these were compared with the control made with anhydrous milk fat (AMF) (Barrantes, 1993; Barrantes *et al.*, 1994d). The finished yoghurts had total solids contents that ranged between 14 and $15.6\,g\,100\,g^{-1}$. The lactic acid was mainly produced by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* and the presence of these fat substitutes in milk did not affect their metabolic activity (Barrantes and Tamime, 1992).

The microbiological quality of these low calorie yoghurts was excellent and the coliform and yeast and mould counts were $<10\,cfu\,g^{-1}$ in fresh and stored products; both starter organisms were recovered in high numbers (streptococci $\times 10^8\,cfu\,g^{-1}$ and lactobacilli $\times 10^5\,cfu\,g^{-1}$). All the yoghurts were rated acceptable by the taste panellists, except P-Fibre 150 C and 285 F products which were not favoured when fresh or after storage (Barrantes *et al.*, 1994b). The flavour and aroma scores of the yoghurts were higher after storage, suggesting that the fat substitutes would not affect the quality of yoghurt during storage and distribution (Barrantes *et al.*, 1994b). Also these same yoghurts (with the exception of P-Fibre fat substitutes) were assessed by typical consumers ($n = 182$), but with the products sweetened with $1\,g\,100\,g^{-1}$ sugar and flavoured with strawberries (Barrantes *et al.*, 1993; Ronchetti, 1995). The results suggested that (a) the order of the presentation of the yoghurts was significant ($P < 0.05$) and the products tasted first and last tended to score higher than the other yoghurts, and (b) aspects such as sex, yoghurt consumption habits, age or nationality of the consumer did not significantly influence yoghurt preference; however, overseas consumers (i.e. about 3%) had a higher preference for yoghurts made with AMF and N-Oil®II fat-substitute and lower preference for Lycadex® yoghurts than the U.K. consumers. This latter aspect should be studied separately and with a higher proportion of overseas consumers if the products are to be marketed in foreign countries.

Serum separation and firmness of all these fat substitute yoghurts were very similar with the exception of the product made with P-Fibre 150 C in which the least amount of syneresis was observed during storage (Barrantes *et al.*, 1994c). There was a linear plus quadratic effect (i.e. decrease in serum separation or increase in

firmness) with time. Some statistically significant correlations ($P < 0.05$) were observed when certain variables were combined, protein content, viscosity of the milk, serum separation and firmness). In addition, SEM and TEM studies revealed subtle differences in the microstructure of set-style yoghurts due to the different starch-based fat substitutes used (Tamime *et al.*, 1996). Spikes and hairline structures were evident around the casein micelles in the milk base; they were lightly stained when compared with the caseins. Their detection in the yoghurt was very difficult and they were only seen clearly with the P-Fibre 150C and 285F substitutes (Fig. 5.20a); with the other substitutes, spikes could not be detected even when the concentration of the compound was increased to $5\,g\,100\,g^{-1}$. Yoghurt made with Lycadex® 100 was more porous and had slightly larger void spaces filled with milk serum. The use of higher concentration ($5\,g\,100\,g^{-1}$) of fat substitutes increased firmness, but impaired the flavour and mouthfeel of the yoghurts.

In a separate study, Barrantes *et al.* (1994e) reported on the effect of adding protein-based fat substitutes or microparticulated whey proteins (Simplesse® 100 in wet and dry forms) to yoghurts, and compared the end products with yoghurt containing AMF ($1.5\,g\,100\,g^{-1}$). The quality of whey protein-based yoghurts (at a $1.5\,g\,100\,g^{-1}$ level of addition) was high and similar to that of the control samples containing AMF. However, serum separation was higher and firmness lower for yoghurts containing microparticulated whey protein compared with those containing AMF. The differences between yoghurts containing AMF and microparticulated whey protein were most marked when the wet type was incorporated on an equivalent dry matter basis to AMF. The sensory panel identified significant differences ($P < 0.05$) between products containing AMF and microparticulated whey protein only in terms of sour odour and perceived serum separation. The microstructure (i.e. TEM) of these yoghurts revealed that homogenisation of AMF produced fat globules which interacted with milk proteins present in the yoghurt base and thus the fat becomes an integral part of the yoghurt microstructure (Tamime *et al.*, 1995b). Similar integration was observed with the fat substitute, the particles of which ($0.1–3\,\mu m$ in diameter) were found to form part of the casein micelle chains or span adjacent chains (Fig. 5.20b). These chains were found to be somewhat shorter (no statistical assessment was carried out) in the yoghurts made with the fat substitutes in wet or dry forms than in the yoghurt made with AMF.
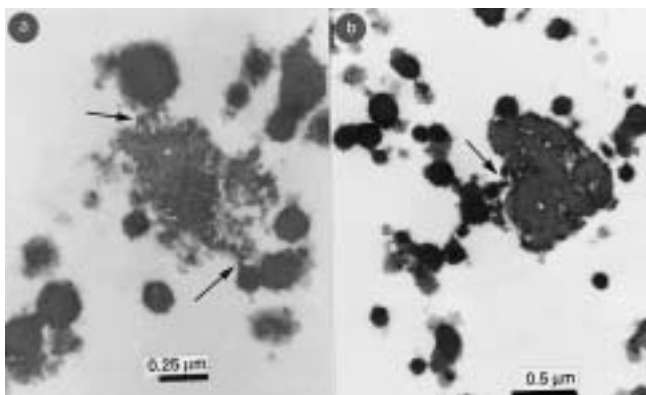
Fig. 5.20 Casein particle chains (TEM) in yoghurt are attached (arrows) to P-fibre 150C (a) and microparticulated protein particle of Simplesse® fat substitute (b)

5.12 Vegetable oil yoghurt

In developing countries, 'filled' milk products are manufactured from reconstituted skimmed milk powder and the milk fat is replaced by vegetable fats or oils. The use of these indigenous fats and oils is primarily aimed at avoiding the cost of imported fat (i.e. unsalted butter or AMF), while maintaining a wide range of dairy products. Although filled milk products are not supported by the International Dairy Federation, they have been produced for more than 30 years. There is no doubt that these products benefit consumers in developing countries and to satisfy the nutritional requirements of filled milk products, Newstead *et al.* (1979) have recommended the addition of vitamins A and D. Incidentally, dietetic acidophilus milk has been produced in the former U.S.S.R. from skimmed milk fortified with $2\,g\,100\,g^{-1}$ maize oil, whilst in the U.S.A., Metzger (1962b) has patented a process for the manufacture of yoghurt containing unsaturated fat or vegetable oils.

Awareness of consumers in many countries with regard to the dietary aspects of food in relation to cardiovascular disease has increased over the past few decades and the general consensus among the medical profession is that an increased intake of unsaturated fats or oils would be welcome; hence vegetable oil yoghurt may provide an alternative product for consumers. Little data are available on fermented milks made with vegetable oils, but the development of two vegetable fats which can be used in filled yoghurt has been reported (Anon., 1985a). Mouniqua (1986) has patented a base comprising 93% fermented milk (i.e. made using a starter culture of *L. delbrueckii* subsp. *bulgaricus* and *L. helveticus*), $3\,g\,100\,g^{-1}$ oil (e.g. groundnut, maize or soya) and $3\,g\,100\,g^{-1}$ modified starch for the manufacture of low fat and low energy sauces. Whilst Shamanova *et al.* (1989) developed a special yoghurt containing 2.4 and $0.8\,g\,100\,g^{-1}$ dairy fat and vegetable oil, respectively and the product was declared suitable for 1–6 year old children. A mango flavoured filled bio-yoghurt was made successfully from a milk base (18 g and $4.5\,g\,100\,g^{-1}$ SNF and vegetable oil, respectively), processed and inoculated with a yoghurt starter culture and *B. bifidum* (Asgar and Thompkinson, 1994).

Al-Saleh and Hammad (1992) reported that the sensory properties of yoghurt made by substituting milk fat with maize and sunflower oil were characterised as being inferior when compared with equivalent products made with either cow's or camel's AMF or butter. Similarly, Barrantes *et al.* (1996a) reported that the sensory panel had identified significant differences ($P < 0.05$) between natural flavoured yoghurt containing $1.5\,g\,100\,g^{-1}$ AMF and vegetable oils (olive, maize, groundnut or sunflower) in terms of perceived whey separation and some flavour and aroma attributes (e.g. acidic, oxidised, unclean and aftertaste). However, when the same yoghurts were sweetened and flavoured with processed strawberry fruit (Barrantes *et al.*, 1994a), the results of a consumer survey ($n = 80$) suggested that: (a) the yoghurt preference did not appear to be influenced by the amount of yoghurt consumed per week by the consumers (i.e. <3, 4–5 or >5 pots per week) or nationality (Scottish region, elsewhere in the U.K. or from overseas), (b) the yoghurts containing AMF and groundnut oil were rated significantly higher ($P < 0.05$) by females than males, and (c) all age groups (<20, 20–30 and >30 years) rated the AMF yoghurt highest with the sunflower yoghurt lowest; however, only the 20–30 years age group detected any appreciable differences between the other three types of vegetable oil yoghurt.

The stability of the oil emulsion during the manufacture of vegetable oil yoghurt

was reported by Barrantes (1993) and Barrantes *et al.* (1996a). Milk bases (14 g TS 100 g^{-1}) containing 1.5 g 100 g^{-1} of either olive, groundnut, sunflower or corn oil were subjected to homogenisation at 60°C using three different pressures (17.3, 20.7 and 24.1 MPa) and processed in the manner normal for the manufacture of yoghurt. The processed milks, containing no starter cultures, were dispensed into 150 ml plastic cups at 42°C for 3 hours. The oil content of the top and bottom layers was analysed at 0 and 3 hours; any differences in these determinations is indicative of an unstable emulsion. Three hours was chosen because during production it resembles normal yoghurt making, the starter culture would have reduced the pH to a value at which the gel starts to form and, as a consequence, oil droplets would be prevented from rising to the surface. Separation of the milk base was not observed for any homogenisation pressure used and hence no emulsifier was required. Also, no statistically significant difference (variance–ratio test) between the size of AMF or oil globules was observed and the particle sizes in all milk samples were finely dispersed (Barrantes *et al.*, 1996a).

The rheological properties and microstructure of set-type natural yoghurt containing different vegetable oils were reported by Barrantes *et al.* (1996b). They concluded that whey separation was higher and firmness was lower for all the vegetable oil-based yoghurts than for the product containing AMF and microscopy studies (SEM) suggested that the microstructures of all the yoghurts were similar (i.e. porosity of the protein matrices); TEM examinations revealed that both the milk fat and all the vegetable oil globules interacted with the casein micelles and participated in the formation of the gel matrices. Figure 5.21 illustrates these effects. The yoghurts were made with 10 g 100 g^{-1} AMF, cream or vegetable oils (corn, olive, ground nut and sunflower) to show the incidences of membrane formation around the fat/oil globules and its attachment to the casein micelle particles.
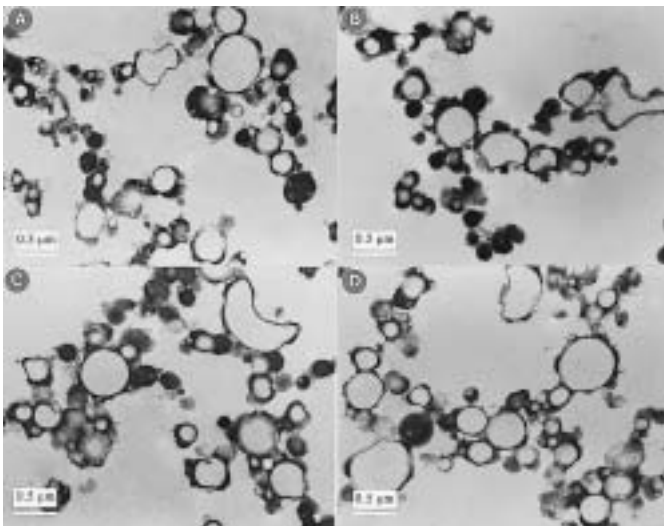
Fig. 5.21 Illustrations of the microstructure (TEM) of yoghurts made with different milk fats or vegetable oils

A, Cream; B, olive oil; C, sunflower oil; D, corn oil.

5.13 Chemically acidified yoghurt

The addition of organic acids (ascorbic, acetic, fumaric, malic, lactic, tartaric, citric, succinic, oxalic and phosphoric) or glucono-δ-lactone (GDL) to milk can result in the formation of a coagulum at pH < 4.6. The end product is referred to as directly or chemically acidified yoghurt, and while it resembles yoghurt in its appearance, delicate gel, body and texture, it lacks the typical aroma, flavour and the therapeutic qualities of cultured yoghurt. The manufacture of this type of yoghurt is included in this section merely for comparison. The principles of this technique are discussed in various patents (Morgan *et al.*, 1970; Edwards, 1976; Igoe, 1979b; Takahata, 1980; Kulkarni *et al.*, 1980; Manabe and Miyake, 1985; Budolfsen and Nielsen, 1994) and details of the kinetics of colloidal aggregation of milk using GDL have been given by Banon and Hardy (1991, 1992).

The parameters selected for the production of directly acidified milk desserts were reported by Schwab (1996) and the milk base (g 100 g^{-1}) consisted of protein 4, fat 3.4, and sugar 8. However, acidification with lactic rather than citric acid was recommended and this process provides scope for continuous production (see also Hammelehle *et al.*, 1997). The use of calcium gluconate (i.e. as a means of enhancing the calcium content of cultured yoghurt) affected the gel firmness and syneresis of the product (Flinger *et al.*, 1988). However, Akbulut and Kinik (1991) recommended the use of 1 g 100 g^{-1} GDL in conventional yoghurt production to shorten the incubation period by 45% and give increased gel strength (see also Gregurek *et al.*, 1996; El-Etriby *et al.*, 1997). A similar observation was reported by Fly *et al.* (1997). Furthermore, Bayoumi and Madkor (1988) and Bayoumi and Reuter (1989) have reported that the combined use of starter culture and 1 g 100 g^{-1} GDL in yoghurt making improved the organoleptic properties of the product and a good quality yoghurt could be made from non-homogenised milk.

In a recent study, Vlahopoulou and Bell (1995) compared the gelation processes of fermented milks using EPS and non-EPS starter cultures and GDL acidification of cow's and goat's milk, and they concluded that fermented cow's milk produced a gel with about half the firmness of the equivalent GDL gel, the gel of goat's milk yoghurt was 8 to 10 times than that of the GDL product and the EPS cultures formed weaker gels in both types of milk base than the equivalent non-EPS cultures and GDL.

5.14 Soy-milk yoghurt

Owing to the worldwide shortage of food, attempts have been made to find alternative sources of protein, particularly for the developing countries where malnutrition exists. Since soybeans are plentiful, relatively inexpensive and rich in protein (see Table 5.17), some effort has been devoted to exploiting them for the manufacture of more acceptable and palatable food products. Thus the main objections to soybean products from the consumer are associated with the beany flavour and the phenomenon of flatulence (i.e. production of carbon dioxide, hydrogen and methane by the intestinal flora during the breakdown and/or metabolism of oligosaccharides present in the soybean). It is possible that these problems can, of course, be overcome by various processing techniques and/or fermentations and two current approaches to the production of fermented food are the use of soy-milk for the

Table 5.17 Comparative chemical analysis (g 100 g^{-1}) of soy-milk and cow's milk

Component	Milk	
	Soy	Cow
Protein	3.6	3.3
Fat	1.9	3.9
Carbohydrate	2.8	4.7
Ash	0.4	0.7
Water	91.3	87.4

Data compiled from Angeles and Marth (1971) and Table 2.1.

manufacture of a yoghurt-like product and the extension of mammalian milk with soy extracts for the manufacture of yoghurt.

Over time, many researchers in different countries have studied and developed many fermented products, such as soy-milk yoghurt and a bibliography of published work since 1910 has been reported by Aoyagi (1994). Some relevant aspects of soy-milk fermentation have been reported by Mital and Steinkraus (1976, 1979) and Gupta *et al.* (1987); whilst Yamanak *et al.* (1969), Fridman (1976), Emura and Ohba (1989), van Oosten and Verhue (1990) and Karmas and Bachmann (1991) have patented different methods for the preparation of yoghurt from soy-milk.

The production of yoghurt from soy-milk was evaluated by Pinthong *et al.* (1980a–c), who concluded that: (a) using *L. delbrueckii* subsp. *bulgaricus* alone, an acceptable yoghurt-like product can be manufactured from soy-milk, (b) optimum quality of the fermented product was observed at about 1.15% lactic acid, which resulted in the formation of a homogeneous, firm curd without whey separation, and an improved flavour compared with soy-milk, (c) the flavour of fermented soy-milk was directly related to the levels of *n*-pentanal and *n*-hexanal; *S. thermophilus* produces the former compound, while *n*-hexanal is naturally present in soy-milk and (d) the reduction in the level of oligosaccharides was insignificant.

An example of the alternative approach is the fortification of cow's or buffalo's milk with soy extract (basically protein) for the manufacture of zabadi. This introduction of soy-protein into the milk base aimed to alleviate the existing shortage of mammalian milk in Egypt, and when Abou-Donia *et al.* (1980) evaluated the quality of this zabadi they concluded that:

- The level of acidity, total nitrogen and volatile acids increased gradually in both cow's and buffalo's milk, as the level of soy extract was raised from 10 to 50 g 100 ml^{-1}.
- In general, the organoleptic assessment of these soy yoghurts was, in terms of body and texture, appearance and acidity, similar to the controls, but the major difference was in the flavour; a score of only 25 points out of 45 was recorded for zabadi with 10 g 100 ml^{-1} soy extract, as against 40 for the control (no soy extract added).
- The use of soy extract concentrations above 10 g 100 g^{-1} imparted a beany flavour which was not accepted by the taste panel.

- The method used for the preparation of the soy extract in this study could not be recommended, since other methods can eliminate the beany flavour altogether.

Khader *et al.* (1983) recommended that defatted soy-milk (i.e. 45%) could be used to replace buffalo's milk for the manufacture of zabadi; whey syneresis decreased dramatically in the product after 24 hours storage at 5°C. However, El-Sayed and El-Sayed (1988) concluded that the addition of soy-milk to buffalo's milk should not exceed 10% because the starter culture counts decreased with increasing soy-milk concentation and the acceptability of zabadi decreased due to the detection of a beany flavour. Choprea and Prasad (1992) observed a reduced rate of acid development in soy-milk fermented with *S. thermophilus* when compared with buffalo's skimmed milk. Yoghurt made from a mixture of buffalo's and soy-milk at a ratio of 65:35 was rated acceptable by a sensory panel; the addition of Na-alginate $(0.2 \text{ g } 100 \text{ g}^{-1})$ improved the texture of the product when compared with the control or with the use of carboxymethyl cellulose.

Dimov *et al.* (1982) produced a dietetic product, which was claimed to be suitable for the prevention of allergic diseases, by mixing yoghurt at 10°C with an equal amount of uncultured soy-milk; this approach could overcome the reduced acid development by starter cultures in soy-milk, and/or their survival during storage. Although the growth of *L. acidophilus, L. delbrueckii* subsp. *bulgaricus* and a yoghurt starter were similar in cow's, UF cow's and soy-milk, and counts did not alter in cow's products during storage, but decreased greatly in fermented soy-milk, the latter products had a distinctive "pulse" off-flavour (Krsev, 1983).

The rate of fortification of cow's milk with soy-milk varies greatly in the reported literature. de Souza *et al.* (1990, 1991) claimed that soy-milk yoghurt containing 10–15% cow's milk was acceptable, especially when flavoured, whilst Caric *et al.* (1983) made yoghurt from milk that was fortified with soy isolate or dried soy-milk; the addition of sugar, coffee and caramel was recommended to mask the soy flavour. Alternatively, a mixture of soy-milk and skimmed milk (i.e. at a ratio of 80:20) plus $1 \text{ g } 100 \text{ g}^{-1}$ sucrose fermented with a mixed culture consisting of *S. thermophilus* and *L. acidophilus* produced a firm yoghurt with no beany flavour (Chopra *et al.*, 1984). However, a mixture of 50:50 was recommended by Miyamoto *et al.* (1983) and the best yoghurt was made with *L. delbrueckii* subsp. *bulgaricus* alone (see also Hardi and Novakovic, 1994; Kinik and Akbulut, 1996; Radwan, 1996). Other reported formulations have been claimed to improve the flavour and consistency of the fermented product. (a) The use of cow's milk fortified with 20% whey protein concentrate (WPC) and 2% soy protein concentrate improved the quality of zabadi (El-Neshawy and El-Shafie, 1988). (b) Soy-milk fortified with 6–8% SMP produced an acceptable yoghurt with good flavour, firm body and smooth texture (El-Gazzar and Hafez, 1992), whilst Patel *et al.* (1989) recommended the use of 2–3% SMP. (c) Soy-milk fortified with caseinates or casein hydrolysate, but not whey protein hydrolysate, and later made into yoghurt was similar to a product made from cow's milk in terms of lactic acid content, key volatile compounds, flavour and texture (Karleskind *et al.*, 1991; Yadav *et al.*, 1994; Granata and Morr, 1996). (d) Soy-milk fortified with cheese whey or whey solids and SMP or dried whey and oat flour were used successfully to produce acceptable yoghurts (Rossi *et al.*, 1984, 1993; Paolielo *et al.*, 1987; Shirai *et al.*, 1992a, b).

Yoghurt made from soy protein contentrates alone had an unacceptable taste and mouthfeel and was yellowish in colour. Such defects could be minimised using

different additives and/or processing methods and some examples include: (a) the addition of glucose or fructose to the milk base (Hasenmaile, 1993) or the use of lactose and citrate (Patel and Gupta, 1982); Buono (1989) and Buono *et al.* (1990c) reported that soy-milk yoghurt was not widely acceptable, (b) enzyme treatment of soy protein concentrate with protease or papain and fortified with $1\,g\,100\,g^{-1}$ glucose enhanced the growth of *L. acidophilus* and slightly improved the sensory properties of the fermented product (Ko, 1990; Kim *et al.*, 1990), (c) the addition of sucrose, stabilisers, Na-citrate and/or Ca-sulphate helped to improve the flavour and sensory properties of a soy-based product (Paolielo *et al.*, 1987; Shelef *et al.*, 1988; Vargas *et al.*, 1989; Nsofor and Chukwu, 1992; Rossi *et al.*, 1993); however, Cheng *et al.* (1990) observed no improvement in the quality of sogurt made from soy-milk fortified with Ca-acetate, gelatin and lactose, and (d) carbon-treated soy-milk, later fortified with SMP and WPC, produced a product that compared well with yoghurt except for flavour; the treatment did not remove the phenolic compounds present in soy-milk (Lee *et al.*, 1990b; see also Trindade *et al.*, 1998).

The kinetics of carbohydrate utilisation by the yoghurt organisms were studied by Buono *et al.* (1990a, b) who concluded that: (a) the performance of a mixed culture based on a weight:weight ratio was better than that selected on a cell:cell ratio of 1:1, and (b) cultures stored in soy-milk ≥168 hour were able to hydrolyse stachyose. de Valdez and de Giori (1993) observed that the presence of *S. thermophilus* in soy-milk cultured with *L. acidophilus* reduced the viability of the lactobacilli in the product. However, Wang *et al.* (1995) reported that the best flavour in soy-milk yoghurt was obtained when the milk base was sweetened with sucrose and later cultured with *L. acidophilus* and *S. thermophilus*; the presence of *B. bifidum* stimulated the growth of both yoghurt organisms in cultured soy-milk (Murti *et al.*, 1993). Shehata *et al.* (1984) studied the growth behaviour of a wide range of lactic acid bacteria in soy-milk (i.e. mesophilic lactococci, the yoghurt organisms, *L. paracasei* subsp. *paracasei* and *L. helveticus*), and they concluded that the growth was improved when soy-milk was heated at 60°C for 15–60 min in the presence of glucose and lactose. These contrasting observations could be influenced by strain(s) variation, and possibly by the type of soy-milk used.

Bacteria cultured in the exudates of cassava and corn reduced the pH of soy-milk at a faster rate than cow's milk and the culture that originated from corn produced the most acceptable yoghurt-like aroma in cultured soy-milk (Nsofor *et al.*, 1992). It may be that different organisms should be used for cultured soy-milk rather than the conventional yoghurt starter cultures in order to minimise the beany taste of soy. Finally, a soy-milk drinking yoghurt was produced from blanched soy bean cotyledons ground with buttermilk (i.e. slurry consisting of soy solids and buttermilk solids in a ratio of 2:1 or 1:1) and then processed into an acceptable cultured and sweetened product resembling lassi or dahi (Deka *et al.*, 1984; Deka and Rajor, 1988; Rajor, 1990).

5.15   Miscellaneous yoghurt products

A wide range of yoghurt products appear in different markets of the world. While some products may have been commercialised or a limited market has been established, other yoghurt products have been developed in order to generate dietetic/therapeutic yoghurts for medicinal purposes and to provide a wider range of retail products for display in shops or supermarkets than may appeal to special

Table 5.18 Selection of yoghurt-based products for medicinal and product development purposes

Product	References
Cholesterol-free	Metzger (1962b)
Vitamin enriched, Vitana	Metzger (1962a), Anon. (1977d), Primatesta (1981)
Low calorie	Grabs (1979), Munk (1980)
Wheat bran or fibre	Costamanga and Rossi (1980), Anon. (1983c)
Antibiotic therapy, dietetic and pharmaceutical tablets[a]	Anon. (1974, 1977e), Kisza *et al*. (1978), Rossi *et al*. (1978), Kanbe (1987), Tamime and Marshall (1997)
Infant feed	Ivanov *et al*. (1973), Ilyin *et al*. (1982), Rasic *et al*. (1982), Anon. (1983a), Morales de Leon *et al*. (1988), Cavaliere *et al*. (1994b)
Yoghurt for sportsmen	Baltadzhieva *et al*. (1981)
Humanised cultured milk	Kochkova and Spasov (1981)
Guar yoghurt	Robinson and Khan (1978), Khan (1980)
Dietetic soy-milk yoghurt	Dimov *et al*. (1981)
Liqueur or wine	Morris (1983), Anon. (1985b), Kao (1987), Anon. (1990c), Celestino and Dulay (1990)
Sour porridge (uji)	Mbugua *et al*. (1984)
Salad dressing	Main (1991), Suhaj and Krkoskova (1995)
Yoghurt cakes	Yuan *et al*. (1993), Altman and Landis (1995), Saintain (1995)
Pet food	Heinemann and Fedder (1995)
Fermented sausages	Swartz (1982)

[a] Product(s) may contain bio-cultures and yoghurt microflora.

consumers. Some examples are shown in Table 5.18 and some reviews of these products have been published by Mann (1978, 1985c).

A yoghurt product which may have market potential is the mousse, foamed or whipped type-yoghurt. In order to maintain air bubble stability in the foamed product, a combination of stabilisers and emulsifiers are used (Kozhev and Tsonkova, 1986; Zeller, 1986; Dalziel *et al.*, 1989). A typical example of an aerated yoghurt formulation (kg) consisted of: yoghurt 5.2, sugar 0.7, cream 3.3 (i.e. double cream about 48 g 100 g^{-1} fat), Hamulsion SPR 0.34 (obtained from G.C. Hahn and dissolved in 1.7l of boiling water at 90°C and added to the mix at 60°C) and fruit (25%). The mix is aerated using a Mondomix machine with an overrun around 65–70% (Tamime, unpublished data).

5.16 Future developments and conclusion

It is evident that some traditional products, for example concentrated/strained yoghurt, have been adapted by technologists for manufacture with mechanised processing equipment and with minimum modification of the quality of the product. Achievements in this area ensure that the products can be commercialised and, at the same time, modified to suit the preferences of consumers in different markets.

Thus it is safe to assume that prior to 1950s yoghurt was virtually unknown outside the Middle East and the Balkan region, but sweetening and the addition of fruits to yoghurt have increased its popularity and acceptability worldwide. It is most likely that some yoghurt-based products may follow these developments, especially products such as dried yoghurt or kishk and related products that may offer nutritional benefits.

5.17   References

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6

Microbiology of yoghurt and "bio" starter cultures

6.1 Introduction

The first bacteriological study of yoghurt was made by Grigoroff (1905) who observed three different micro-organisms present, namely a diplostreptococcus, a rod/coccal-shaped *Lactobacillus* and a rod-shaped *Lactobacillus*. The same observation was also reported by Lüerssen and Kühn (1908). However, the popularity of yoghurt could be attributed to Metchnikoff (1910), who postulated the theory that prolongation of life would follow ingestion of a lactic acid bacterium named as *Bulgarian bacillus*. The presence of this organism in yoghurt was supposed to inhibit the growth of putrefactive organisms in the intestine.

The *Bulgarian bacillus* is, in fact, *Thermobacterium bulgaricum* (Orla-Jensen, 1931), later designated as *Lactobacillus bulgaricus* (currently known as *L. delbrueckii* subsp. *bulgaricus*). However, Rettger and Cheplin (1921) and Rettger *et al.* (1935) found that *Thermobacterium acidophilin (Lactobacillus acidophillus)* is the lactic acid bacterium that can establish itself in the intestine, and furthermore, that the main therapeutic value of yoghurt is observed when *L. acidophilus* is one of the bacteria present in the starter culture. The classification of the lactic acid bacteria by Orla-Jensen (1931) is still recognised as the standard method for distinguishing these organisms, i.e. the sphere shape was *Streptococcus* and the rod forms were *Thermobacterium*, *Streptobacterium* and *Betabacterium*. According to Orla-Jensen (1931), the yoghurt starter organisms were thermophilic lactic acid bacteria capable of growing at 40–45°C. These organisms were designated as *Thermobacterium bulgaricum*, *Thermobacterium jugurti* (*Lactobacillus jugurti*) and *Streptococcus thermophilus*. According to the seventh edition of Bergey's Manual (1957), all the lactic acid bacteria were grouped into one family, the Lactobacillacceae, which was subdivided into the Streptococceae (ovoid or spherical in shape) and the Lactobacilleae (rod-shaped). But this classification was reorganised in the eighth edition of Bergey's Manual (1974) to give two separate families, the Streptococcaceae and the Lactobacillaceae, whilst in the latest edition of Bergey's Manual (1986) the same

organisms are grouped in different sections. For example, the Gram-positive cocci consist of two families where the genus *Streptococcus* is grouped in family II, i.e. Deinococcaceae. However, the genus *Lactobacillus* is grouped in a separate section known as regular, non-sporing, Gram-positive rods. The group-N lactic streptococci (i.e. the mesophilic type) are now known as *Lactococcus* species, and *S. thermophilus* (i.e. a thermophilic organism) has retained its nomenclature.

6.1.1 Historical background and classification

The taxonomic status of *S. thermophilus* reported by Orla-Jensen (1931) has fluctuated since the 1980s due to the close relationship between this organism and *Streptococcus salivarius* and, as a consequence, it was denoted as a subspecies (e.g. *S. salivarius* subsp. *thermophilus*). In 1991, a separate species status was reproposed on the basis of both genetic and phenetic criteria; for further detail see the reviews by Hardie and Whiley (1992, 1995). Selected characteristics of *S. thermophilus* are shown in Table 6.1. Other characteristics may include:

- Spherical or ovoid cell morphology, <1 μm in diameter and forming chains or occurring in pairs.
- Absence of growth at 15°C, whilst growth at 45°C may give rise to irregular cells and segments; most strains are able to grow at 50°C or survive heating for 30 min at 60°C.
- Bacteria are Gram-positive, anaerobic homofermentative lactic acid and produce L(+) lactate, acetaldehyde and diacetyl from lactose in milk.
- Some strains produce exopolysaccharide (EPS), and require B vitamins and some amino acids for enhanced growth rates.
- Absence of growth in methylene blue (0.1 g 100 ml^{-1}) or at pH 9.6.
- The cell wall peptidoglycon type is Lys-Ala$_{2-3}$, and 16S rRNA sequence data have demonstrated close association between *S. thermophilus*, *S. salivarius* and *Streptococcus vestibularis*.
- A group antigen for serological identification has not been demonstrated (see also Nour *et al*., 1989; Ehrman *et al*., 1992).

The situation is different when certain *Lactobacillus* spp. are considered with regard to classification and nomenclature. The standard method proposed by Orla-Jensen (1931) (i.e. *Thermobacterium*, *Streptobacterium* and *Betabacterium*) has been replaced using group I, II or III in the latest edition of Bergey's Manual (1986); however, the history of the group and the redefinitions of the lactobacilli have been reviewed by Bottazzi (1988), Collins *et al*. (1991), Hammes *et al*. (1992), Hertel *et al*. (1993), Pot *et al*. (1994) and Hammes and Vogel (1995). Studies of the guanine plus cytosine (G + C) content of deoxyribonucleic acid (DNA), DNA–DNA hybridisation and enzyme homology have shown that *L. jugurti* is a biotype of *Lactobacillus helveticus* and there is no reassociation between *L. bulgaricus* and *L. jugurti* (Simonds *et al*., 1971; Nakamura and Anzai, 1971). The DNA homology between *L. jugurti* and *L. helveticus* is about 80–100%, and the former which is considered to be a maltose-negative variant of *L. helveticus* is not recognised any more (London, 1976). However, because of the high phenotypic and genomic similarities between *Lactobacillus delbrueckii*, *leichmanni*, *lactis* and *bulgaricus*, only *L. delbrueckii* has been retained as a separate species, whilst the other organisms are subspecies. Both *L. lactis* and *L. leichmanni* are grouped as *L. delbrueckii* subsp. *lactis* and

Table 6.1 Selected characteristics of some lactic acid bacteria[a] associated with yoghurt

Characteristic	Streptococcus spp.		L. delbrueckii subsp.			Lactobacillus spp.		
	thermophilus	salivarius	delbrueckii	bulgaricus	lactis	acidophilus	helveticus	jugurti[b]
G + C[c] mean (%)	37–40	39–42	49–51	49–51	49–51	34–37	38–40	39
Lactic acid isomer(s)	L(+)	L(+)	D(−)	D(−)	D(−)	DL	DL	DL
Growth at 10/45°C	−/+	−/+	−/+	−/+	−/+	−/+	−/+	−/v
Requirement for								
Thiamine			−	−	−	−	−	−
Riboflavine			+	+	+	+	+	+
Pyridoxal			−	−	−	−	+	+
Folic acid			+	+	+	+	−	−
Thymidine			+	+	+	−	−	−
Vit. B$_{12}$			+	+	+	−	−	−
Carbohydrate utilisation								
Aesculin	+	+	−	−	+	+	−	−
Amygladin	+	−	−	−	+	+	−	−
Cellobiose	+	−	d	−	d	+	−	−
Fructose	+	+	+	+	+	+	d	
Galactose	−	d	−	−	d	+	+	+
Lactose	+	+	−	+	+	+	+	+
Maltose	+	−	d	−	+	+	d	−
Mannose			+	−	+	+	d	
Melezitose			−	−	−	−	−	
Melibiose	−	d	−	−	−	d	−	−
Raffinose	−	d	−	−	−	d	−	
Ribose	−	d	−	−	−	−	−	
Salicin	+	−	−	−	+	+	−	−
Sucrose			+	−	+	+	−	−
Trehalose	d	−	d	−	+	d	d	d

[a] None of the organisms produce gas from gluconate and glucose or NH_3 from arginine. [b] *L. jugurti* is included for comparative purposes. [c] Mean % of guanine and cytosine of DNA.

+, Positive reaction by 90% or more strains; −, negative reaction by 90% or more strains; d, positive or weak reaction by 11–89%;, empty spaces indicate no data available.

Data compiled from Hansen (1968), Rogosa and Hansen (1971), Bergey's Manual (1974, 1986), Ottogalli *et al.* (1979), Accolas *et al.* (1980), Tamime (1990), Hammes and Vogel (1995) and Hardie and Whiley (1995).

L. bulgaricus is currently known as *L. delbrueckii* subsp. *bulgaricus*. Table 6.1 illustrates the overall differences between these various lactobacilli. Other characteristics of *L. delbrueckii* subsp. *bulgaricus* are:

- It is represented in Group I or Aa – the obligately homofermentative lactobacilli; the letter a indicates the affiliation to the *L. delbrueckii* group.
- The cells are rods and rounded ends, of 0.5–0.8 × 2–9 µm, and occur singly or in short chains.
- This organism ferments fewer sugars, produces D(+) lactate and acetaldehyde from lactose in milk, and some strains produce EPS.
- Slight growth occurs at <10°C and most strains are able to grow at 50–55°C.
- The cell wall peptidoglycan type is Lys-DAsp (see also Park *et al.*, 1991; Sungil *et al.*, 1996).

In view of the wide range of technical data available on yoghurt and other dairy starter cultures, it is recommended that the reader consults some selected publications for general information (Accolas and Auclair, 1983; Auclair and Accolas, 1983; Bianchi-Salvadori, 1983; Sriranganathan *et al.*, 1985; Chassy, 1986; Terre, 1986; Marshall, 1986, 1987, 1993; Kashket, 1987; Daly, 1987; IDF, 1988a; Roginski, 1988; Lücke *et al.*, 1990; Schleifer *et al.*, 1991; Gasser, 1994; Roussis, 1994; Stiles, 1996).

According to Mitsuoka (1992), *L. acidophilus* was first isolated from faeces of bottle-fed infants and named *Bacillus acidophilus*, but in 1959, Rogosa and Sharpe gave a detailed description of *L. acidophilus* based on their own observations and those of Tittsler *et al.* (1947) and Rogosa *et al.* (1953). Later, Lerche and Reuter (1962) subdivided the species into five biotypes based on fermentation patterns of trehalose, melibose and raffinose, while Mitsuoka (1969) expanded the number of biotypes to ten based on variations in the fermentation of ribose and lactose. More recently, the phylogenetic approach based on 16S rRNA adopted by Collins *et al.* (1991) and Fujisawa *et al.* (1992) has cast doubt on some of these earlier groupings but, even so, the identity of *L. acidophilus* as proposed by Gasser and Mandel (1968) remains intact. As a consequence, the description of the species by Hansen and Mocquot (1970) based on a specific strain (ATCC 4356) is still valid.

The taxonomic status of *L. acidophilus* has not fluctuated over the years and whilst some characteristics of this organism are shown in Table 6.1, some other aspects may include:

- It is presented in Group I or Aa – the obligately homofermentative lactobacilli; in the same group as *L. delbrueckii* subsp. *bulgaricus*.
- The cells are rods with rounded ends, of 0.6–0.9 × 1.5–6 µm, occurring singly, in pairs and in short chains; cells are non-motile and non-sporulating and proteins in the cell wall may be important in attaching the bacterium to the intestinal wall (Bhowmik *et al.*, 1985; Brennan *et al.*, 1986).
- This organism requires riboflavin, pantothenic acid, folic acid and niacin for growth, but not the other B vitamins.
- Recent studies (i.e. electrophoresis of cellular proteins or lactate dehydrogenase and DNA–DNA reassociation) suggest that *L. acidophilus* strains include six genomospecies.
- No growth occurs at <15°C, most strains grow about 35–45°C and the optimum pH for growth is 5.5–6.0.
- The cell wall peptidoglycan type is Lys-DAsp.

According to Mital and Garg (1992), the growth requirements of most strains of *L. acidophilus* are quite complex and, as the normal habitat of *L. acidophilus* is attached to the walls of the small intestine of mammals, such requirements can usually be met quite easily. The ability of the species to utilise carbohydrates *in vitro* is shown in Table 6.1 and although *L. acidophilus* is the best known of the health-promoting lactobacilli, other species of human intestinal origin are often used in fermented milk and comparable data for some of these species has been included as well. In addition, strains of *L. acidophilus* may require fatty acids, minerals, peptides and amino acids, nucleic acid derivatives and vitamins of the B-complex to grow successfully and, given these requirements, it is not surprising that most strains grow only poorly in bovine milk. The final value of lactic acid is within the range of 0.3–1.9 g 100 g^{-1} lactic acid suggested by Rasic and Kurmann (1978) but, while some strains can secrete these high levels of acid, few strains are sufficiently acid tolerant to survive such conditions for more than a few days; the optical rotation of the lactic acid is DL.

The alleged health-promoting properties of *L. acidophilus* are discussed elsewhere and it is relevant that, in addition to secreting lactic acid, some strains of the species may produce antibiotic-like substances as well. Some authors have suggested that such compounds could be important in preventing the growth of pathogens in the intestine (Shahani *et al.*, 1976), but it could be that intrageneric activity could be equally relevant. Thus, Barefoot and Klaenhammer (1983) and Barefoot *et al.* (1994a, b) purified a bacteriocin compound from a strain of *L. acidophilus* and found it to be active against a range of other *Lactobacillus* spp. If this inhibitory activity happens in the intestine as well, then it might provide an additional mechanism whereby indigenous strains of *L. acidophilus* could retain dominance on the epithelial surfaces.

However, the taxonomic and nomenclature situation of the bifidobacteria have changed, and in the eighth edition of Bergey's Manual (1974) they were classified as *Lactobacillus* spp., whilst in latest edition of Bergey's Manual (1986) the same organisms are grouped in a different section, and known as *Bifidobacterium* spp.

Currently, 30 different strains of bifidobacteria have been identified which have been isolated from different sources such as the faeces of humans, animals, birds and sewage, the human vagina, bees and dental caries. Only six species of bifidobacteria have attracted attention in the dairy industry for the manufacture "bio" ferment dairy products. These organisms are known as *Bifidobacterium adolescentis*, *breve*, *bifidum*, *infantis*, *lactis* and *longum*, and these species have been isolated from human subjects for the manufacture of fermented milk. This restriction is based on the assumption that, if an isolate is of human origin, then it should become implanted on the walls of, and/or metabolise in, the colon of another human. The validity of this idea remains open to debate, for there is some evidence that, while an ingested strain may dominate the colon walls of a patient with a low count, the strains that are indigenous to that patient will, in time, overgrow the invading culture. It is relevant also that non-human strains of *Bifidobacterium animalis* can adhere to human cells in tissue culture, so that the question of which species should be permitted in bio-yoghurts is a matter of some debate.

The differentiating characteristics have been reviewed in Bergey's Manual (1986) and by Biavati *et al.* (1992), Sgorbati *et al.* (1995), Tamime *et al.* (1995), Kok *et al.* (1996), Meile *et al.* (1997) and Ballongue (1998). Other characteristics may be considered.

- Bacteria are Gram-positive, anaerobic heterofermentative, non-motile, non-spore-forming rods (0.5–1.3 × 1.5–8 μm).
- Cell morphology of these bifidobacteria grown anaerobically in trypticase-phytone-yeast (TPY) medium have distinctive shapes and arrangements (e.g. "amphora-like"; specific epithet, thin and short; very elongated, thin with slight irregular contours and rare branching).
- The cell wall peptidoglycan varies among the species, but this complex material consists of linear chains of *N*-acetylmuramic acid and *N*-acetylglucosamine molecules alternating along the length of the chain.
- Different species utilise different types of carbohydrates (see Table 6.2) and such fermentations are used for identification purposes. One key enzyme involved is fructose-6-phosphate phosphoketolase (F6PPK) known as "bifidus shunt", and this enzyme can be used to identify the genus; it should be noted that not all strains produce enough F6PPK for it to be detectable. The fermentation of two molecules of glucose leads to two molecules of lactate and three molecules of acetate.
- The guanine plus cytosine molecular percentage of the DNA of this genus ranges between 54 and 67.
- A wide range of components have been identified as bifidogenic growth stimulators.

The rods of bifidobacteria often have an irregular shape, with a slightly concave central region and swollen ends (i.e. having the appearance of a dog's bone in a Disney cartoon). It is, however, not unusual to encounter cells that are coccoid or appear as very long or short bacilli of varying widths, or the cells may be V, Y or X-shaped depending on the constituents of the medium on which the colony is growing. It is believed that, under adverse growing conditions, the cell morphology changes to produce more branched cells; for example, in a medium deficient in β-methyl-D-glucosamine, the cells become more branched, while the addition of certain amino acids (e.g. serine, alanine or aspartic acid) can transform X- or Y-shaped cells into curved rods (Glick *et al.*, 1960). Similarly, Samona and Robinson (1994) transformed coccoid cells of *B. bifidum* into the Y-shaped form through the addition of sodium chloride to a medium, but noted that neither *B. longum* nor *B. adolescentis* reacted in the same way. The same authors recorded also that the pattern of carbohydrate fermentation changed as the morphology altered, suggesting perhaps that the permeability of the cell membrane to certain sugars was being modified in parallel with the structural changes taking place in the wall.

Notwithstanding this tendency of some species to alter in shape, the cell morphology of species of bifidobacteria grown anaerobically in stabs of TPY extract medium showed a tendency to adopt distinctive cellular shapes. For example, *B. bifidum* forms groups of amphora-like cells, the cells of *B. breve* are the thinnest and shortest among bifidobacteria, while *B. longum* appears as very elongated, relatively thin cells with slightly irregular contours.

A summary of cell wall and DNA contents of the important species of bifidobacteria species are shown in Table 6.2. The principal component of the cell wall is peptidoglycan, also known as murein. This is a macromolecule that consists of linear polysaccharide chains (glucose, galactose and rhamnose) which are linked to each other by tetrapeptide bridges (Ballongue, 1998).

Table 6.2 Some selected characteristics of bifobacteria used for the manufacture of bio-yoghurt[a]

Characteristic	*Bifidobacterium* spp.					
	adolescentis	*bifidum*	*breve*	*infantis*	*lactis*[b]	*longum*
G + C mean (%)	58.9	60.8	58.4	60.5	61.9	60.8
Type of peptidoglycan	Lys(Orn)-D-ASP	Orn(Lys)-D-Ser-D-ASP	Lys-Gly	Orn(Lys)-Ser-Ala-Thr-Ala	Lys(Orn)-Ala-(Ser)-Ala$_2$	Orn(Lys)-Ser-Ala-Thr-Ala
Carbohydrate utilisation						
Arabinose	+	−	−	−	d	+
Cellobiose	+	−	d	−	−	−
Fructose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Gluconate	+	−	−	−	−	−
Inulin	d	−	d	d	+	−
Lactose	+	+	+	+	+	+
Maltose	+	−	+	+	+	+
Mannitol	d	−	d	−	−	−
Mannose	d	−	+	d	−	d
Melezitose	+	−	d	−	−	+
Melibiose	−	d	−	+	+	+
Rafinose	+	−	+	+	+	+
Ribose	+	−	+	+	+	+
Salicin	+	−	+	−	−	−
Sorbitol	d	−	d	−	+	−
Starch	+	−	−	−	−	−
Sucrose	+	d	+	+	+	+
Trehalose	d	−	d	−	+	−
Xylose	+	−	−	d	d	d

[a] For identification of symbols see Table 6.1. [b] After Kok *et al.* (1996) and Meile *et al.* (1997).
Data compiled from Bergey's Manual (1986), Biavati *et al.* (1992) and Sgorbati *et al.* (1995).

6.1.2 Modification of starter cultures

The characteristics of the various species shown in Tables 6.1 and 6.2 are based essentially on what are referred to as type cultures. These are strains of the species that have been: (a) isolated and grown as pure cultures in one of the internationally recognised culture laboratories, (b) examined for a range of characteristics, such as temperature of growth and/or rate of acid production (Zanatta and Basso, 1992), fermentation of selected sugars (Hickey *et al.*, 1986), enzyme profiles (Bianchi-Salvadori *et al.*, 1995), DNA base-pair characteristics (Sriranganathan *et al.*, 1985), DNA hybridisation reactions (Lick *et al.*, 1996), plasmid homology and/or profiles (Girard *et al.*, 1987; El-Soda *et al.*, 1989) and DNA fingerprinting (Ramos and Harlander, 1990), and then (c) designated as a distinct species. This procedure means that there is held somewhere in a deep frozen (−196°C) or freeze-dried state, a culture which displays all the characteristics of one recognised species and, once these characteristics have been recorded in an authoritative reference source (e.g. Bergey's Manual, 1986) anyone in the dairy industry or elsewhere can identify, with a reasonable degree of certainty, any cultures that may be isolated from cheese or a fermented milk.

For many years, this approach to bacterial taxonomy has worked well, but since about 1990, the degree of strain variability within species has increased because taxonomists have begun to employ increasingly sophisticated techniques for identification, for example, 16S RNA sequencing (Davidson *et al.*, 1996) and the use of DNA probes to isolate individual strains (Delley *et al.*, 1990; Colmin *et al.*, 1991; Neve and Soeding, 1997), and the number of cultures available from commercial suppliers has increased.

Some of this variability has arisen as a natural process of change, because the selective pressures on a culture of *S. thermophilus* employed in a dairy in the Middle East, for example, might well be different from those operational in a plant in North America (Nunez de Kairwuz *et al.*, 1983; Yoast *et al.*, 1994; Teixeira *et al.*, 1994). The same species isolated from a cheese factory in Italy might well be different again, so that the precise definition of a species becomes, in some respects, more difficult (Sandine, 1987; Mercenier and Lemoine, 1989). A good example of this situation can be found for the mesophilic starters for cheese, in that while the type culture of *Lactococcus lactis* subsp. *cremoris* differs widely from *Lactococcus lactis* subsp. *lactis* with respect to the sugar fermentation pattern, a culture of *L. lactis* subsp. *cremoris* purchased today may well display the same sugar utilisation profile as *L. lactis* subsp. *lactis* (de Vos, 1996).

Although this complicated situation may, in part, be the result of culture evolution as a result of mutation (Mollet and Delley, 1990; see also Germond *et al.*, 1995), conjugation (Kleinschmidt *et al.*, 1993; Soeding *et al.*, 1993), transformation (Mollet *et al.*, 1993b) and intercellular and/or plasmid transduction (Mercenier *et al.*, 1988a, b; Heller *et al.*, 1995; Neve and Heller, 1995a, b), the deliberate genetic manipulation of cultures has become increasingly important (Yu *et al.*, 1984; Chassy, 1987; Romero *et al.*, 1987; Knol *et al.*, 1993a, b; Sasaki, 1994; Mercenier *et al.*, 1994). Thus, genetic engineering or recombinant DNA technology can now be employed to modify the properties of various organisms to generate genetically modified organisms (GMOs) (Herman and McKay, 1986; de Vos and Simons, 1988; Somkuti and Steinberg, 1988, 1991; Lee *et al.*, 1990b; Gasson, 1997).

To avoid potential conflicts with consumers, bacteria to be used in the manufacture of foods should be subject only to so-called food grade genetic modifications,

which means that the GMO must contain only DNA from the same genus and, possibly, small stretches of imported DNA (Johansen *et al.*, 1995). Thus, a *Lactococcus* GMO would only contain DNA from the genus *Lactococcus* plus a small amount of imported DNA (Mollet *et al.*, 1993a; Griffen and Gasson, 1995). These small stretches of non-lactococcal DNA are usually no longer than 50 base pairs and act as recognition sites for the restriction enzymes used in the actual construction process (Solaiman and Somkuti, 1991, 1995, 1997a–c; Somkuti and Solaiman, 1997; Satoh *et al.*, 1997). It is essential, of course, that none of the imported DNA should provide a code for RNA, and specific DNA probes should be constructed to check that no additional genetic material has been introduced (Lick and Teuber, 1992).

However, pressure is mounting within the dairy industry for permission to exchange DNA between any genus of micro-organism associated with food fermentation (Langella and Chopin, 1989), provided that the donor bacterium can be described as generally recognised as safe (GRAS). Whether or not it is appropriate for microbiologists to borrow this definition from the chemists has not been challenged, but it is relevant that food-grade GMOs can usually be used in the United States without specific regulatory approval.

6.1.3   Potential genetic modifications

Genes can be deleted from a strain to avoid the release of an undesirable metabolic product into a food, or the gene can be replaced with the homologous gene from another strain (Sasaki, 1994; Ito and Sasaki, 1994). For example, if a strain of *Lactococcus* has a particularly useful characteristic, such as the secretion of a desirable flavour component, but the level of β-galactosidase activity is low, then this latter deficiency could be corrected by introducing a more active copy of the gene from another strain (Yu *et al.*, 1983; Kochhar *et al.*, 1992). Genes could be inserted into a strain to expand the range of carbohydrates utilised (Branny *et al.*, 1993, 1996) or increase resistance to a wider spectrum of bacteriophage or, alternatively, a useful gene within the existing genome can be copied, so doubling the beneficial activity (Mollet and Delley, 1991).

An example of the potential offered by these techniques relates to the production of diacetyl, a major flavour component of buttermilk and kefir and a compound that is usually derived via pyruvate. If genes coding for α-acetolactate synthase, an enzyme involved in the conversion of pyruvate to diacetyl, could be inserted into a food-grade culture, diacetyl production would increase and the same approach could be employed in the synthesis of EPS by *S. thermophilus* or *L. delbrueckii* subsp. *bulgaricus*. The relevant genes have been identified from several strains and GMOs with altered texture-producing properties could be constructed (Gasson, 1997).

Exactly how far and fast the construction of GMOs will proceed – or will be allowed to proceed – remains to be seen, but it seems likely that: (a) the identification of species within starter cultures is going to become increasingly imprecise as the borderlines between, for example, *L. delbrueckii* subsp. *bulgaricus* and *L. delbrueckii* subsp. *lactis* become blurred as a result of genetic manipulation, and (b) future generations of yoghurt makers will be able to request the supply of starter cultures with quite specific characteristics.

In view of the wide range of technical data available on the genetic modifications of the yoghurt and bio starter cultures, it is recommended that the reader consults

some selected publications for general information (Nicholson and Sanders, 1988; le Bourgeois *et al.*, 1989; Schmidt *et al.*, 1989; Miteva *et al.*, 1991; Yohda *et al.*, 1991; Schroeder *et al.*, 1991; Leong-Morgenthaler *et al.*, 1991; Janzen *et al.*, 1992; Pébay *et al.*, 1992; Delcour *et al.*, 1993; Poolman, 1993; Mustapha *et al.*, 1995).

6.2 Characteristics of growth

Yoghurt and the many fermented milks known across the world have been traditionally made by the spontaneous growth of indigenous micro-organisms present in milk. At present, carefully controlled microbial processes have been developed using selected combinations of cultures and the technology required for large-scale production has evolved from the knowledge of the physiology and biochemistry of the micro-organisms involved (refer to Chapter 7).

Since the late 1970s much work has been done on the biochemistry and molecular biology of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. Catabolism is not the only important consideration for a successful fermentation to produce yoghurt of good quality in terms of flavour and stability, but anabolic pathways also have a role in providing texture-modifying polysaccharides and providing other compounds which have preservative and health-promoting properties.

6.2.1 Milk as a medium for microbial growth

Lactic acid bacteria are widely distributed in nature and their nutritional requirements are very complex. Table 6.1 shows the fermentation ability and growth temperatures of the yoghurt starter cultures and some of these characteristics are used to differentiate the genera and species. *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* and many other lactic acid bacteria are unable to synthesise a full complement of amino acids and this deficiency dictates their natural habitat. Milk is a nutritionally rich medium which will support the growth of many micro-organisms, but the processing of milk provides control over the type of growth necessary to achieve a desirable product (see Chapter 2; Chandrakanth *et al.*, 1993).

The metabolic activity of an organism is indicative, to some extent, of its growth rate, and one of the most popular tests for monitoring starter cultures is the development of acidity in the growth medium. Autoclaved, reconstituted skimmed milk (10–12 g total solids (TS) $100 g^{-1}$) is mainly used and the milk must be free from any inhibitory substances, for instance antibiotics. The activity of a typical yoghurt starter culture and the isolated strains of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* is illustrated in Fig. 6.1 which shows a marked difference in the rate of acid development by the mixed starter compared with the isolated single strains. It is also noticeable that the rate of acid development of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* increases with increase in incubation temperature, up to maxima of 40°C and 45°C, respectively; the former organism is initially more active than *L. delbrueckii* subsp. *bulgaricus* in relation to acid production. Although the activity of mixed strains is optimum at 45°C, it is recommended that, in order to maintain and/or achieve a ratio of 1:1 between *S. thermophilus* and *L. delbrueckii*
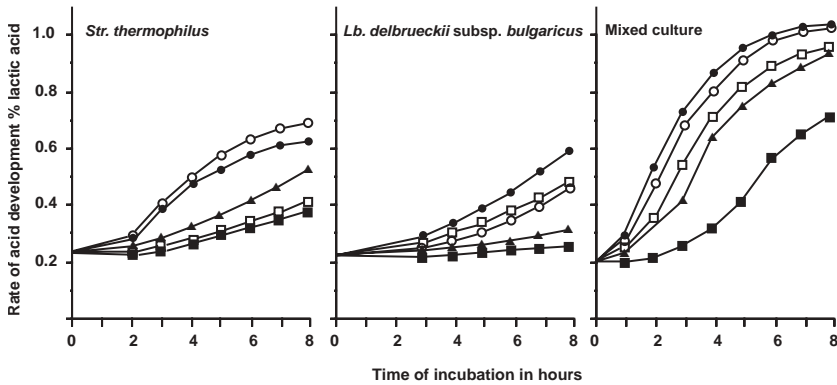
Fig. 6.1 Behaviour of single and mixed strain yoghurt cultures propagated at different temperatures in autoclaved skimmed milk ($10\,g\,TS\,100\,g^{-1}$) at $2\,ml\,100\,ml^{-1}$ inoculation rate ■, 30°C; ▲, 35°C; ○, 40°C; ●, 45°C; □, 50°C.

Note: Test organisms is Chr. Hansen's (CH-1).

Adapted from Tamime (1977a).

subsp. *bulgaricus*, the organisms should be propagated together at 42°C using a $2\,ml\,100\,ml^{-1}$ inoculation rate (Kurmann, 1967; Tamime, 1977a) or direct-to-vat inoculation (DVI).

6.2.2 Associative growth

The growth association between the two organisms *(S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus)* of the yoghurt starter culture used to be termed a symbiosis and this relationship has been reported by many workers; the earliest record dates back to the work of Orla-Jensen (1931). This association could be briefly described as each organism providing compounds which benefit the other. Since both *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* can grow in milk as single cultures, the term symbiosis should be replaced by associative growth instead. Pette and Lolkema (1950a) observed that the rate of acid development was greater when mixed yoghurt cultures of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* were used compared with the single strains (see Fig. 6.2; Lee *et al.*, 1990a). Furthermore, they also observed that the numbers of *S. thermophilus*, as recorded by the Breed smear method, were much higher in mixed cultures than when the organism was grown alone, although no such differences in numbers of *L. delbrueckii* subsp. *bulgaricus* were noted. This observation was not true with respect to *L. delbrueckii* subsp. *bulgaricus* as reported by Tamime (1977b). The findings of Pette and Lolkema (1950b) led them to postulate that the interaction between these two organisms was mainly dependent on the production of valine by *L. delbrueckii* subsp. *bulgaricus*. However, due to variations in the chemical composition of milk during the year, other amino acids may also be deficient and hence Pette and Lolkema (1950c) suggested that during the spring months, *S. thermophilus* required amino acids leucine,
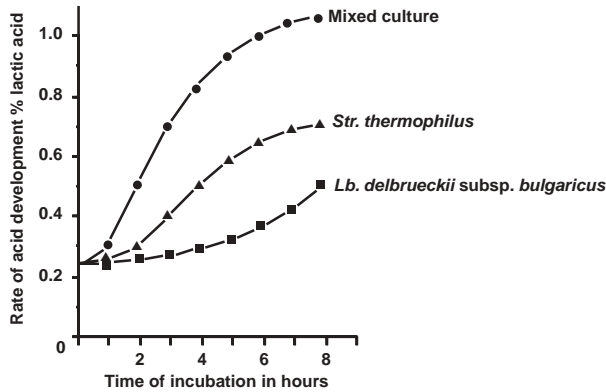
Fig. 6.2 Behaviour of single and mixed strain yoghurt cultures propagated at 40°C in autoclaved skimmed milk $(10\,g\,TS\,100\,g^{-1})$ at $2\,ml\,100\,ml^{-1}$ inoculation rate

Note: Test organism is Chr. Hansen's (CH-1).

Adapted from Tamime (1977a).

lysine, cystine, aspartic acid, histidine and valine. During the autumn/winter months, glycine, isoleucine, tyrosine, glutamic acid, methionine, as well as the six amino acid mentioned above, were essential.

Bautista *et al.* (1966) also investigated the associative growth theory and supported the view that *L. delbrueckii* subsp. *bulgaricus* stimulates *S. thermophilus* by releasing glycine and histidine into the growth medium; they concluded that histidine rather than valine was the most important requirement. However, the stimulation by glycine and histidine, as reported by Bautista *et al.* (1966), was very poor in comparison with the various amino acids observed by Pette and Lolkema (1950b). Accolas *et al.* (1971) reported that the stimulation of *S. thermophilus* by milk culture filtrate of *L. delbrueckii* subsp. *bulgaricus* was due to the presence of valine, leucine, isoleucine and histidine. Bracquart *et al.* (1978) and Bracquart and Lorient (1979) concluded that depleting the growth medium of valine, histidine, glutamic acid, tryptophan, leucine and isoleucine reduced the stimulation of *S. thermophilus* by 50%. Similar findings were reported by Higashio *et al.* (1977a), where methionine was also included as a stimulant amino acid; however, by far the most effective amino acid was valine (see also Shankar, 1977; Shankar and Davies, 1978; Hemme *et al.*, 1981; Rao *et al.*, 1982; Marshall, 1983).

It is well established that *L. delbrueckii* subsp. *bulgaricus* possesses more proteolytic enzymes than *S. thermophilus* (see Chapter 7; Rajagopal and Sandine, 1990; Abu-Tarboush, 1996) and El-Soda *et al.* (1986) reported that crude cell-free extracts of the yoghurt lactobacilli stimulated the growth of *S. thermophilus*; they concluded that acid production was enhanced by the addition of peptone, amino acids and, to a lesser extent, water-soluble vitamins, purines and pyridines. A similar view was reported by El-Abbassy and Sitohy (1993) and Neviani *et al.* (1995), whilst Carmi-

nati *et al.* (1994) concluded that a skimmed milk medium deprived of soluble nitrogen inhibited the growth of *S. thermophilus*. Other amino acids, which are not the result of proteolysis by the yoghurt organisms, that have stimulated the growth of *S. thermophilus* are: (a) peptides containing lysine (Desmaseaud and Hermier, 1972), (b) hepta- or pentapeptides containing histidine and free non-aromatic amino acids (Desmaseaud and Hermier, 1973; Hayashi *et al.*, 1974), (c) tripeptides containing histidine, methionine and glutanic acid (Bracquart and Lorient, 1979), (d) casein hydrolysate (Marshall and Mabbitt, 1980; Marshall *et al.*, 1982; Nakamura *et al.*, 1991), and (e) the addition of magnesium (Amouzou *et al.*, 1985). However, the transport of branched amino acids in *S. thermophilus* is energy dependent and optimum activity was between 30°C and 45°C for leucine, valine and isoleucine (Akpemado and Bracquart, 1983). Other technical data available on the associative growth of the yoghurt organisms have been reported by Radke-Mitchell and Sandine (1984), Matalon and Sandine (1986), Juillard *et al.* (1987), Berkman *et al.* (1990), Kneifel *et al.* (1993) and Oberg and Broadbent (1993) (see also Champagne *et al.*, 1990; Klaver *et al.*, 1992; Franzetti *et al.*, 1997).

Thus, the streptococci benefit from the stronger activity of the lactobacilli and in return provide certain compounds which stimulate the growth of *L. delbrueckii* subsp. *bulgaricus*. However, glutamic acid uptake in *S. thermophilus* was energy dependent (e.g. lactose, glucose and sucrose), but aspartic acid exhibited an inhibitory effect (Benateya *et al.*, 1986; Bracquart *et al.*, 1989).

Galesloot *et al.* (1968) investigated the opposite side of the associative growth relationship between *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. They concluded that, under anaerobic conditions, the former organism produces a stimulatory factor for *L. delbrueckii* subsp. *bulgaricus* that is equal to or can be replaced by formic acid. Furthermore, the same workers looked at the effect of various heat treatments on milk, and found that in intensively heated milk (i.e. autoclaved and UHT) the stimulation was masked on account of a compound which could be replaced by formic acid. However, after the normal heat treatment of milk used for yoghurt manufacture (e.g. 85–90°C), *L. delbrueckii* subsp. *bulgaricus* definitely needs the stimulatory factor produced by *S. thermophilus*. The normal presence of this stimulatory factor in autoclaved milk (Auclair and Portman, 1957; Shankar, 1977; Marshall, 1983), appears to have been overlooked by both Pette and Lolkema (1950b) and Bautista *et al.* (1966).

The production of formic acid by *S. thermophilus* was confirmed by Veringa *et al.* (1968), and Bottazzi *et al.* (1971) demonstrated that the presence of formic acid in milk increases the ratio of rods to cocci at concentrations between 30 and 50 γ ml^{-1}. This compares with the stimulation of *L. delbrueckii* subsp. *bulgaricus* by formate at 20–30 µg ml^{-1} (Galesloot *et al.*, 1968; Shankar, 1977; Marshall, 1983) and 40–600 µg ml^{-1} (Accolas *et al.*, 1971; Pulsani and Rao, 1984; Kikuchi *et al.*, 1985; El-Abbassy and Sitohy, 1993; Moreira *et al.*, 1997). This variation in the level of formate required to promote activity could be attributed to the use of different strains of *L. delbrueckii* subsp. *bulgaricus*. Also, the amount of formate production by *S. thermophilus* is dependent on strain, culture medium and growth temperature (Perez *et al.*, 1990, 1991); the streptococci produce formic acid in milk only if the level of oxygen ≤ 4 mg O$_2$ l^{-1} (Driessen *et al.*, 1983).

Some *L. delbrueckii* subsp. *bulgaricus* strains grown in milk heated to 100°C for 15 min showed an abnormal cell elongation, and septum staining indicated that the septum had not yet formed. However, such morphological behaviour was not

observed in autoclaved milk and/or milk heated to 100°C for 15 min (Suzuki *et al*., 1986). Furthermore, the presence of sodium formate (40 μg ml⁻¹) in milk induced the proteolytic activity of *L. delbrueckii* subsp. *bulgaricus* so that it became able to hydrolyse β-Lg, α_{s1}- and β-casein compared to only β-casein without the added formate (Moreira *et al*., 1997).

Carbon dioxide, which is produced by *S. thermophilus* (Ascon-Reyes *et al*., 1995) had been reported by Driessen *et al*. (1982) to stimulate the growth of *L. delbrueckii* subsp. *bulgaricus* because part of the CO_2 produced by the streptococci disappears during mixed growth with the lactobacilli. CO_2 is produced as a result of urea hydrolysis and can be measured using an indirect conductance technique (Ascon-Reyes *et al*., 1995; see also Lanzanova *et al*., 1993), whilst measuring partial pressure of dissolved CO_2, the concentration of viable cells of the yoghurt micro-organisms could also be determined (Spinnler *et al*., 1987). CO_2 production in dahi incubated at 42°C using 1 ml 100 ml⁻¹ starter culture amounted to about 450 μl, and Warsy (1983) suggested that the gas produced may contribute to the sensory quality of the product. However, in a recent study, Louaileche *et al*. (1993, 1996) reported that CO_2 and sodium bicarbonate stimulated the growth of *S. thermophilus*, and exerted a marked influence on the metabolic activities of the micro-organism, a phenomenon that has not been reported before.

Other compounds produced by *S. thermophilus* that stimulate the growth of *L. delbrueckii* subsp. *bulgaricus* are pyruvate and HCO_3 (Higashio *et al*., 1977b, 1978; Juillard *et al*., 1987). Other added compounds that stimulated the growth of the lactobacilli are purine, adenine, guanine, uracil and adenosine (Weinmann *et al*., 1964; Cogan *et al*., 1968), monosodium orthophosphate and sodium tripolyphosphate (Yu and Kim, 1979), oxaloacetic and fumaric acid (Higashio *et al*., 1977b) and cysteine at ≤50 mg l⁻¹ (Dave and Shah, 1997). Nevertheless, the action of psychrotrophic bacteria in milk, fortification of the solids of the milk base and/or heating of the milk can also promote the growth of the yoghurt starter culture (Tramer, 1973; Cousins and Marth, 1977a, b; Sellars and Babel, 1985; Slocum *et al*., 1988a, b; for further information refer to Chapter 2).

It can be concluded from the data available, therefore, that the release of stimulatory factors by the yoghurt starter cultures takes place during the incubation period and, while *L. delbrueckii* subsp. *bulgaricus* provides essential nutrients (i.e. amino acids) for *S. thermophilus*, the latter produces formate which promotes the growth of the lactobacilli. Alternatively, the growth characteristics of the yoghurt organisms can be increased through the application of an electromagnetic field (Blicq and Murray, 1994) or the use of surface methodology to evaluate some variables affecting the growth behaviour of the yoghurt organisms (Torriani *et al*., 1996).

Amoroso and Manca de Nadra (1990) observed the mutual stimulation in milk, while in LAPT medium (containing yeast extract, peptone, tryptone and Tween) with different sugars, only the stimulatory effects of the *Streptococcus* on the *Lactobacillus* were observed. This is an expected result as the nitrogen sources in LAPT medium are readily available and not dependent on proteolytic activity (the mechanism for stimulation of the *Streptococcus* is the release of peptides by the lactobacilli); thus, the medium used could demonstrate only one side of the partnership. This underlines the importance of understanding the special qualities of milk as a growth medium; it has an ample supply of a simple disaccharide and an ample, but complex source of nitrogen. It is also important to remember that both organisms

grow perfectly well in milk. Indeed, many of the mild bio-yoghurts are prepared with mixed cultures, some of which include *L. delbrueckii* subsp. *bulgaricus* for a successful fermentation (see also Marshall and Tamime, 1997).

Although in the 1980s, *S. thermophilus* was temporarily included as a subspecies of *Streptococcus salivarius* (Farrow and Collins, 1984), a separate species was proposed by Schleifer *et al*. (1991); *S. salivarius* fails to grow in milk in the presence of *L. delbrueckii* subsp. *bulgaricus* and is not suitable for the manufacture of yoghurt because of poor flavour, aroma and texture (Marshall *et al*., 1985). Such observations may also justify the revival of the species, *S. thermophilus*, even though both species have similar DNA base compositions and belong in the same DNA homology group. Nevertheless, associative growth was reported between *S. thermophilus* and *L. helveticus* or *L. acidophilus* (Yoon *et al*., 1988; Kim *et al*., 1992) and bifidobacteria stimulated the growth of yoghurt starter cultures (Kumar *et al*., 1995).

6.3 Factors affecting slow growth of starter cultures

Yoghurt microflora can easily grow in milk and the rate of acid development is faster due to the growth associated with *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (see Fig. 6.2 and Section 6.2). Nevertheless, the fermentation conditions and the presence of certain agents or substances in milk may either reduce the rate of acid development or inhibit growth of the culture and these aspects are summarised in the following section.

6.3.1 Compounds that are naturally present in milk

There are various antimicrobial systems present in milk and their major role is the protection of the suckling animal against infection and disease. These inhibitory systems have been reviewed by Reiter (1978) and their presence in milk can inhibit the growth of lactic acid bacteria. Auclair and Hirsch (1953) and Auclair and Berridge (1953) reported the inhibition of starter organisms by raw milk and that pasteurisation and boiling of the milk improved culture activity. The inhibitory compounds, known as lactenins, are heat sensitive, and are destroyed by heating the milk to 68–74°C (Auclair, 1954). Patel (1969) reported that *S. thermophilus* showed a growth inhibition in fresh raw buffalo's milk during the first 1–2 hour of incubation, but a resumption of growth followed. He proposed that the loss of inhibitory action was due either to adaptation of the organism to the lactenins or to the destruction of the lactenins.

Another bactericidal component found naturally in milk is the peroxidase system, which consists of lactoperoxidase/thiocyanate/hydrogen peroxide [LP/SCN⁻/H₂O₂ abbreviated as LPS]. Reiter (1978) reported on the sources of these compounds.

- LP is synthesised in the mammary gland and milk may contain up to $30\,\mu g\,ml^{-1}$ peroxidase which is sufficient to activate the LPS (Reiter, 1985; Nichol *et al*., 1995).
- SCN⁻ anion is widely distributed in animal secretions and possibly derived from a rhodanese catalysed reaction with thiosulphate in the liver and kidney; the SCN⁻ concentration in milk may reach up to $10–15\,\mu g\,g^{-1}$ (Reiter and

Härnulv, 1984; Reiter, 1985; Haddadin *et al.*, 1996; see also Prasad and Sukumaran, 1992).

- H_2O_2 does not occur naturally in milk (Piard and Desmazeaud, 1991; Nichol *et al.*, 1995), but its presence in milk is the result of metabolic activity of the lactic bacteria or from anaerobic growth of other micro-organisms.

In this system, the inhibitory compound is the result of an oxidation reaction where, in the presence of H_2O_2, the LP catalyses the oxidation of thiocynate to non-inhibitory compounds (SO_4^{2-}, CO_2 and NH_3) followed by further oxidation to form intermediate inhibitory substances, such as hypothiocyanate or higher oxyacids (Piard and Desmazeaud, 1991; Björck, 1992; Dionysius *et al.*, 1992; Grieve *et al.*, 1992). However, the inhibition is reversible in the presence of some reducing compounds (e.g. cysteine and dithionite; Reiter, 1978). In general, most starter organisms are resistant to LP systems, but some lactic cultures can give rise to sensitive mutants (Auclair and Vassal, 1963). Alternatively, continual propagation of starter cultures in autoclaved milk can affect the susceptibility of the organisms to the LP system (Jago and Swinbourne, 1959). A preventive measure is the addition of peroxidase to autoclaved milk (Reiter, 1973), or the addition of reducing agents, like cysteine and dithionite (Reiter, 1978). Incidentally, the LP system is inactivated by heating milk at 85°C for 16s (Feagan, 1959a, b), so that heat treatment of yoghurt milk (85°C for 30min or 90–95°C for 5–10min) and the bulk starter milk (93°C for $1\frac{1}{2}$–2 hours) are sufficient to destroy the natural inhibitors (Storgards, 1964; Pearce and Bryce, 1973; Ekstrand *et al.*, 1985; Farkye, 1992). Thus, since H_2O_2 does not occur naturally in milk, the mechanism(s) involved in production and inhibition of the yoghurt organisms is given in detail in Section 6.3.4.

Other inhibitory systems which may warrant some consideration are: (a) bacterial agglutinin which can cause agglutination of the starter organisms, thus affecting their metabolic activity and growth, and (b) certain types of forage, such as mouldy silage, turnips or vetch, which may result in a milk containing inhibitory substances which can reduce the rate of acid production of the yoghurt starter culture, even after heating the milk at 90°C for 15min (see the review by Tamime and Deeth, 1980).

6.3.2 Effect of incubation temperature and inoculation rate

The growth behaviour of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (i.e. as single and/or mixed cultures) has been shown in Fig. 6.1 and it is evident that when the starter culture is incubated at 40–50°C, the optimum rate of acid development is obtained within a very short period. However, in industrial situations, yoghurt is produced over a short or long period using incubation temperatures at 30°C or 45°C, respectively. In the former method of production, a reduced rate of acid development becomes inevitable and, although this effect is governed by processing conditions, the quality of the end product could be affected. Some published data are available and it is recommended that the reader consult the following publications for general information (Tayeb *et al.*, 1984; Mohanan *et al.*, 1984; Radke-Mitchell and Sandine, 1986; Jayaram and Gandhi, 1987; Cho-Ah-Ying *et al.*, 1990; Béal and Corrieu, 1991; Lankes *et al.*, 1998).

The inoculation rate can also affect the rate of acid develoment during the manufacture of yoghurt. For example, an additional rate of 2–3 ml100ml^{-1} bulk

starter culture is recommended, whilst a DVI inoculation rate may range between 2.5 and $70 \, g \, 100 \, l^{-1}$ depending on the starter culture blend used (i.e. standard or bio culture). Thus, an inaccurate rate of starter addition to the milk base can affect the rate of acid development of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*.

6.3.3 Mastitis milk and somatic cell count

Gajdusek and Seleba (1973) reported a 35% reduction in the activity of a yoghurt culture in milk containing large numbers of somatic cells; however, boiling the milk for 2 min, or heating to 90°C for 20 min, inactivates the cells completely. Whilst somatic counts of $4.0 \times 10^5 \, cells \, ml^{-1}$ cause some inhibition of growth of the yoghurt organisms with *S. thermophilus* less resistant than *L. delbrueckii* subsp. *bulgaricus*, complete inhibition of both organisms occurs at counts $>1.0 \times 10^6 \, cell \, ml^{-1}$ (Mitic *et al.*, 1982). However, Marshall and Bramley (1984) and Okella-Uma and Marshall (1986) reported stimulation of *S. thermophilus*, but inhibition of *L. acidophilus*, when these organisms were grown in mastitic milk containing high somatic cell counts. The stimulation was attributed to increased proteolysis and the inhibition to increased phagocytic activity of the polymorphonuclear leukocytes. However, Fang *et al.* (1993) observed only reduced growth activity of *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus* and *Lactobacillus paracasei* subsp. *paracasei* in mastitic milk. These reported differences in the growth behaviour of *L. acidophilus* could be strain related.

The quality of yoghurt made from skimmed milk containing a somatic count of $\leq 2.5 \times 10^5 \, cells \, ml^{-1}$ was organoleptically superior to a parallel product made from milk of $\geq 2.5 \times 10^5 \, cells \, ml^{-1}$ (Mitchell *et al.*, 1985; Rogers and Mitchell, 1994). Thus, from the limited data available in this field, it is recommended that yoghurt producers should use milk with a low somatic cell count as reported by Rogers and Mitchell (1994) (see also Auldist and Hubble, 1998).

6.3.4 Hydrogen perxoide (H₂O₂)

Hydrogen peroxide is added to raw milk produced in hot countries to improve its quality during storage. The recommended rate to activate LPS system is $3 \, mg \, 100 \, g^{-1}$ of sodium percarbonate $(2Na_2CO_3 \times 3H_2O_2)$ and $1.4 \, mg \, 100 \, g^{-1}$ of sodium thiocyanate (NaSCN) (IDF, 1988b). However, the natural presence of H_2O_2 in milk and activation of LPS, which can inhibit the growth of lactic acid bacteria and other micro-organisms, is the result of sugar metabolism during fermentation. A wide range of reactions and catalysing enzymes are involved and these have been recently reviewed by Condon (1987) and Piard and Desmazeaud (1992).

Oxygen uptake activity and aerobic metabolism of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* have been reported by Smart and Thomas (1987), Teraguchi (1987), Teraguchi *et al.* (1987) and Condon (1987). H_2O_2 produced by the *Lactobacillus* in the presence of glucose at pH values of 6.5 and 5.0 was apparently due to the action of cytosolic NADH oxidase (Kot *et al.*, 1996, 1997). Schuts *et al.* (1982) reported that the amount of H_2O_2 (0.8 to $1.8 \, mg \, 100 \, ml^{-1}$) produced by *L. delbrueckii* subsp. *bulgaricus* was influenced by the strain, growth medium and the type of added sugars; the highest amount of H_2O_2 was obtained in UHT milk. However, lactic acid bacteria can rid themselves of H_2O_2 formed only by their NADH peroxidase (Piard and Desmazeaud, 1991). The ability of the yoghurt organisms to

consume oxygen in milk was about $0.4 \, mg \, 100 \, ml^{-1}$ in 24 hour at 25°C (Langeveld and Bolle, 1985), whilst the influence of dissolved O_2 on acid production in buffalo's milk by *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus* and lactococcal species has been studied by Shekar and Bhat (1983). However, *L. acidophilus*, *S. thermophilus* and some bifidobacterial strains, but not *L. delbrueckii* subsp. *bulgaricus*, could transport Fe^{2+} into the cell where it is partially oxidised to the ferric form (Kot *et al.*, 1995); *L. delbrueckii* subsp. *bulgaricus* could only oxidise extracellular Fe^{2+} through the elaboration of H_2O_2 in the presence of glucose and air.

Therefore, the LPS system can be activated in the presence of H_2O_2 via two possible routes, the first due to the metabolic activity of the starter cultures and the second, by the addition of thiocyanate and H_2O_2. Zall *et al.* (1983) reported that when the latter approach was used with rates of 0.2 mM and 0.25 mM, respectively, it extended the shelf life of raw milk up to 8 days without substantially increasing the total viable count, but when such milk was used for the manufacture of buttermilk, Cheddar cheese or yoghurt, culture activity was reduced. Nichol *et al.* (1995) reported self-induced inhibition of *S. thermophilus* by activation of LPS, whilst activation of LPS system by adding H_2O_2 and thiocyanate suppressed acid production during the manufacturing stages and refrigerated storage of yoghurt (Mehanna and Hefnawy, 1988; Kumar and Mathur, 1989; Basaga and Dik, 1994; Sarkar and Misra, 1994; Nakada *et al.*, 1996). In a simulated system, *L. acidophilus* (one strain) and *L. delbrueckii* subsp. *bulgaricus* (three strains) were inhibited in the presence of lactoperoxidase and thiocyanate indicating their ability to produce H_2O_2 to complete the LPS system, whilst *S. thermophilus*, *L. helveticus* and *Lac. lactis* subsp. *lactis* (one strain) required an external source of H_2O_2 to cause inhibition by the LPS system (Guirguis and Hickey, 1987a). The same authors also reported that one strain each of *L. delbrueckii* subsp. *bulgaricus*, *L. lactis* subsp. *lactis* and *Enterococcus faecium* were resistant to LPS system.

It is evident that the LPS system may inhibit or act as a bacteriostatic agent of the yoghurt starter cultures. Such effects may possibly depend on the rate of accumulation and/or reduction of the H_2O_2 (i.e. the activities of NADH oxidase and NADH peroxidase) in the bacterial cell. Therefore, screening of the yoghurt organisms in relation to the effect of the LPS system may help to overcome production problems at certain period(s) of the year, stages of lactation, or thiocynate and H_2O_2 must be used at lower levels than recommended by IDF (1988b).

6.3.5 Antibiotic residues

Antibiotics and/or other antimicrobial agents are used for the treatment of diseases. One of the major diseases in the dairy cow, which can affect the quality and yield of milk, is mastitis. Today there are known to be about 1000 different types of antibiotic and the following antimicrobial compounds (penicillin, streptomycin, neomycin, chloramphenicol, tetracycline, sulphonamide, cloxacillin and ampicillin) are widely used in the United Kingdom for the treatment of mastitis. The presence of these antibiotics in milk can either inhibit the growth or reduce the activity of the yoghurt starter cultures. The sensitivity of these organisms (i.e. single strains or mixed culture) to these various compounds is shown in Table 6.3 (see also Park *et al.*, 1984; Sinha, 1984; Hsu *et al.*, 1987; IDF, 1987, 1991a; Herian *et al.*, 1990; Milashki, 1990; Schiffmann *et al.*, 1992; Celik, 1992; Brindani *et al.*, 1994).

Table 6.3 Sensitivity of the yoghurt starter cultures to various antibiotics (ml^{-1})

Antibiotics	Micro-organisms		
	S. thermophilus	*L. delbrueckii* subsp. *bulgaricus*	Mixed culture (IU)
Penicillin	0.004–0.01 IU	0.02–0.1 IU	0.01
Streptomycin	0.38 IU	0.38 IU	1.0
	12.5–21.0 μg	6.6 μg	NR
Tetracycline	0.13–0.5 μg	0.3–2.0 μg	1.0
Chlortetracycline	0.06–1.0 μg	0.06–1.0 μg	0.1
Oxytetracycline	0.4 IU	0.7 IU	0.4
Bacitracin	0.04–0.12 IU	0.04–0.1 IU	0.04
Erythromycin	0.3–1.3 mg	0.7–1.3 mg	0.1
Chloramphenicol	0.8–13.0 mg	0.8–13.0 mg	0.5

IU, international units; NR, not reported.

Data compiled from Tamime and Deeth (1980), Loussouarn (1983), Schiffmann (1993) and Lim *et al*. (1995).

During the intramammary injection of antibiotics for the treatment of mastitis in the dairy cow, these antimicrobial compounds are retained in the udder tissues and gradually diffuse into the milk.

Thus, milk from treated cows must be withheld for 72 hours for two main reasons. First, residual antibiotics in milk are a potential public health hazard and second, low levels can affect the behaviour and activity of the starter culture (see Table 6.3), resulting in a poor yoghurt and/or economic loss for the manufacturer. Hence, a number of governments have introduced a payment penalty scheme for milk containing >0.004 International Units (IU) of penicillin ml^{-1}; among the test methods are the disc assay, the 2,3,5-triphenyltetrazolium chloride (TTC), bromocresol purple (BCP) or the Charm test (see IDF, 1991a and Chapter 10). Some of these methods use *S. thermophilus* as the test organism because of its sensitivity to antibiotics (see Table 6.3), but unfortunately the available methods are prone to certain drawbacks:

- The sensitivity of *S. thermophilus* can vary in relation to the strain used (see Reinbold and Reddy, 1974).
- The above test methods may have certain limitations, for example, Cogan (1972) observed that *L. delbrueckii* subsp. *bulgaricus* is more sensitive than *S. thermophilus* to streptomycin, and to cause a 50% inhibition of growth, 1.6–4.45 and 7.3–13.00 μg ml^{-1} of streptomycin were required, respectively. Thus, a milk which passes the antibiotic test may contain enough streptomycin to inhibit the growth of *L. delbrueckii* subsp. *bulgaricus* (see also Park *et al*., 1984). However, comparative growth of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in milk containing streptomycin showed that the latter micro-organism was more sensitive (Ramakrishna *et al*. (1985); again strain differences appear to be important.

The major effect of antibiotic residues in yoghurt milk is to cause a breakdown in the associative growth between *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus,* or a slow down in the rate of acid development (i.e. longer processing time)

and this can, in turn, lead to syneresis or wheying-off. To combat such problems, the following measures have been recommended:

- The use of milk for the manufacture of yoghurt that is free from antibiotics.
- The addition of penicillinase or penicillinase-producing organisms, e.g. *Micrococcus* spp., to milk in order to inactivate residual penicillin contamination (Reiter *et al.*, 1961; Vazquez and Reiter, 1962).
- Heat treatment of milk can reduce the potency of some antibiotics. Tramer (1973) reported an 8% inactivation of penicillin at 72°C for 15 s, or 20% at 87.7°C for 30 min, or 50% at commercial sterilisation temperatures; tetramycin lost 2/3 of its potency at 85°C for 30 min, but streptomycin and chloramphenicol remained stable and unaffected.
- Lowering the water activity of the growth medium with glycerol for *S. thermophilus* (A_w from 0.992 to 0.995) and *L. delbrueckii* subsp. *bulgaricus* (A_w from 0.992 to 0.985) improved the resistance of these organisms against penicillin, but not gentamycin (Larsen and Anon, 1989b).

It is most likely that the inhibitory effect on these organisms is influenced by the mode of action of the antibiotics and, in view of the immense number of antimicrobial drugs used in veterinary medicine, an attempt has been made to classify only the most widely used antibiotics. The overall characteristics of this group and their possible effect on the yoghurt starter cultures is shown in Table 6.4. Furthermore, depending on the type of antibiotic used, the mode of action of these drugs on *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* can be summarised as follows: (a) interference with the cell membrane structure and permeability, (b) interference with cellular metabolism of proteins, carbohydrates and lipids, (c) interference with energy-yielding transformations in the cell, (d) inhibition of various enzymes and phosphorylation systems, and (e) blocking the synthesis of DNA and RNA during cell division.

Antibiotic-resistant yoghurt strains (see Table 6.5) have been induced to resist higher concentrations of antibiotics by repeated subculturing in milk containing varying concentrations of the antibiotics (Babu *et al.*, 1989a; see also Yondem *et al.*, 1989; Bozoglu *et al.*, 1996). However, the quality of yoghurt produced by such strains was not reported, but Babu *et al.* (1989a) reported the penicillin-resistant *L. delbrueckii* subsp. *bulgaricus* showed almost 50% reduction in acetaldehyde production, whilst the streptomycin-resistant cultures exhibited appreciable depression in flavour production. Thus, these developed cultures may have different characteristics, such as reduced rates of acid and flavour production, or the inability to ferment certain carbohydrates, and these changes could adversely affect the performance of a culture during commercial production (see Babu *et al.*, 1989a, b; Chirica *et al.*, 1998). Furthermore, genes for drug-resistance play an important role as genetic markers, and spontaneous frequencies of mutation to antibiotic resistance interfere with genetic research for the improvement of starter cultures for fermentation (Curragh and Collins, 1992).

6.3.6 Detergent and disinfectant residues

Detergents and disinfectants are widely used in the dairy industry for cleaning and sanitising dairy equipment on the farm and in the creamery (see Chapter 4). The general specification and classification of these preparations is discussed elsewhere,

Table 6.4 Classification and mode of action of some antibiotics

Source or origin	Antibiotics produced	Production (%)	Possible function and mode of action on the yoghurt starter culture
Microbial			
Streptomyces spp.	Streptomycin		* Protein synthesis inhibitors
	Tetracyclines		** Protein synthesis inhibitors
	Neomycin		* Protein synthesis inhibitors
	Erythromycin	58	* Protein synthesis inhibitors
	Chloramphenicol		** Protein synthesis inhibitors
Nocardia spp.	Ristocetin		* Cell wall inhibitors
Micromonospora spp.	Gentamicin		* Protein synthesis inhibitor
Penicillin notatum	Penicillin Xanthocillin		* Cell wall inhibitors
Fusidium coccineum	Fusidic acid	18	Nucleic acid inhibitors
Aspergillus fumigatus	Fumagillin		
Bacillus licheniformis	Bacitracin		* Cell wall inhibitors
Bacillus brevis	Gramicidins		* Alter cell membrane permeability
	Tyrocidin	9	* Disorganise cell membrane structure
Bacillus polymyxa	Polymyxin		* Disorganise cell membrane structure
Synthetic	Sulphonamide		Reaction or site inhibited is folate synthesis
	Penicillin	12	* Cell wall inhibitors
	Chloramphenicol		** Protein synthesis inhibitors
Plant extracts	Alkaloids		
Miscellaneous	Drugs extracted from algae, lichens and animals	3	

* Bactericidal. ** Bacteriostatic.
Adapted from Garrod *et al.* (1973) and Edwards (1980).

but basically, the detergent formulations contain alkali compounds (e.g. sodium hydroxide), while the sanitising agents are quaternary ammonium compounds (QAC) or iodine or chlorine-based compounds.

Inorganic acids are also used for cleaning and disinfecting purposes. Therefore, residues of these compounds in milk can be attributed to two main causes. First negligence, bad management or a faulty cleaning-in-place (CIP) system (i.e. on the farm or at the factory); the latter is more likely to occur on the farm or in milk tankers.

Table 6.5 Development of yoghurt starter cultures resistant to different antibiotics

Antibiotics	Achieved resistance (ml^{-1})	References
Penicillin	3 IU	Hargrove *et al.* (1950)
Streptomycin	500 µg	
Chlortetracycline	70–120 µg	Solomon *et al.* (1966)
Chloramphenicol	40–50 µg	
Streptomycin	500 µg	
Ampicillin	50 µg	Ferri *et al.* (1979)
Cephalexin	150 µg	
Chlortetracycline	≤50–150 µg	
Penicillin	0.25 IU	Babu *et al.* (1989a, b)
Streptomycin	500 µg	

Table 6.6 Sensitivity of the yoghurt starter cultures to various detergent disinfectants and pesticides (mg l^{-1})

Inhibitory substances	Micro-organisms		
	S. thermophilus	*L. delbrueckii* subsp. *bulgaricus*	Mixed culture
Disinfectant/detergent			
Chlorine compounds	5–100	2.5–100	50–>2500
QAC	100–500	0.5–100	>250
Ampholyte			>1000
Iodophore	10–60	60	>2000
Alkaline detergent			500–1000
Insecticides			
Malathion			200
N-methylcarbanate			20

Adapted from Tamime and Deeth (1980), Guirguis and Hickey (1987b) and Petrova (1990).

Second, it is the practice of some milk producers overseas to add biocidal compounds (e.g. H_2O_2) to milk in order to improve its keeping quality. This latter approach is not recommended for public health reasons and the presence of such compounds in milk can adversely affect, or totally inhibit, the growth of starter cultures.

It can be observed from Table 6.6 that the susceptibility of *S. thermophilus* and *L. debrueckii* subsp. *bulgaricus* to cleaning residues is increased in monocultures compared with mixed cultures and this variation could be attributed to:

- differences or variations in the strains of bacteria being used by different researchers (Liewen and Marth, 1984; Guirguis and Hickey, 1987b; El-Zayat, 1987; Mäkelä *et al.*, 1991);
- variation between batches of the commercial detergents and disinfectants tested;
- variation in the test method used to measure the levels of inhibition (see Lanzanova *et al.* (1991) for the use of a conductimetry technique to evaluate the effects of disinfectants and detergents on the activity of starter cultures);
- greater resistance as a result of associative growth relationships.

Another possible source of detergent and/or sterilant residues is the glass bottle washer, for in some countries, glass bottles are still used for packaging stirred or set yoghurt. In the latter type of yoghurt, Nikolov (1975) concluded that if the milk contained above 2.5% of bottlewash liquid, consisting of 1% sodium hydroxide and hypochlorite (i.e. the chlorine concentration $>100\,mgl^{-1}$), the concentration was high enough to inhibit the growth of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*.

6.3.7 Environmental pollution

Incidents of insecticide residues in milk have been reported and this occurrence could well be due either to post-milking contamination, or to feeding cattle with fodder that has been sprayed with an insecticide to combat disease. Milk containing malathion ($200\,mgl^{-1}$) or *N*-methylcarbamate ($20\,mgl^{-1}$) will inhibit the growth of the yoghurt organisms (see Table 6.6). However, Deane and van Patten (1971) observed that $100\,mgl^{-1}$ of malathion or trichlorphon in milk had little effect on the rate of lactic acid development by yoghurt cultures, but some variation in cell morphology did occur after several culture transfers. When viewed under a light microscope (using ordinary staining techniques) the recorded changes included a decrease or increase in cell size and the formation of longer chains. In addition, Deane and Jenkins (1971) propagated *L. delbrueckii* subsp. *bulgaricus* alone in milk containing the same insecticides and observed various morphological changes under the electron microscope. The rod cells were longer, wider or narrower and showed a compact protoplasm and frequent flaking of the cell wall material, and there were fewer cross-walls produced.

In the 1980s, Egyptian scientists intensified their research into the fate of different pesticides (e.g. aldicarb, chlorpyrifos, deltamethrin, lindane, fenvalerate (pyrethroid), malathion and DDT) during the manufacture of zabadi and cheese, and on the growth behaviour of starter cultures (Shaker *et al*., 1985, 1988; Ismail *et al*., 1987; Magdoub *et al*., 1989; Zidan *et al*., 1990; see also Misra *et al*., 1996). The results of these studies could be summarised as follows:

- The pesticide concentration decreased in freshly made zabadi.
- Gelation time of the milk increased and the cheeses had many holes.
- Cells of *L. delbrueckii* subsp. *bulgaricus* floculated into clumps in milk containing aldicarb and the cell count was lower than the control.
- Heating of the pesticide-contaminated milk and fermentation contributed towards the degradation of pesticides.
- Reduced growth rates of *S. thermophilus* in the presence of fenvalerate or DDT were observed, whilst *L. delbrueckii* subsp. *bulgaricus* was sensitive to malathion and DDT.

6.3.8 Bacteriophages

Bacteriophages (phages) are viruses which can attack and destroy the yoghurt organisms and the resultant failure of lactic acid production leads to poor coagulation of the process milk. The occurrence of such viruses in mesophilic dairy starter cultures (e.g. cheese starters) was first reported by Whitehead and Cox (1935) and, for the past few decades, research work on the phages of mesophilic lactic acid

bacteria has been intensified, primarily because of the economic importance of cheese in the dairy industry. However, interest in bacteriophages that can attack thermophilic lactic acid bacteria (i.e. the yoghurt cultures) has been aroused first because world production figures of yoghurt have increased significantly and product failure results in great economic loss to the industry; second because the manufacture of yoghurt is more centralised and bacteriophage attack could become a major problem; and third because strains of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* are widely used in the manufacture of high temperature scalded cheese (e.g. the Swiss varieties) and hence bacteriophage problems could result in both a slow "make" and a low quality cheese. As a consequence, research work on bacteriophages has intensified and a large number of publications are available. However, some selected reviews on bacteriophages of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* are recommended for further information (Reinbold and Reddy, 1973; Sozzi *et al.*, 1981; Stadhouders *et al.*, 1984; Thunell and Sandine, 1985; Ackermann and DuBow, 1987; Mata and Ritzenthaler, 1988; Sechaud *et al.*, 1988; Rajagopal and Sandine, 1989; Jarvis, 1989; Cogan and Accolas, 1990; Coffey *et al.*, 1994; Sable and Lortal, 1995; Gasson, 1996; Neve, 1996; Auvray *et al.*, 1997; Josephsen and Neve, 1998).

The general morphology of a bacteriophage consists of a head and protruding tail, and the type capable of infecting lactic acid bacteria may consist of a double strand of DNA in a linear form which is located in the head (Lawrence *et al.*, 1976; Sandine, 1979; Neve, 1996). The guanine plus cystine (G + C) content of the bacteriophage is somewhat similar to the G + C composition of the bacterial hosts' chromosomes; thus in principle, such similarity may explain the close relationship between the bacteriophage and the host. Over the years different methods have been proposed to classify bacteriophages (Pette and Kooy, 1952; Bradley, 1967; Lawrence *et al.*, 1976; Soldal and Langsrud, 1978; Koroleva *et al.*, 1978; Mullan, 1979), but they were not accepted universally. However, a recent approach to bacteriophage taxonomy, which is accepted universally, has identified three groups known as bacteriophage families, namely the *Myoviridae*, *Podoviridae* and *Siphoviridae* (Ackermann and DuBow, 1987; Francki *et al.*, 1991). Bacteriophages of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* belong to the *Siphoviridae* family (Neve, 1996; Josephsen and Neve, 1998), and Fig. 6.3 illustrates an example of an isometric head structure of a bacteriophage of *S. thermophilus*. The overall morphology of bacteriophages of the yoghurt starter cultures are described as having an isometric head with non-contractile tails. Some bacteriophages may have a collar situated under the head and a base plate at the terminal tail structure including spikes (see Soldal and Langsrud, 1978). Bacteriophages are classified into two main categories depending on the growth responses in the bacterial host, and these types are virulant or lytic bacteriophages (i.e. those that can infect and lyse the host cell) and temperate, prophage or lysogenic bacteriophages (i.e. those that do not lyse the bacterial host, but instead insert their genome in the host chromosome) (Neve, 1996).

The lytic cycle of a bacteriophages involves several stages known as adsorption to the bacterial host, injection of bacteriophage DNA, bacteriophage maturation and lysis of the bacterial cell. The lysogenic cycle primarily involves only the first two stages since rather than the bacteriophage maturing in the bacterial host, the bacteriophage DNA is inserted into the bacterial chromosome. According to Neve (1996) and Josephsen and Neve (1998), this action occurs by a single reciprocal
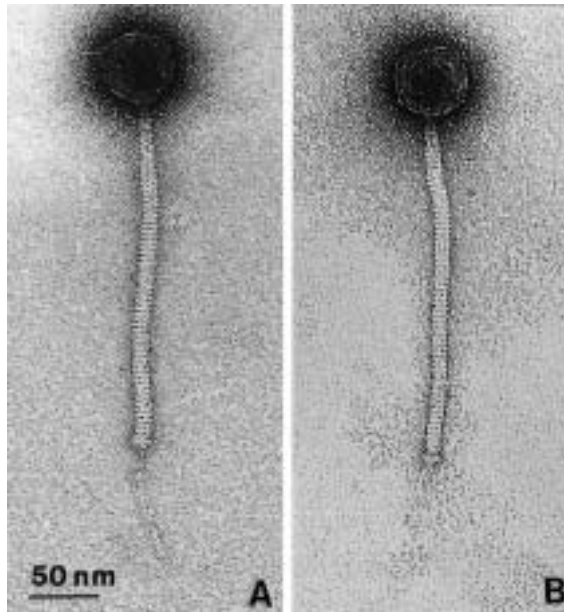
Fig. 6.3 Illustration of a transmission electron micrograph showing different morphological characteristics of a virulent bacteriophage of *S. thermophilus* with (A) and without (B) a tail fibre

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recombination event taking place at a specific region of homology between the bacteriophage DNA and the bacterial host DNA which is known as an attachment site (i.e. attP in the bacteriophage genome and attB in the bacterial host). Thus, bacterial host lysis does not occur and the bacteriophage DNA is now known as probacteriophage and is replicated simultaneously with bacterial host DNA, giving rise to a progeny of lysogenic cells. This bacteriophage is known as a temperate bacteriophage. Over the years, many researchers have used electron microscopy to observe the morphology of bacteriophages of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (see Table 6.7).

Accolas and Spillmann (1979a) observed that six out of seven *S. thermophilus* bacteriophages were similar, that is the head, which was polyhedral or possibly octahedral, was 49–53 nm in diameter, the tail length ranged from 200 to 224 nm (with the exception of one, i.e. 130 nm) and the tail width from 8 to 9 nm; the tail tip had a small plate covered with short prongs or a fibrous mass; the seventh type of phage had no specific tail-tip structure. However, a recent study by Krusch *et al.* (1987) suggested that streptococcal bacteriophages obtained from different research laboratories in Europe have different morphological sizes (see Table 6.7). The distinctive characteristics of *S. thermophilus* bacteriophages can be summarised as follows:

- The sensitivity of the organism to bacteriophage attack was described by Pette and Kooy (1952) under one of three headings: bacteriophage-insensitive, bacteriophage-tolerant (i.e. carriers of the particles) and bacteriophage-sensitive (i.e. results in complete lysis of the host cell).

Table 6.7 Morphology (range) of bacteriophages of yoghurt starter cultures

| Micro-organism | Head | | Tail | Tail Tip | $(n =)^b$ | References |
	Structure	Size[a] (nm)	Length (nm) × diameter (nm)			
S. thermophilus	Hexagonal	50–60	217–239 × 4.8	−	2	Sarimo and Moksunen (1978)
	Polyhedron	40–60	220–420 × 8	−	2	Koroleva *et al.* (1978)
	Polyhedral or octahedron	49–50	130–224 × 8–9	+	14	Accolas and Spillmann (1979a)
	NR	60–65	236–290 × 10	+	3	Reinbold *et al.* (1982)
	Polyhedral	48–70	213–265 × 11–12	±	59	Krusch *et al.* (1987)
	Isometric	57	234 × 9.5 (mean)	+	50	Carminati *et al.* (1994)
	Hexagonal	45–65	220–245 × NR	+	120	Fayard *et al.* (1993)
	Isometric	65	230–260 × NR	+	24	Brüssow *et al.* (1994)
L. delbrueckii subsp. *bulgaricus*	Hexagonal	56–62	205–215 × NR	+	1	Peake and Stanley (1978)
	Polyhedral or octahedron	44–55	116–160 × 8–9	±	7	Accolas and Spillmann (1979b)
	NR	50–59.4	175–198 × 5–6.6	−	3	Reinbold *et al.* (1982)
	Hexagonal	47	159 × NR	−	1	Auad *et al.* (1997)

n = Number of strains tested. NR: not reported.

- A similar classification was proposed by Sarimo and Moksunen (1978), but they incorporated some morphological features as well. Russian workers (Koroleva *et al*., 1978) divided the bacteriophages of *S. thermophilus* into two groups based on morphological observations: regular polyhedron head 40 nm in diameter and others, i.e. head size 65 nm in diameter.
- All virulent bacteriophages of *S. thermophilus* belong to one DNA homology group (e.g. genome size 37–44 kb (Kivi *et al*., 1987; Neve *et al*., 1989; Larbi *et al*., 1990, 1992; Fayard *et al*., 1993; le Marrec *et al*., 1997) and based on the protein profiles and degree of homology of these bacteriophages, they were classified into two or three subgroups (see also Prevots *et al*., 1989; Benbadis *et al*., 1990; Sebastiani and Jäger 1992, 1993; Brüssow *et al*., 1994; Bruttin *et al*., 1997a, b).
- Larbi *et al*. (1992) identified three different mechanisms of bacteriophage resistance in the bacterial host, one of which exhibited a temperature-dependent response.
- Expression of a *Lac. lactis* subsp. *lactis* plasmid-encoded bacteriophage defence mechanism in *S. thermophilus* increased the bacteriophage resistance in the *Streptococcus* (Moineau *et al*., 1995).
- The conductance measurement technique and spot test method have been used successfully for bacteriophage detection in *S. thermophilus* and a yoghurt culture, respectively (Carminati and Neviani, 1991; Champagne and Gardner, 1995).
- Lysogenic strains and many temperate bacteriophages of *S. thermophilus* may have an endogenous origin (Carminati and Giraffa, 1992).

Virulent bacteriophages attacking *S. thermophilus* host cells result in lysis of the cell wall by an enzyme, lysin, which releases newly formed bacteriophages into the growth medium. A typical illustration of which can happen to such a culture before and after infection with a bacteriophage is shown in Fig. 6.4.

In the 1970s, some of the distinctive morphological features of *L. delbrueckii* subsp. *bulgaricus* bacteriophages were reported by Peake and Stanley (1978) and Accolas and Spillman (1979b) and, in brief, they are (a) shorter in overall length in comparison with *S. thermophilus* bacteriophages (e.g. 116–198 nm) with the exception of those phages studied by Peake and Stanley (1978), where the length varied from 205 to 215 nm, (b) the presence of a "collar" structure, and (c) the appearance of up to ten "cross-bar" structures intersecting the tail at intervals (see Table 6.7). More recent characterisations of the lactobacillar bacteriophages have included the following:

- Both lytic and temperate bacteriophages have been found in *L. delbrueckii* subsp. *bulgaricus* and subsp. *lactis*, and their classification has been reported by Cluzel *et al.* (1987a, b), Sechaud *et al*. (1988) and Lahbib-Mansais *et al*. (1988).
- A temperate bacteriophage infecting *L. delbreuckii* subsp. *bulgaricus* had a circularly permuted and terminally redundant genome with a unique sequencing of 36 kb, and was capable of infecting *L. delbrueckii* subsp. *lactis* (Boizet *et al*., 1990; Lahbib-Mansais *et al*., 1992; Auad *et al*., 1997); a virulent bacteriophage has a linear genome of 35 kb (Chow *et al*., 1988).
- Vescovo *et al*. (1990) reported on the sensitivity to bacteriophages of morphological variants of *L. delbrueckii* subsp. *bulgaricus* (e.g. curved or straight cells) and suggested that the physiological reactions were influenced by calcium and magnesium.
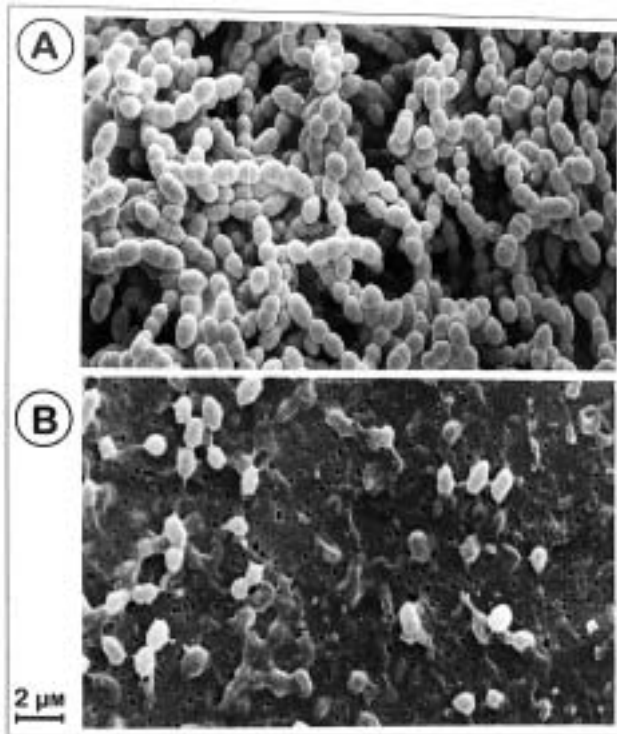
Fig. 6.4   Scanning electron micrograph illustrating (A) a healthy *S. thermophilus* culture and (B) the lysis of cells after infection with a virulent bacteriophage

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No data are available on infection of *L. acidophilus* and *Bifidobacterium* species with bacteriophages. It could be argued that the presence of these cultures in bio-yoghurt is for the provision of probiotic cells in the product rather than for the production of acid for the gellation of milk. However, the interest generated in using these organisms as starter cultures may initiate research work on their bacteriophages. Research work on the bacteriophages of thermophilic lactic starters has increased substantially since the 1970s and Table 6.7 reviews the morphology of those bacteriophages of the yoghurt organisms that have been reported in the literature up to the present time. Figure 6.5 shows some of the morphological characteristics of the bacteriophages that can infect *L. delbrueckii* subsp. *bulgaricus*.

It is also relevant, concerning the viruses attacking *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, that:

- If milk is the origin of bacteriophage contamination, then heat treatment at 85°C for 20 min ensures their destruction (Stolk, 1955); raw milk could be the source of bacteriophages, thus causing problems during the manufacture of some traditional cheeses from raw milk in Europe.
- The optimum temperature of bacteriophage proliferation is the same as the optimum growth temperature of the host, i.e. *S. thermophilus* phages at 39–40°C
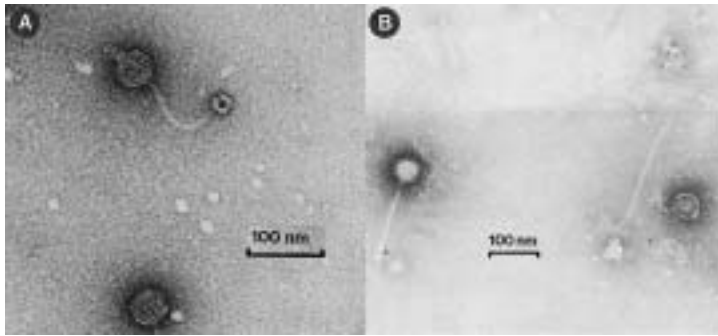
Fig. 6.5 Electron micrograph showing the morphology of two bacteriophages of *L. delbrueckii* subsp. *bulgaricus*

(A) Bacteriophage c5 of strain LT4; note the isometric head, non-contractile flexible and regularly striated tail and large basal plate. (B) Bacteriophage y5 of strain Y5; note the isometric head with large triangular "facets", a small fibrous collar and tail tip composed of short fibres.

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and *L. delbrueckii* subsp. *bulgaricus* bacteriophages at 42–43°C (Sozzi *et al.*, 1978).

- Chemical sterilisation of equipment using 0.1% QAC, 70–90% ethanol, 0.5–1.0% potassium permanganate or 50–100 mg l^{-1} of available chlorine causes the destruction of *S. thermophilus* phages (Ciblis, 1966); peracetic acid (120–300 mg l^{-1}) and active chlorine (≥ 2.6 mg l^{-1}) were recommended by Langeveld and van Montfort-Quasig (1995, 1996) for inactivating yoghurt starter culture bacteriophages (see also Neve *et al.*, 1996).
- Phages are species and/or strain specific, i.e. phages of mesophilic lactic starters do not attack thermophilic starter cultures, and furthermore, *S. thermophilus* phages do not attack *L. delbrueckii* subsp. *bulgaricus* strains.
- The lysis of *Lactobacillus* species including *L. delbrueckii* subsp. *bulgaricus* in the vagina was due to the action of bacteriocins produced by certain lactobacilli and bacteriophages (Tao *et al.*, 1997).

It is evident, therefore, that one or more of the following precautionary measures should be practised in order to eliminate or control phage attack (see also IDF, 1991b):

- use of aseptic techniques for the propagation and production of starter cultures;
- ensure effective sterilisation of utensils and equipment;
- ensure proper heat treatment of the milk;
- restrict movement of plant personnel in starter handling room, and locate starter room far away from production area;
- check filtration of air into the starter room and production area;
- "fog" the atmosphere in the starter room with hypochlorite solution (not to be encouraged) or use laminar-flow cabinets for small-scale culture transfers;
- grow starter culture in bacteriophage inhibitory medium (BIM);
- use a daily rotation of bacteriophage unrelated strains (or phage-resistant strains) of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (Havlova and Jicinska, 1985);

- produce the bulk starter culture or even the retail product using a direct-to-vat system;
- the use of turpine (obtained from aromatic plants by steam distillation) at a rate of $500 \, mg \, 100 \, l^{-1}$ or black pepper oil inhibited bacteriophage infection but not the growth of *L. delbrueckii* subsp. *bulgaricus* (Wolf *et al.*, 1983);
- growth of the yoghurt organisms in soy-milk stopped bacteriophage infection (Farhat *et al.*, 1984).

6.3.9 Bacteriocins

Antibacterial substances (usually segregated from antibiotics) are produced by a wide range of bacteria including dairy starter cultures. They were termed colicin-like, but currently they are known as bacteriocins. For further information refer to the following reviews (Piard and Deamazeaud, 1992; Nettles and Barefoot, 1993; Barefoot and Nettles, 1993; Hoover and Steenson, 1993; de Vuyst and Vandamme, 1994; Nes *et al.*, 1996; Marshall and Tamime, 1997). In general, Tagg *et al.* (1976) characterised bacteriocins as follows:

- proteinaceous in nature
- bactericidal rather than just bacteristatic
- capable of linking to specific binding sites on the bacterial cells and showing different activity from other antimicrobial substances
- plasmid-mediated
- Active against bacteria of the same genera.

At present, around 70 different types of bacteriocins have been identified and produced by lactic acid bacteria. Table 6.8 summarises some selected characteristics of the bacteriocins produced by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, and careful selection of the streptococci strains of the starter culture blend is important to minimise their inhibition. However, other lactic acid bacteria including *Propionibacterium* species can produce bacteriocins that are slightly inhibitory to *L. delbrueckii* subsp. *bulgaricus* (see Table 6.9). The use of such organisms beside the yoghurt starter culture is aimed at controlling over- or postacidification in the product (Weinbrenner *et al.*, 1997). It could be of practical relevance that a bacteriocin produced by *S. thermophilus* affected the growth of *L. delbrueckii* subsp. *bulgaricus* only in M17 broth and not in milk (Cilano *et al.*, 1991; see also Sikes and Hilton, 1987).

Limited data have been published on the mode of action of bacteriocins produced by lactic acid bacteria that can affect the yoghurt starter cultures. For example, lacticin B is bactericidal to sensitive cells, but it does not cause cellular lysis of host cells. It adsorbs non-specifically to sensitive and insensitive lactobacilli because it is a highly hydrophobic peptide and the mode of action may be similar to nisin and pediocin AcH (de Vuyst and Vandamme, 1994).

6.3.10 Miscellaneous factors

6.3.10.1 UF milk

The associative growth by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was lower in ultrafiltered (UF) milk than in milk (Tayfour *et al.*, 1981). A similar

Table 6.8 Some selected characteristics of bacteriocins produced by yoghurt starter cultures

Starter organisms and strain	Bacteriocin name	Molecular mass (kDa)	Sensitivity	Comments
S. thermophilus	NR	<0.7	NR	Antimicrobial compound is heat stable (100°C for 10 min) and and displayed inhibitory activity to Gram-negative and Gram-positive bacteria.
STB 40 and 78	STB 40 and 78	10–20	Lipase, α-chymotrypsin, trypsin and pronase	Both bacteriocins are stable between pH 2 and 12 and are heat resistant; they are active against *Enterococcus* spp. and *S. thermophilus* strains.
ST 10	ST 10	>100	Proteolytic enzymes and α-amylase	Only active against *S. thermophilus* and heat stable at 121°C for 15 min.
SFI 13	Thermophilin 13	~4.0	NR	Thermophilin is heat stable (100°C for 1 hour) and active in the pH range 1.6–10.
L. delbrueckii subsp. *bulgaricus* DDS 14	Bulgarican	NR	NR	Thermostable (120°C for 60 min) and only active at acidic pH; displayed a wide spectrum of inhibiting Gram-positive and Gram-negative bacteria.
7994	NR	<0.7	NR	Still active at pH 4 and thermostable for 1 hour at 100°C; it is active against *Pseudonomas* and *Staphylococcus* species.
CFR 2028	NR	NR	NR	Active principal of the bacteriocin is proteinaceous in nature; stable at pH 3.8–5.0 and heat for 75°C for 30 min; active against *Bacillus cereus*.

NR, not reported.

Data compiled from Abdel-Bar *et al*. (1987), Cilano *et al*. (1990, 1991), Marshall and Tamime (1997) and Balasubramanyam and Varadaraj (1998).

Table 6.9 Inhibition of yoghurt starter cultures by bacteriocins produced by different micro-organisms

Micro-organisms	Bacteriocin name/molecular mass (kDa)	Comments and references
L. delbrueckii subsp. *lactis*	Lacticin A & B kDa (NR)	Inhibited growth of *L. delbrueckii* subsp. *bulgaricus*[a] (Toba *et al*., 1991)
	Lactobacillin G4 kDa (NR)	As above (Giraffa *et al.* 1989, 1990)
	No name <1 kDa	As above (Hara *et al*., 1995)
Lac. lactis subsp. *lactis*	No name 1–10 kDa	As above (Su and Lin, 1990)
	Lacticin 481 1.3–2.9 kDa	As above (Piard and Desmazeaud, 1992)
L. acidophilus	Acidophilucin A kDa (NR)	As above (de Vuyst and Vandamme, 1994)
	Lactacin B 6.2–8.1 kDa	
	Lactacin F 2.5–6.3 kDa	
L. helveticus	Helveticin J 37 kDa	
Propionibacterium jensenii	Jenseniin G	As above (Weinbrenner *et al*., 1997)
Lactobacillus reuteri	Reutericin 6 2.7 kDa	As above (Kabuki *et al*., 1996)
Lac. lactis subsp. *lactis*	Lactococcin DR 2.3–2.4 kDa	Inhibited growth of *S. thermophilus* (de Vuyst and Vandamme, 1994)
	Lacticin 481 1.3–2.9 kDa	

NR, not reported.

[a] Lacticin A is active against this micro-organism.

observation was recently reported by Radulovic and Obradovic (1997), but they observed that the lactobacilli showed better acid development than the streptococci. Ozen and Ozilgen (1992) reported that the kinetic analysis clearly illustrated that the contribution of each microbial species of the yoghurt organisms to the mixed culture growth changed drastically when the substrate concentration was about $15 \, g \, 100 \, g^{-1}$.

6.3.10.2 Added flavours
The addition of coffee (*Coffee robusta*) extract, ginseng saponins and garlic extract to the milk base before fermentation reduced acid development during the manufacture of yoghurt, dahi and acidophilus milk, or in milk inoculated with single strains of lactic acid bacteria (Kim *et al*., 1987; Gandhi and Ghodekar, 1988; Fardiaz, 1995).

6.3.10.3 Lysozyme
This compound is sometimes added to cheese milk to control or inhibit the growth of clostridia. Most of the *L. helveticus* strains have been found to be sensitive to

lysozyme at low concentrations of 10 or 20 $\mu g\,ml^{-1}$, but not the yoghurt starter organisms (Neviani *et al.*, 1988a). However, a strain of *L. delbrueckii* subsp. *bulgaricus* that was sensitive to lysozyme was cultured eight times in the presence of $100\,\mu g\,g^{-1}$ of lysozyme; it developed some resistance but lost it on subsequent culturing in milk (Neviani *et al.*, 1988b); lysozyme resistance is thought to be plasmid related (see also Mercenier *et al.*, 1988a, b).

6.3.10.4   Diet of the cow
At certain times of the year (i.e. June to August in Italy), the acidification rate of the yoghurt organisms is reduced, but activity is retained when the milk is supplemented with paraffin, vitamin E or Fe^{2+} and Zn^{2+}; the problem may also be reduced by supplementing the cow's diet with vitamins (Maianti *et al.*, 1996).

6.3.10.5   Nitrates (NaNO$_3$) and nitrites (NaNO$_2$)
The presence of nitrites in some dairy products is permitted at a level of 0.01 mg $100\,ml^{-1}$ (Baranova *et al.*, 1997). However, the addition of the nitrates or nitrites to the milk base reduced the rate of acid development by yoghurt cultures (Korenekova *et al.*, 1997; Baranova *et al.*, 1997) and the resulting products had low viscosities. Changes in the $NaNO_3$ content in yoghurt, including interactions with caseins, have been reported by Steinka and Przyblowski (1994, 1997).

6.3.10.6   Radioactive materials (^{131}I)
Contamination of milk with such components is undesirable, but in view of the Chernobyl accident, Greek scientists studied the effect of adding ^{131}I to milk during the manufacture of yoghurt and labneh (Vosniakos *et al.*, 1991, 1992, 1993; see also Section 5.7 in Chapter 5; Micic *et al.*, 1985). An ^{131}I content in milk amounting to 6–12 $kBq\,kg^{-1}$ reduced the counts of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* by 45–52% in set yoghurt and labneh; lactococcal species were reduced by 30% in cheese and buttermilk and 26% in ripened butter.

6.3.10.7   Aflatoxins
Aspergillus flavus and *parasiticus* have been identified as producing toxins ($AFB_{1\&2}$ and $AFG_{1\&2}$) that have been implicated as acute toxicants and heptacarcinogens in the human (El-Nezami and Ahokas, 1998). Their presence in yoghurt is discussed in Chapter 10, but research work regarding the role of lactic acid bacteria in controlling the growth of *Aspergillus* species is very limited. However, certain mesophilic and thermophilic starter cultures are capable of detoxifying aflatoxin (El-Nezami and Ahokas, 1998).

Mohran *et al.* (1985) showed that whereas AFB, added to skimmed milk (up to $0.44\,\mu g\,ml^{-1}$), did not affect the growth of *S. thermophilus* and lactococcal species, *L. delbrueckii* subsp. *bulgaricus* and *L. paracasei* subsp. *paracasei* were inhibited, but Kalra *et al.* (1977) observed the opposite effect on the yoghurt organisms; the yoghurt starter cultures were very effective in the detoxification of $0.5\,mg\,l^{-1}$ ochratoxin A present in milk (Rasic *et al.*, 1991; Skrinjar *et al.*, 1996).

6.3.10.8   Sweetening agents
The addition of sugar $\geq 9\,g\,100\,g^{-1}$ to the milk may cause inhibition or delay in the fermentation period, as will the addition of artificial sweeteners. For further details refer to section 2.6 in Chapter 2 (see also Lacroix and Lachance, 1988a, b, 1990; Larsen and Anon, 1989a, 1990; Latrille *et al.*, 1992).

6.3.10.9 Cadmium (Cd)

As a result of environmental pollution, Cd may be found in cow's milk at low levels up to $160 \mu g \, kg^{-1}$ with typical values $<0.5 \mu g \, kg^{-1}$ (Walstra and Jenness, 1984). An inhibition of the decrease in pH was observed for *S. thermophilus* $>5 \mu g \, Cd \, l^{-1}$ (Korkeala *et al*., 1984), but not at lower levels.

6.3.10.10 Phosphates

Bacteriophage inhibitory media (BIM) for lactococci contain high levels of phosphates which chemically bind the free calcium in milk, thus preventing bacteriophage replication (Zottola and Marth, 1966). However, the growth of *L. delbrueckii* subsp. *bulgaricus* in phosphated milk (i.e. added phosphate or commercially available BIM) was inhibited and cellular morphology was altered in milk containing about $3 \, g \, 100 \, g^{-1}$ phosphate (Wright and Klaenhammer, 1983, 1984). Shalaby *et al*. (1986) observed no effect on growth of four strains of *S. thermophilus* in phosphated media and when milk containing sodium citrate + sodium phosphate, yeast extract and infected with bacteriophage was used, the rate of acid production was not reduced either; the presence of the buffering agents was effective in suppressing bacteriophage attack. However, Champagne and Gange (1987) observed that the starter activity of three strains of *S. thermophilus* growth in Phase 4 and In-sure (i.e. a commercially available BIM) was influenced by two factors: (a) the age of the culture, for example, the starter cultures lost their activity in milk after 16–24 hour, whilst in BIM retained their activity for 40–48 hours, and (b) the heat treatment used for preparation of the BIM and agitation during growth affected *S. thermophilus* activity in relation to the BIM used (i.e. In-sure but not Phase 4).

6.3.10.11 Preservatives

In some countries, the addition of preservatives (e.g. K- or Na-sorbate, benzoic acid or nisin) is permitted in fruit yoghurt, but not in natural yoghurt (for details refer to Section 2.7.2 in Chapter 2). These compounds are mycostatic agents and, at the same time, they can affect the activity of the starter cultures (see Table 2.12; Gupta and Prasad, 1988; Kebary and Kamaly, 1991; Rajmohan and Prasad, 1994).

6.3.10.12 Miscellaneous compounds

The concentration ($mg \, l^{-1}$) of fatty acids (1000), ethylenedichloride and methylsuphone (10–100 each) and acetonitrile, chloroform or ether (10 each) had an inhibitory effect on *S. thermophilus* (see also Tamime and Deeth, 1980; Antonopoulou *et al*., 1996).

6.4 Conclusion

It is evident that milk is an excellent growth medium for yoghurt starter cultures, but the rate of growth in milk is influenced by a multitude of factors. Thus, using milk free from these inhibitory agents, providing hygienic standards during the preparation of starter culture and production of yoghurt and using the right combination of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* will lead to successful growth. Future development of these cultures in terms of resistance against bacteriophage and/or inhibitory agents will help to minimise culture failure during production.

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7

Biochemistry of fermentation

7.1 Introduction

Micro-organisms sustain their life cycles via a large number of interrelated/complex metabolic pathways covering both biosynthetic and energy-yielding functions. Each individual metabolic pathway consists of many reactions which, in turn, are regulated by different enzyme systems, and hence it is the level of enzyme synthesis and activity which maintains and controls the functions of the microbial cell (Stanier *et al.*, 1987). One regulatory (or feedback) mechanism is derived from low molecular weight compounds which result from the breakdown of nutrients (carbohydrates, proteins, lipids and other minor constituents) present in the growth medium. The composition of this medium is, therefore, important in relation to the build-up and division of the microbial cells but, in the case of yoghurt, its effect on the metabolism and growth of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (including the "bio" cultures) also influences the properties and characteristics of the product. For this reason, the biochemical reactions initiated by the yoghurt and bio organisms are fundamental to the manufacture of a high quality product, and hence it is pertinent to consider them in some detail.

7.2 Carbohydrate metabolism

Microbial cells derive their energy requirements via different systems; the cytochrome system for harnessing energy from electrons of NADH, the enzymes that operate the anaplerotic pathways, the tricarboxylic acid cycle or by fermentation. The lactic acid bacteria (i.e. the lactococci, leuconostoc, lactobacilli, streptococci and bifidobacteria), however, do not possess any of the former three systems and energy can only be supplied by the fermentation of carbohydrates (Lawrence *et al.*, 1976). The energy is largely obtained via substrate-level phosphorylation and the adenosine triphosphate enzymes (ATPases) of the cytoplasmic membrane (see also Nannen and Hutkins, 1991b). In general, dairy starter cultures metabolise car-

bohydrate (i.e. lactose is the main sugar present in milk) either through the homo- or heterofermentative metabolic pathways. *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus* and *Lactobacillus acidophilus* ferment lactose homofermentatively, whilst *Bifidobacterium* spp. ferment the same sugar heterofermentatively; the metabolic pathways of these micro-organisms are as follows.

7.2.1 Homolactic fermentation

Since the catabolism of lactose takes place inside the microbial cell, the key step in this metabolic pathway is at the entry of lactose into the cell. In the lactococci and certain strains of *L. acidophilus* (Kanatani and Oshimura, 1994; Marshall and Tamime, 1997a) a specific system is involved in lactose transport and the sugar is phosphorylated by phosphoenolpyruvate (PEP) during translocation by the PEP-dependent phosphotransferase system (PTS) as described by McKay *et al.* (1969) (see also Lawrence *et al.*, 1976). This mechanism is known as PEP:PTS and four proteins (in sequential order: enzyme II, III, I and HPr) are involved in translocating the lactose from outside to the inside of the cytoplasmic membrane and into the microbial cell to become lactose phosphate (Dills *et al.*, 1980; Zourari *et al.*, 1992b; Cogan and Hill, 1993; Monnet *et al.*, 1996). Lactose-6-phosphate is hydrolysed by β-phosphogalactosidase (β-Pgal) into its monosaccharide components. The galactose and glucose are then catabolised via the Tagatose and Emden–Meyerhof–Parnas EMP) pathways, respectively (Monnet *et al.*, 1996; Marshall and Tamime, 1997a). However, dephosphorylation of galactose may take place and it will remain unmetabolised and excreted from the microbial cell. Nevertheless, in both pathways the glucose and galactose converge at dihydroxyacetone phosphate and glyceraldehyde-3-phosphate where the three-carbon sugars become further oxidised to phosphoenolpyruvate and then produce lactic acid (see Fig. 7.1).

Homolactic fermentation by *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus* and *L. acidophilus* follows the EMP pathway *mainly* for glucose catabolism. However, an alternative system for lactose transport into the cells of these starter cultures including *Bifidobacterium* spp. involves cytoplasmic proteins (permeases) that translocate lactose without chemical modification. Such a sugar transport mechanism could be similar to the lactose permease system in *Escherichia coli*. After the lactose enters the cell via a permease as an unphosphorylated disaccharide, it is hydrolysed by β-galactosidase (β-gal) to non-phosphorylated glucose and galactose. Glucose is catabolysed to pyruvate (see Fig. 7.1) and the galactose is secreted from the cell. When all the glucose is depleted, *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus* and *L. acidophilus* will utilise the galactose via the Leloir pathway (Fig. 7.1) with galactokinase as the first enzyme of the metabolic pathway (Kandler, 1983; Hutkins *et al.*, 1985b; Hickey *et al.*, 1986; Thompson, 1988; Benito de Cardenas *et al.*, 1989; Poolman *et al.*, 1989, 1990, 1995; le Bras *et al.*, 1991; Hutkins and Ponne, 1991; Collins and Thompson, 1992; Zourari *et al.*, 1992a; Poolman, 1993). However, Cogan and Hill (1993) suggest that some strains can metabolise galactose only when low (4mM) concentrations of lactose are present; this may be due to an antiporter proton motive force (PMF) transport system involving galactose, but the details have yet to be established (see also Thomas and Crow, 1984; Hutkins *et al.*, 1985a).

Thus, it appears the carbohydrate metabolism by *S. thermophilus* differs from lactococcal species. Characterisation of the metabolic activity of this organism has been

LACTOSE IN MILK

Environment

Cell membrane

(Permease)   (Permease)

Cytoplasm

Lactose   Lactose

| HOMOLACTIC FERMENTATION | | HETEROLACTIC FERMENTATION |

Glucose   Galactose   Glucose

ATP   ATP   ATP
ADP   ADP   ADP

Galactose -1-phosphate

Glucose-6-phosphate ◄── Glucose-1-phosphate ──► Glucose-6-phosphate

Fructose-6-phosphate   **LELOIR PATHWAY**

Fructose-6-phosphate   Fructose-6-phosphate

Fructose-1,6-bisphosphate   (P)

Erythrose-4-phosphate   Acetyl phosphate

Dihydroxyacetone ⇌ Glyceraldehyde-3-phosphate   ADP
phosphate   ATP

(P)   4 ADP
       4 ATP   Heptose phosphate + Triose phosphate   Acetate
       2 NAD   (sugar phosphate rearrangements)
       2 NADH

Pyruvate   2 Acetyl phosphate   2 Glyceraldehyde-3-phosphate
       2 NADH
       2 NAD   2 ADP   4 ADP
Lactate   2 ATP   4 ATP
                                                  2 NAD
| EMBDEN-MEYERHOF-   2 Acetate   2 NADH | BIFIDUS PATHWAY |
| PARNAS PATHWAY | 2 Pyruvate
                                                  2 NADH
                              Acetate   Formate   2 NAD

                                                  2 Lactate

Cytoplasm

(H⁺ Lactate symport)   (H⁺ Lactate and acetate symport)

Cell membrane

Environment

Lactate in milk   Lactate and acetate in milk

Fig. 7.1 Homolactic and heterolactic fermentation of lactose by the yoghurt and bio starter cultures after translocation by a permease

Note: The dotted line sequence may indicate an alternative pathway to account for excess acetate observed in fermentation by some *Bifidobacterium* strains.

Adapted from Monnet *et al.* (1996) and Marshall and Tamime (1997a).

reported by Hemme and Nardi (1980), Hemme *et al.* (1980) and in the reviews by Hutkins and Morris (1987), Ramos and Harlander (1990) and Arihara and Luchansky (1995).

It is worth pointing out that the presence of CO_2 during the fermentation of milk stimulates the growth of *L. delbrueckii* subsp. *bulgaricus* (see Chapter 6) and, if it is accepted that *S. thermophilus* can metabolise galactose via the Leloir pathway, this may explain the presence of CO_2 in the milk (see Fig. 7.1); however, an alternative route for the production of CO_2 is the hydrolysis of urea (Tinson *et al.*, 1982a–c).

Lactate dehydrogenase is also important in the control of carbohydrate metabolism. The enzyme in *Lactococcus* spp. is activated by fructose 1,6-bisphosphate aldolase and by tagtose 1,2-bisphosphate aldolase (see the reviews by Monnet *et al.*, 1996; Marshall and Tamime, 1997a). The homolactic fermentation of *Lactobacillus* spp. may be different, as the enzyme from many species has been found to have constitutively high activity which is independent of the presence of fructose 1,6-bisphosphate aldolase. Sequencing the lactate dehydrogenase gene from *S. thermophilus* shows it to have 328 amino acid residues (Taguchi and Ohta, 1991, 1993), whilst 332 amino acid residues were reported by Kochhar *et al.* (1992d) for the equivalent gene from *L. delbrueckii* subsp. *bulgaricus* (see also Kochhar *et al.*, 1992a–c). Branny *et al.* (1996) observed that the gene encoding for pyruvate kinase and for phosphofructokinase from *L. delbrueckii* subsp. *bulgaricus* formed a bicistronic operon transcribed into 2.9 kb RNA (see also Branny *et al.*, 1998). Somkuti and Steinberg (1991) reported that sucrose (suc⁻) mutants strains isolated after treating *S. thermophilus* with *N*-methyl-*N*-nitroso-*N*′-nitroguanidine were able to utilise lactose, but not sucrose, and retained the ability to synthesise β-fructofuranosidase (see also Hosono *et al.*, 1989); characterisation of a Mn-containing superoxide dismutase in *S. thermophilus* has been reported by Chang and Hassan (1997). Details of further aspects of sugar metabolism and the synthesis of L(+) and D(−) lactic acid by the yoghurt micro-organisms isolated from commercial products in Argentina, South Africa and Canada have been given by Malan (1987), Amoroso *et al.* (1988, 1989, 1992), Sinha *et al.* (1989) and Amoroso and Manca de Nadra (1991) (see also Richmond *et al.*, 1987).

7.2.2 Heterolactic fermentation

In the present context, only the bifidobacteria ferment lactose and glucose via a heterofermentative pathway (Fig. 7.1). The catabolism of glucose produces no CO_2 because there is no early step involving a decarboxylation. As mentioned earlier, lactose is transported into the cell by means of permease and, in turn, it is hydrolysed into glucose and galactose. Aldolase and glucose-6-phosphate dehydrogenase are absent in this species. Hexoses are catabolised by a fructose-6-phosphate shunt and the pathway involves fructose-6-phosphate phosphoketolase. The products of fermentation by *Bifidobacterium* spp. are lactate and acetate, and the fermentation of two molecules of glucose yields three molecules of acetate and two molecules of lactate.

7.2.3 Lactase activity

β-Galactosidase from the yoghurt organisms has been identified as an important enzyme in fermented milk processing and is mainly involved in lactose catabolism.

However, the enzyme from *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* has also been characterised as an alternative source of lactase for commercial developments.

The optimum activity of streptococcal β-gal has been observed as follows: (a) neutral pH, (b) temperature at 55°C in buffer, (c) more heat stable than a similar enzyme from yeasts, (d) Mg^{2+} stimulated enzyme activity, whilst EDTA caused inhibition, and (e) the presence of oxgall ($0.15\,ml\,100\,ml^{-1}$) increased the activity of β-gal (Greenberg and Mahoney, 1982; Noh and Gilliland, 1994; Garman *et al.*, 1996; Gündüz and Rejaee, 1997). Greenberg and Mahoney (1984) observed that the activity of the enzyme was greater in heated (63°C or 85°C for 30 min) milk than in raw milk, whilst the activity in a buffered system was greater than in whey or milk, due to the unfavourable ionic environment in the latter. The stability of β-gal in milk and sweet whey was ≥10-fold that in lactose solution (Greenberg *et al.*, 1985). However, thermal denaturation occurs at ~60°C, but stability can be enhanced by the addition of bovine serum albumin (Chang and Mahoney, 1994). In milk the activation energy for lactose hydrolysis was $35\,kJ\,mol^{-1}$ (Chang and Mahoney, 1989a, b); different strains of *S. thermophilus* demonstrate different β-gal activities (Occhino *et al.*, 1986).

One possible use of β-gal from *S. thermophilus* is the hydrolysis of lactose in milk without concomitant production of lactic acid (Somkuti and Steinberg, 1995; see also Smart *et al.*, 1985; Smart and Richardson, 1987; Smart, 1991; Benateya *et al.*, 1991; Linko *et al.*, 1998) or the immobilisation of β-gal on DEAE-cellulose for the production of low lactose milk (Sharma and Dutta, 1990). From genetic studies (David *et al.*, 1992), the β-gal of the yoghurt organisms and *Leuconostoc lactis* are similar, and *in vivo* activity of β-gal in high lactose yoghurt was much less acid resistant than that in ordinary yoghurt (Kotz *et al.*, 1994); the β-gal activities of three commercial bio yoghurts were reported by Ordonez and Jeon (1995).

The enzyme from *L. delbrueckii* subsp. *bulgaricus* may have a requirement for Mg^{2+} for activity (Adams *et al.*, 1994) and a pH optimum of 6.5–7.0, although the enzyme is stable at pH 5.8 (Gupta *et al.*, 1994). The β-galactosidase of *L. delbrueckii* subsp. *bulgaricus* is made of a dimer consisting of two subunits of identical size (molecular weight 235 kDa). It was rapidly and irreversibly inactivated at pH 4 due to a decline in the number of the exposed tryptophan residues because of the denaturation process (Winters and Batt, 1991). The optimum activity (i.e. pH 7 and 55°C) of the enzyme in autoclaved milk resulted in ~85% of the lactose being hydrolysed (Shah and Jelen, 1991; see also Yoast *et al.*, 1994). The specific activity of β-gal was greater in lactobacilli in group I (i.e. *Thermobacterium*) than in group II (i.e. *Streptobacterium*) (Cesca *et al.*, 1984) and Nader de Macias *et al.* (1986) used the enzyme-linked immunosorbent assay (ELISA) for determining the immunological relationships among β-gal from different lactobacilli (see also Wang *et al.*, 1996) or for arginine dihydrolase activity in the lactobacilli groups I, II and III (Manca de Nadra *et al.*, 1982).

β-Phosphogalactosidase (β-Pgal) has been reported in *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (Permi *et al.*, 1972; Reddy *et al.*, 1973b; Somkuti and Steinberg, 1978, 1979a, b; Farrow, 1980; Toba *et al.*, 1981) so that galactose could be metabolised. However, Cogan and Hill (1993) reported that the enzyme is an artefact derived by formation of *o*-nitrophenyl-β-galactopyranoside (ONPG), the substrate for β-gal, from ONPG phosphate, the substrate for β-Pgal, by a phosphatase.

7.2.4 Production of lactic acid

The catabolism of lactose by *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus* and bifidobacteria results mainly in the production of lactic acid, or lactic and acetic acids when bifidobacteria are used in the starter culture (see Fig. 7.1). Although the conversion process consists of many different biochemical reactions it can be simplified by the following equation:

$$\text{Lactose} + \text{Water} \rightarrow \text{Lactic acid}$$
$$C_{12}H_{22}O_{11} \quad H_2O \quad 4C_3H_6O_3$$

Lactic acid is important during the manufacture of yoghurt for the following reasons.

First, the lactic acid helps to destabilise the casein micelles by progressively converting the colloidal calcium/phosphate complex (in the micelle) to the soluble calcium phosphate fraction which diffuses into the aqueous phase of the milk. This results in the micelles being gradually depleted of calcium, so leading to coagulation of the casein at pH 4.6–4.7 and the formation of the yoghurt gel (refer to Chapter 2 for further details). Once this physical condition has been established, soluble calcium lactate is formed and according to Dyachenko (1971) the destabilisation reaction can be summarised as follows:

$$\text{Ca-caseinate-phosphate} + \text{lactic acid} \rightarrow \text{Casein complex}$$
$$\text{complex} \qquad\qquad\qquad +$$
$$\text{Ca-lactate}$$
$$+$$
$$\text{Ca-phosphate}$$

Second, the lactic acid gives yoghurt its distinctive and characteristic taste (i.e. sharp and acidic). It can also enhance or contribute to the nutty and/or aromatic flavour of the product. Lactic acid bacteria possess the enzyme lactate dehydrogenase (LDH) for the synthesis of lactate from pyruvate (see Fig. 7.1). Lactate is the Latin word for lactic acid derived from milk. Different forms of lactic acid can be produced (e.g. L(+), D(−) or DL(±)) and these isomers differ in the configuration of the second carbon atom, as follows:

```
        COOH                    COOH
         |                       |
HO — C — H              H — C — OH
         |                       |
        CH₃                     CH₃
   L(+) Lactic acid       D(−) Lactic acid
```

In yoghurt starter cultures, *S. thermophilus* produces mainly L(+) lactic acid (Garvie, 1978; Hemme *et al*., 1981) and D(−) lactic acid is produced by *L. delbrueckii* subsp. *bulgaricus* (Gasser, 1970; Gasser and Gasser, 1971; see also the review by Tamime and Deeth, 1980). The LDH enzyme is situated in the cytoplasm of the bacterial cell and according to Garvie (1980) the activity of this enzyme is, in the yoghurt organisms, dependent on nicotinamide adenine dinucleotide (NAD)/reduced nicotinamide adenine dinucleotide (NADH). The former coenzyme is regenerated from NADH during the conversion of pyruvic acid to lactic acid. However, some strains of *S. thermophilus* contain an LDH enzyme which is activated by fructose 1,6-

bisphosphate (FDP) (Wolin, 1964; Garvie, 1980) and such enzymes show an absolute requirement for FDP at physiological pHs; the reaction is virtually non-reversible and the enzyme reacts weakly with lactic acid and NAD (see also Delcour *et al.*, 1993; Bernard *et al.*, 1994, 1995, 1997; Álvarez *et al.*, 1997).

Recently, Vinals *et al.* (1995) described the structure of LDH of *L. delbrueckii* subsp. *bulgaricus* as being constituted of subunits of α/β structure with a catalytic domain (i.e. consisting of a histidine residue along with arginine and phenyalanine) and a coenzyme binding domain.

During the manufacture of yoghurt, *S. thermophilus* grows faster than *L. delbrueckii* subsp. *bulgaricus* (see Fig. 6.2), and hence L(+) lactic acid is produced first followed by D(−) lactic acid. The percentage of each isomer present in yoghurt is an indication of the following:

- Yoghurt, which contains more than 70% of L(+) lactic acid has been inoculated with a starter culture which consists predominantly of *S. thermophilus* (Kunath and von Kandler, 1980), or the fermentation has been carried out at a temperature below 40°C, or the product has been cooled to a low acidity and the cooled yoghurt contains around 0.8 g 100 ml^{-1} or less lactic acid.
- Yoghurt containing more D(−) lactic acid than L(+) lactic acid has been incubated at too high a temperature, i.e. 45°C or more, or for a long period whereby the product has become highly acidic, or has suffered from prolonged storage, or the starter inoculation rate was more than 3%, or the starter contained more rods than cocci.

Yoghurt usually contains 45–60% L(+) lactic acid and 40–55% D(−) lactic acid (Puhan *et al.*, 1973a, b, 1974; Vanderpoorten and von Renterghem, 1974; Kielwein and Daun, 1980; Aleksieva *et al.*, 1981), and the ratio of L(+):D(−) lactic acid could be used to assess the quality of yoghurt. However, Puhan *et al.* (1973b, 1974) examined 269 samples of commercial yoghurt and found that the ratio of L(+):D(−) ranged from as little as 0.34 (very acidic) to 8.28 (i.e. L(+) lactic acid predominant). A ratio of two was suggested by Blumenthal and Helbling (1974) to be consistent with a good yoghurt, but such an approach could be more useful in situations where the quality of yoghurt (i.e. sweet-low in acid or sharp-high in acid) has to be manipulated to meet the demands of consumers in different markets, that is, a sharp and acidic yoghurt must contain a low ratio of L(+):D(−) and vice versa. A combined starter of *Lactobacillus helveticus* and *S. thermophilus* used for the manufacture of yana yoghurt in Bulgaria gave rise to >80% L(+) lactic acid which is suitable for infant foods (Gyosheva *et al.*, 1996). A similar result was obtained by reducing the lactate dehydrogenase activity in *L. delbrueckii* subsp. *bulgaricus* (Germond *et al.*, 1995) (see also Klupsch, 1984).

L. acidophilus produces DL lactic acid, whilst the bifidobacteria produces L(+) acid as the result of lactose metabolism. Marshall and Tamime (1997b) have shown that these organisms do not produce acid at the same rate as *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. Furthermore, most of the bio starter cultures rely on the yoghurt organisms (singly or mixed) for the acidification of milk and hence it was decided not to review the metabolism of bio organisms in detail. One aspect which should not be overlooked, however, is the amount of acetic acid produced in bio yoghurt. High levels will impart a "vinegary" taste which may not be accepted by consumers (see Fig. 7.1).

Nevertheless, the main role of the yoghurt organisms is to acidify the milk by producing lactic acid from lactose. Detailed information on the anaerobic fermentation reacts of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (i.e. as single or mixed strains including different strains of each species) has been reported by Oner and Erickson (1986), Oner *et al*. (1986a–d), Zourari and Desmazeaud (1990, 1991) and Zourari *et al*. (1991). Such characterisation of yoghurt organisms is aimed at blending strains together so that different products can be made for different markets and can include variations in flavour and aroma (see also Nannen and Hutkins, 1991a; Zanatta and Basso, 1992; Hutkins and Nannen, 1993). In addition, it has been reported that the undenatured whey protein in skimmed milk decreased during the incubation period with the yoghurt starter cultures (Vaitheeswaran and Bhat, 1988).

7.2.5 Production of exopolysaccharide (EPS)

Some strains of bacteria utilise the carbohydrates in the growth medium for the production of EPS materials, and examples of such organisms are *Streptococcus mutans*, *Streptococcus bovis* and *Leuconostoc mesenteroides* subsp. *mesenteroides* which have the ability to produce extracellular dextrans (Berkeley *et al*., 1979). Sharpe *et al*. (1972) isolated a similar material, slime, from some heterofermentative *Lactobacillus* spp. and it was found to be a glucan, probably dextran, consisting of α-1-6-glycosidic linkages. At present, isolated strains of *Lactococcus* spp. and thermophilic lactic acid bacteria are used extensively in the manufacture of fermented milks and many EPS-producing lactic acid bacteria have been studied extensively since 1990 (see the reviews by Cerning, 1990, 1994, 1995; Malik *et al*., 1994; Sikkema and Oba, 1998). The role of EPS in the consistency and texture of yoghurt has been discussed elsewhere (Chapter 2 and 10; see also Wacher-Rodarte *et al*., 1993; Uemura *et al*., 1994; Giraffa, 1994; Lira *et al*., 1997; Rawson and Marshall, 1997; Sebastiani and Zelger, 1998).

EPS materials are produced by some yoghurt starter cultures, for example, the RR culture which was developed in The Netherlands to enhance the viscosity of yoghurt (Galesloot and Hassing, 1966; see also Tamime and Robinson, 1978; Luczynska *et al*., 1978). The work of Tamime (1977a, b, 1978) suggested that the chemical composition of the EPS material produced by starter culture RR was a β-glucan which yielded only glucose after acid hydrolysis. However, current studies suggest that the yield and carbohydrate constituents of the EPS materials produced by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* are influenced by many factors such as the growth medium used, the temperature of incubation, the level of acidity in the growth medium and the strain variation (Cerning 1990, 1994, 1995; Petit *et al*., 1991; Gassem *et al*., 1995, 1997a, b; Grobben *et al*., 1995, 1997, 1998; Mollet, 1996).

In general, the amount of EPS material produced by the yoghurt organisms may reach up to $40\,mg\,100\,ml^{-1}$ (Cerning, 1995). Further factors relating to yield and production of EPS are summarised in Table 7.1. No data are available on EPS production by the bio starter cultures. However, Mozzi *et al*. (1995a) reported that optimum yield of EPS from a strain of *L. acidophilus* was $\sim 6\,mg\,100\,ml^{-1}$ after incubation at 37°C or 42°C for 24 hours.

It is evident that a number of strains of the yoghurt starter culture are capable of producing EPS. These are classified as heteropolysaccharides composed of either

Table 7.1 Reported factors that can influence the yield and characteristic of EPS by the yoghurt organism

Micro-organism	Comments	References
S. thermophilus	Ropy strains exhibited an increase in viscosity in milk, but not in whey or synthetic media; they produced more soluble polysaccharides ($\leq$4 mg 100 ml^{-1}) than the non-ropy strains; when grown at 30°C, the ropy strains produced four to eight times more insoluble glucides than the non-ropy strains.	Giraffa and Bergère (1987)
	The amount of EPS produced ranged between 5 and 34 mg 100 ml^{-1} in UF milk enriched with casamino acid or heart extract; compared with skimmed milk, the amount of EPS produced was much lower.	Cerning *et al.* (1990)
	Grown in synthetic media, EPS was produced in the stationary phase; factors that influenced EPS production were type of sugar, temperature and initial pH; at optimal growth rate, EPS production was dependent on lactose concentration.	Gancel and Novel (1994a, b)
	Optimum yield of EPS (~10 mg 100 ml^{-1}) was obtained when the organism was incubated at 30°C for 24 hour.	Mozzi *et al.* (1995c)
L. delbrueckii subsp. *bulgaricus*	Isolates from a commercial yoghurt produced soluble EPS.	Manca de Nadra *et al.* (1985)
	Yield of EPS ranged between 6 and 43 mg 100 ml^{-1}; growth media did not influence amount of EPS produced.	Cerning *et al.* (1990)
	At higher temperature and slower growth, the EPS production per cell was greater; EPS production was increased in the presence of hydrolysed casein early in the growth phase when grown in milk, but was reduced in MRS broth and lactose; preliminary results suggested that the EPS is a glycoprotein, although the protein may be loosely associated with the carbohydrates.	Garcia-Garibay and Marshall (1991)
	Yield of 12 mg 100 ml^{-1} was optimal when the organism was grown at 37°C for 24 hours.	Mozzi *et al.* (1995c)
	Half the amount of EPS was produced in the exponential phase; the yield of EPS in skimmed milk reached 13 mg 100 ml^{-1}.	Bouzar *et al.* (1996)
	Glucose + fructose influenced the yield of EPS and produced 8 mg 100 ml^{-1} which was the highest.	Grobben *et al.* (1996, 1997)
	EPS yield was 35.4 mg 100 ml^{-1}.	Kimmel *et al.* (1998)
Mixed culture	Excessive EPS production when the starter culture was incubated at 32°C for a long time; such yoghurt had a coagulum with decreased relative firmness and apparent viscosity.	Schellaas (1984), Schellaas and Morris (1985)
	A yield of 80 mg 100 ml^{-1} was obtained when both cultures used were EPS producers.	Cerning *et al.* (1990)
	Growth of lactobacilli (EPS producer) with streptococci (non EPS producer) yielded 24 mg ml^{-1} of EPS in skimmed milk.	Bouzar *et al.* (1997)

linear or branched repeating units varying in size from di- to heptasaccharides. The molecular weights of the EPS are rather high ranging from $1–2 \times 10^6$ which is formed by polymerisation of hundreds and possibly thousands of these repeating units. The available data suggest a range of different EPS structures:

- In some mixed cultures, the EPS structure consists of galactose and glucose at a ratio of 2:1 (Schellhaas, 1984; Schellhaas and Morris, 1985), but a ratio of 1:1 was reported by Lemoine *et al*. (1997) for *S. thermophilus*. However, the EPS material produced by *L. delbrueckii* subsp. *bulgaricus* consisted of glucose and fructose (ratio 1:2) and the predominant linkages were α-1,4 and α-1,6-glucosidic linkages at a ratio of 1:1 (Manca de Nadra *et al*., 1985).
- EPS produced by *L. delbrueckii* subsp. *bulgaricus* consisted of galactose, glucose and rhamnose at a ratio of 4:1:1 (Cerning *et al*., 1986), 5:1:3 (Gruter *et al*., 1993) or 7:1:0.8 (Grobben *et al*., 1996); however, Lemoine *et al*. (1997) reported a ratio of 3:1:2 for EPS produced by *S. thermophilus*.
- *L. delbrueckii* subsp. *bulgaricus* produced EPS made up of glucose and galactose with small amounts of mannose (Bouzar *et al*., 1996) or mainly galactose and small amounts of glucose and rhamnose (Bouzar *et al*., 1997; see also Zourari *et al*., 1992a).
- Cerning *et al*. (1988) reported that glucose and galactose were the main saccharides of the EPS material from *S. thermophilus*, along with small amounts of xylose, arabinose, rhamnose and mannose; whilst Ariga *et al*. (1992) reported a ratio of 1:1.47 of rhamnose and galactose in an EPS produced by the same organism.

Nevertheless, the structures of the EPS produced by some yoghurt organisms have been determined by Doco *et al*. (1990, 1991), Gruter *et al*. (1993), Stingele *et al*. (1996) and Lemoine *et al*. (1997). The polymers are based on D-galactose residues connected via $1 \rightarrow 3$ or $1 \rightarrow 4$ glycosidic linkages as follows:
S. thermophilus (i.e. tetrasaccharide)

[→3)-β-D-Gal*p*-(1→3)-[α-D-Gal*p*-(1→6)]-β-D-Glc*p*-(1→3)-α-D-Gal*p*NAc-(1→]*n*
[→3)[α-D-Glc*p*-(1→3)-[β-D-Gal*p*-(1→6)]-β-D-Glc*p*-(1→3)-β-D-Gal*f*-(1→]*n*
[→2)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-D-Gal*p*-(1→3)-[β-D-Gal*p*-(1→4)]-α-L-Rha*p*-(1→]*n*
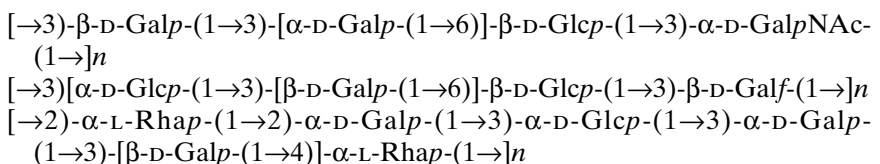
L. delbrueckii subsp. *bulgaricus* (i.e. branched heptasaccharide)

[→2)-[β-D-Gal*p*-(1→3)]-α-D-Gal*p*-(1→3)-β-D-Glc*p*-(1→3)-[β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)]-[α-L-Rha*p*-(1→3)]-α-D-Gal*p*-(1→]*n*
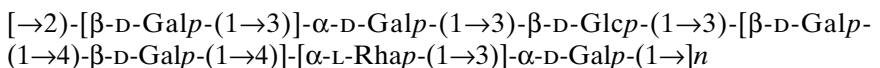
where Gal*p* is galactopyranose, Gal*f* is galactofuranose, Glc*p* is glucopyranose, Rha*p* is rhamnopyranose and NAc is *N*-acetyl-D-galactosamine.

In some strains, EPS production is sometimes unstable (e.g. in *S. thermophilus*) due, perhaps, to the presence of glycohydrolase capable of hydrolysing the EPS material (Zourari *et al*., 1992a).

Other structures of EPS produced by lactic acid bacteria have been reported and some typical examples are *Lactococcus lactis* subsp. *cremoris* (Nakajima *et al*., 1990; Cerning *et al*., 1992; Gruter *et al*., 1992), *Lactobacillus paracasei* subsp. *paracasei* (Robijn *et al*., 1996; Mozzi *et al*., 1994, 1995b, c, 1996, 1997), *Lactobacillus helveticus*
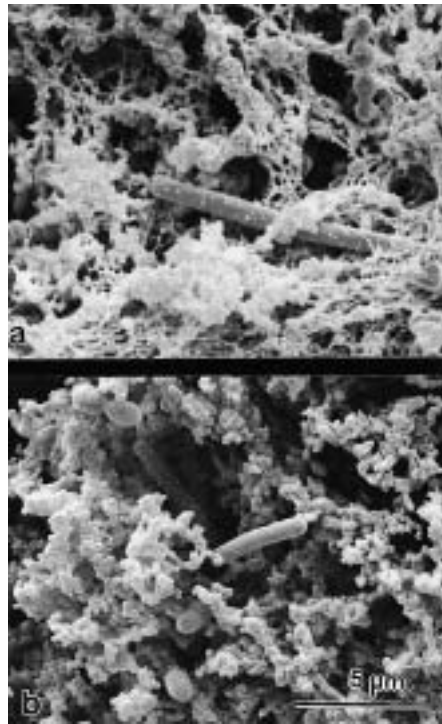
Fig. 7.2 The microstructure (SEM) of stirred yoghurt made with (a) and without (b) EPS starter cultures

After Skriver *et al.* (1995). Reproduced with permission of *Milchwissenschaft*.

(Robijn *et al.*, 1995a), *Lactobacillus sake* (Robijn *et al.*, 1995b), *Bifidobacterium longum* (Roberts *et al.*, 1995; Andaloussi *et al.*, 1995) and *Lactobacillus rhamnosus* (Gamar *et al.*, 1997). However, little is known about the metabolic synthesis of EPS material produced by lactic acid bacteria, including the factors that trigger the mechanisms in the microbial cell. Some hypotheses or possible routes for the synthesis of EPS have been reported by Suzuki (1990), Grobben *et al.* (1996), Stingele *et al.* (1996) and Escalante *et al.* (1998), whilst other researchers have patented EPS producing starter cultures for the manufacture of fermented milks (Vedamuthu, 1982; Gancel *et al.*, 1989; Doco *et al.*, 1989).

As already discussed in Chapter 2, the microstructure of yoghurt consists of a protein matrix composed of casein micelle chains and clusters and the fat globules are embedded in the protein matrix. The production of EPS by the yoghurt starter organisms results in a web of filaments attaching the microbial cell to the protein matrix of the yoghurt (Tamime *et al.*, 1984; Schellaas and Morris, 1985; Bottazzi and Bianchi, 1986; Skriver *et al.*, 1995). However, Skriver *et al.* (1995) reported that the attachment of the filaments to the bacterial cells and the protein could be influenced by the type of yoghurt produced. Figure 7.2 shows such an effect in set-type yoghurt (see also Teggatz and Morris, 1990). The microstructure of stirred yoghurt made at two different laboratories is somewhat different, in that the attachment of these filaments between the microbial cells was not evident, but
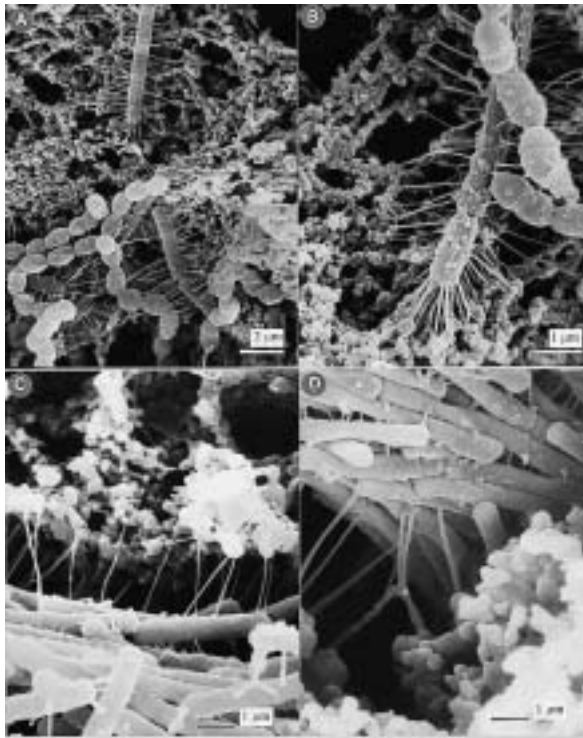
Fig. 7.3 EPS production by *L. delbrueckii* subsp. *bulgaricus* in yoghurt made at different
laboratories

A and B, Tamime and Kalab (Scotland and Canada); C and D, Bottazzi (Italy).

they formed links between the casein micelles of the protein matrix (see Fig. 7.3).
Such minor changes in the microstructure of the yoghurt could be attributed to
mechanical effects that disrupted the attachment of the EPS to the microbial cell
(Skriver *et al*., 1995).

It is evident that some technical data are available on EPS production by the
lactic acid bacteria, but more information is still awaited on the mechanisms that
control the anabolic characteristics. For example, what triggers the starter culture
to polymerise sugars instead of breaking them down for use as an energy source?

7.2.6 Production of flavour compounds

Starter cultures are primarily responsible for the production of the flavour com-
pounds which contribute to the aroma of yoghurt. These compounds may be divided
into four main categories:

- Non-volatile acids (lactic, pyruvic, oxalic or succinic)
- Volatile acids (formic, acetic, propionic or butyric)
- Carbonyl compounds (acetaldehyde, acetone, acetoin or diacetyl)
- Miscellaneous compounds (certain amino acids and/or constituents formed by
  thermal degradation of protein, fat or lactose).

Table 7.2 Production of carbonyl compounds ($\mu g\,g^{-1}$) by yoghurt starter cultures

Organism	Acetaldehyde	Acetone	Acetoin	Diacetyl
S. thermophilus	1.0–13.5	0.2–5.2	1.5–7.0	0.1–13.0
L. delbrueckii subsp. *bulgaricus*	1.4–77.5	0.3–3.2	Trace–2.0	0.5–13.0
Mixed cultures	2.0–41.0	1.3–4.0	2.2–5.7	0.4–0.9

Data compiled from Tamime and Deeth (1980), Abo-Elnaga and Hegazi (1981), Singh *et al*. (1982), Yaygin (1982b), Abou-Donia *et al*. (1984), Ashour *et al*. (1985), Yu and Chung (1986), Hegazi and Abo-Elnaga (1989, 1990), Kneifel *et al*. (1992) and Thomopoulos *et al*. (1993).

There is general agreement in the literature that the aroma and flavour of yoghurt are basically due to the production of non-volatile and volatile acids and carbonyl compounds. For further detail refer to the reviews by Adda (1986), Marshall (1987), Mogensen (1992), Fernandez-Garcia and McGregor (1994), Cogan (1995) and Marshall and Tamime (1997a). Pette and Lolkema (1950) were the first to investigate the flavour of yoghurt and they concluded that the aroma was due to the presence of acetaldehyde and other unidentifiable compounds; however, they also observed that the level of acetaldehyde was much greater in mixed cultures due to the associative growth of the yoghurt organisms, although *L. delbrueckii* subsp. *bulgaricus* played the more important role. This observation has been confirmed by many workers and a summary of these results can be seen in Table 7.2.

Organoleptic assessments of yoghurt by Pette and Lolkema (1950) and Schulz and Hingst (1954) showed that yoghurt was rated best or high by a taste panel when the product contained a low level of acetaldehyde, and they suggested that other carbonyl compounds may be primarily responsible for the typical yoghurt flavour and/or aroma. This view was shared by Bottazzi and Dellaglio (1967) who observed that single strains of *S. thermophilus* produced equal quantities of acetaldehyde and diacetyl, and that a ratio of 1:1 of these compounds typifies the desired aroma of yoghurt. However, in another publication from the same laboratory, Bottazzi and Vescovo (1969) attributed a fullness of yoghurt flavour to a ratio of 2.8:1 of acetaldehyde to acetone, both of which were produced by single cultures of *S. thermophilus*; only a small amount of acetone was produced by *L. delbrueckii* subsp. *bulgaricus*. Incidentally, the same workers did not observe any diacetyl production by these particular test organisms, whereas Dutta *et al*. (1973) obtained $13\,\mu g\,g^{-1}$ of diacetyl (the highest level reported in the literature) from single strains of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (see also Baisya and Bose, 1975; Mutai *et al*., 1972). The production of such high levels of diacetyl and acetoin by single cultures does not appear to correspond with the reported levels of these compounds in yoghurt (see Table 7.2). These discrepancies could be attributed to variations in the strains of streptococci and lactobacilli used, or to differences in the analytical methods employed to detect the level of these carbonyl compounds, and/or to alterations in the level of milk solids, type of milk and degree of heat treatment used during the preparation of the milk base (see Robinson *et al*., 1977; Tamime, 1977a, b; Yaygin, 1982a; Schmidt *et al*., 1983; Ulberth, 1991; Kneifel *et al*., 1992).

Table 7.3 Detectable levels of acetaldehyde in yoghurt produced with different starter cultures

Starter culture	Acetaldehyde	
	($\mu g\,g^{-1}$)	Mean differences
CH-1	37.5	±2.3
Boll-3	27.6	±1.3
RR	10.4	±0.3

A colorimetric test method was used which was non-specific for acetaldehyde as it measured the total content of ketones and aldehyde constituents.

Figures are the mean of 10 samples and the acidity ranged from pH 4.0–4.1 or 1.1–1.2 g $100\,g^{-1}$ lactic acid.

After Tamime (1977a) and Robinson *et al*. (1977).

It could be argued, of course, that the presence of these carbonyl compounds is not essential, for instance, in fruit and flavoured yoghurts, but a high level of acetaldehyde is desirable for the typical aroma of natural or plain yoghurt and Suzuki *et al.* (1979) concluded that yoghurt, which contained only $7\,\mu g\,g^{-1}$ acetaldehyde, did not have sufficient of the desirable yoghurt flavour. Furthermore, the same workers detected high levels of diacetyl in fermented milks only in the presence of *Lactococcus lactis* biovar *diacetylactis*, a view supported by many authors (see Table 7.2 for level of diacetyl production by mixed yoghurt cultures and Chapter 5 for the production of dahi).

Robinson *et al*. (1977) and Tamime (1977a) assessed, both organoleptically and for the presence of carbonyl compounds, samples of natural yoghurt made using different strains of starter culture (CH-1 (normal), Boll-3 (viscous) and RR (EPS producer) – the former two cultures were obtained from Chr. Hansen's Lab. A/S, Denmark and culture (RR) from NIZO, The Netherlands). The judging panel consisted of Mediterranean and non-Mediterranean nationalities. The preference trend was for yoghurt made by culture (CH-1) (i.e. sharp and acidic), followed by (Boll-3), and the least preferred, especially by the Mediterranean nationalities, was the yoghurt made by starter (RR). The level of acetaldehyde in these yoghurts is illustrated in Table 7.3, where it can be observed that starter culture (CH-1) produced the highest level of acetaldehyde, followed by (Boll-3) and finally (RR). Hence, these results tend to confirm that the typical aroma and flavour of natural or plain yoghurt is directly associated with the presence of carbonyl compounds, mainly acetaldehyde, in the product.

Aroma profiles and sensory studies have been correlated with a view to classifying yoghurt made with different starter cultures in Bulgaria by Gyosheva (1985) and Beshkova *et al*. (1998) and in Austria by Ulberth and Kneifel (1992). The aroma compounds that were identified in typical Bulgarian yoghurts were acetaldehyde, acetone, ethyl acetate, butanone, diacetyl and ethanol (Kondratenko and Gyosheva, 1985; Gyosheva, 1986). However, in Switzerland, 91 components were identified in yoghurt, of which 21 had a major impact on the flavour (Ott *et al*., 1997; see also Reps *et al*., 1987; Laye *et al*., 1993; Imhof *et al*., 1994, 1995).

During the manufacture of yoghurt, the production of acetaldehyde becomes evident only at a certain level of acidification (i.e. pH 5.0), reaches a maximum at pH 4.2 and stabilises at pH 4.0. Fortification of the milk base with milk solids, and certain heat treatments of the yoghurt milk, can significantly increase the acetaldehyde content of the yoghurt (Gorner et al., 1968). In acidified milk products, the partition coefficients (i.e. between air and aqueous phases) of carbonyl compounds (acetaldehyde and diacetyl) and ethanol were higher at 50°C than at 30°C, and increased as the solids-not-fat (SNF) ($12\,g\,100\,g^{-1}$) and fat ($20\,g\,100\,g^{-1}$) concentrations increased in the milk base (Lee et al., 1995); the pattern of partition coefficients was acetaldehyde > diacetyl > ethanol. The production of diacetyl and acetoin in fresh milk (cow's or buffalo's) was more than in reconstituted dried whole milk (Ismail et al., 1980). However, comparative studies of flavour development are limited; for example, more volatile acids were found in goat's milk than in cow's milk, whilst more acetaldehyde was produced by the yoghurt starter cultures in cow's milk than in goat's milk (Manjunath et al., 1983; Rysstad and Abrahamsen, 1987). Yaygin (1982a) and Yaygin and Mehanna (1988) reported the contents ($\mu g\,g^{-1}$) of aroma compounds (i.e. range) in yoghurt made from different mammalian milks as follows:

Milk	Acetaldehyde	Acetone	Ethanol
Cow	4–26	3–25	19–365
Sheep	7–30	5–30	10–255
Goat	5–19	3–40	25–355
Buffalo	6–28	5–30	5–195

Note: traces of diacetyl were detected in some samples.

Acetaldehyde production by pure cultures of L. acidophilus and S. thermophilus was maximum at 42°C and 37°C, respectively, and in heated milk at 85°C for 15 min and 65°C for 30 min, respectively, whilst mixed cultures showed more activity in milk steamed for 30 min (Singh, 1983; see also Singh et al., 1982).

Losses of acetaldehyde from yoghurt, after storage for 24 hours, are dependent on the type of milk used for processing, that is, yoghurt made from full fat or whole milk showed little change in acetaldehyde content, while in skimmed milk yoghurt the level decreased (Yu and Nakanishi, 1975a, b). Furthermore, the production of acetaldehyde in yoghurt made from milk of different species can vary. Thus, Gorner et al. (1971) observed that acetaldehyde levels, after 3 hour incubation, were highest in yoghurt made from cow's milk, followed by goat's milk and finally sheep's milk; the GLC peak heights of acetaldehyde in these yoghurts were 400, 23 and 2 mm, respectively. The same observation was reported by Abrahamsen et al. (1978), where $17.1\,\mu g\,g^{-1}$ of acetaldehyde were present in yoghurt processed from cow's milk, compared to 4.7–$5.5\,\mu g\,g^{-1}$ in goat's milk after 3 hours incubation. The behaviour of the yoghurt starter cultures in these different types of milk is not well established, but one of the reasons for the observed changes in metabolism may be that both ewe's and goat's milk contain a substance which blocks the formation of a precursor required by the starter organism for the production of acetaldehyde (see later).

The fate of carbonyl and aroma compounds in yoghurt during storage could be summarised as follows: (a) the levels of acetaldehyde, ethyl acetate and diacetyl in

sheep's milk yoghurt decreased, but the acetone and ethanol contents found in the initial milk showed no change during the fermentation period or storage of the product (Stefanova and Gyosheva, 1985; Georgala *et al*., 1995), (b) acetaldehyde content ($\mu g\,g^{-1}$) decreased in yoghurts made from milk (14.8 to 13.1), milk fortified with SMP (22.8 to 16.5) and UF milk (25.0 to 20.6) (Estevez *et al*., 1988), and (c) the concentration of acetaldehyde decreased in yoghurts stored for 10 days at 4°C or 10°C, whilst the diacetyl and ethanol contents increased (Hruskar *et al*., 1995). However, Kang *et al*. (1988) measured flavour compounds in yoghurt during storage using a dynamic gas-purging headspace technique with a Tenax-GC precolumn or ether extract on a Porapak-Q column, and both methods showed increased acetaldehyde in the product; an observation which was not reported by any other researchers.

Other compounds which could be associated, perhaps indirectly, with flavour enhancement, or act as precursors for the formation of the major aroma compounds in yoghurt, are:

- volatile fatty acids e.g. acetic, propionic, butyric, isovaleric, caproic, caprylic and capric acids (Turcic *et al*., 1969; Dumont and Adda, 1973)
- amino acids e.g. serine, glutamic acid, proline, valine, leucine, isoleucine and tyrosine (Groux, 1976; Grozeva *et al*., 1994)
- products of thermal degradation of milk constituents (i.e. 80–90°C for 15–30 min; Viani and Horman, 1976), for example: (a) from fat (keto acids (acetone, butanone, hexanone), hydroxy acids (v-valerolactone, δ-caprolactone, δ-caprilactone), and miscellaneous (2-heptanone, 2-nonanone, 2-undecanone, pentane)), (b) from lactose (furfural, furfuryl alcohol, 5-methylfurfural, 2-pentylfuran), (c) from fat and/or lactose (benzyl alcohol, benzyladehyde, methylbenzoate), and (d) from protein (methionine (dimethylsulphide), valine (isobutyraldehyde), or phenylalanine (phenylacetaldehyde) (Haesoo *et al*., 1996))
- *n*-pentaldehyde and 2-heptanone produced by *L. delbrueckii* subsp. *bulgaricus* (Yu and Nakanishi, 1975a, b; Groux and Moinas, 1974).

As mentioned earlier, the formation of acetaldehyde and other aromatic compounds by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in yoghurt takes place during the fermentation, and the final levels are dependent on the presence of specific enzymes which are able to catalyse the formation of carbonyl compounds from the different milk constituents. Lees and Jago (1978a, b) reviewed in detail the role of lactic acid bacteria in terms of flavour production in cultured dairy products, but more is now known of the metabolic mechanisms which lead to the production of flavour and aroma compounds (Zourari *et al*., 1992a; Marshall and Tamime, 1997a). Thus, the possible metabolic pathways of acetaldehyde synthesis are described in the following.

7.2.6.1 *Embden–Meyerhof–Parnas pathway*
This generates pyruvate (see Fig. 7.1) which in turn is catalysed by α-carboxylase with the formation of acetaldehyde (see also Seneca *et al*., 1950; Lees and Jago, 1966; Keenan and Bills, 1968). Alternatively, the action of pyruvate dehydrogenase on pyruvate results in the formation of acetyl-CoA which can be catalysed/reduced by an aldehyde dehydrogenase to generate acetaldehyde (see also Lees and Jago, 1966,

1976a, b, 1977). Lees and Jago (1978a, b) reported aldehyde dehydrogenase activity in four strains each of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, and only two strains of streptococci had alcohol dehydrogenase activity, whilst deoxyriboaldolase was found in one strain of *S. thermophilus*. Nevertheless, Raya *et al*. (1986a) tested two strains of each species of the yoghurt organisms and detected no activities of aldehyde dehydrogenase, phosphoketolase or alcohol dehydrogenase, and only traces of pyruvate decarboxylase activity. Similar observations were reported by Manca de Nadra *et al*. (1988) from the same research laboratory and no deoxyriboaldolase or α-carboxylase activities were detected. Therefore, from these results it is difficult to suggest that acetaldehyde is formed from pyruvate, as the metabolic pathway occurs only rarely in *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. However acetate kinase and phosphotranacetylase were found in some strains (Raya *et al*., 1986a), whilst aldehyde hydrogenase was found only in *S. thermophilus* (Manca de Nadra *et al*., 1987, 1988). From these observations it would be difficult to suggest that acetaldehyde could be formed via the hexose monophosphate shunt.

Incidentally, *L. acidophilus* possesses alcohol dehydrogenase activity capable of reducing acetaldehyde so that only a slight yoghurt flavour is found in milk fermented with this culture (Marshall and Cole, 1983). However, diacetyl is produced by *Lactobacillus paracasei* biovar *shirota* from citrate (Marshall, 1987), possibly via the same route of citrate metabolism in *Lactococcus* and *Leuconostoc* species; this *Lactobacillus* is widely used for making yakult (a Japanese fermented milk product).

Benito de Cardenas *et al*. (1991) reported that *L. acidophilus* utilises pyruvate as a carbon source in glucose medium and produces diacetyl; however, diacetyl production is higher in pyruvate medium at 45°C. Greater amounts of acetoin than diacetyl are produced at all temperatures especially 37 and 45°C.

7.2.6.2 Threonine aldolase
This catalyses the cleavage of threonine to acetaldhyde and glycine and both *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* possess this enzyme. However, threonine aldolase is more active in the lactobacilli than in the streptococci (Lees and Jago, 1976a, b, 1977). According to Lees and Jago (1978a) the interconversion of threonine to acetaldehyde and glycine is as follows:

$$^+H_3N - CH - COO^- \xrightarrow[\text{aldolase}]{\text{Threonine}} CH_3 - CH + {}^+H_3N - CH - COO^-$$

Threonine   Acetaldehyde   Glycine
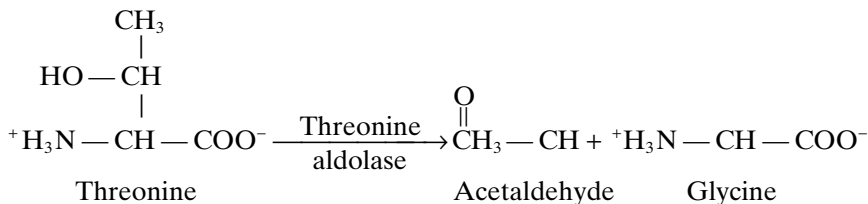
Threonine aldolase activity was detected in two strains of *L. delbrueckii* subsp. *bulgaricus*, but not in the two strains of *S. thermophilus* tested (Raya *et al*., 1986a, b). Other researchers beside Lees and Jago have reported threonine activity in both the yoghurt organisms (Sandine and Elliker, 1970; Wilkins *et al*., 1986a, b; Marranzini *et al*., 1989). Streptococcal threonine aldalase activity decreases as the growth temperature increases from 30–42°C, but remains the same in the lactobacilli; since yoghurt is made at ~40–45°C, it is most likely that the acetaldehyde is produced by *L. delbrueckii* subsp. *bulgaricus* (see Zourari *et al*., 1992a). However, threonine aldolase activity is influenced by glycine level, salts and some divalent cations such
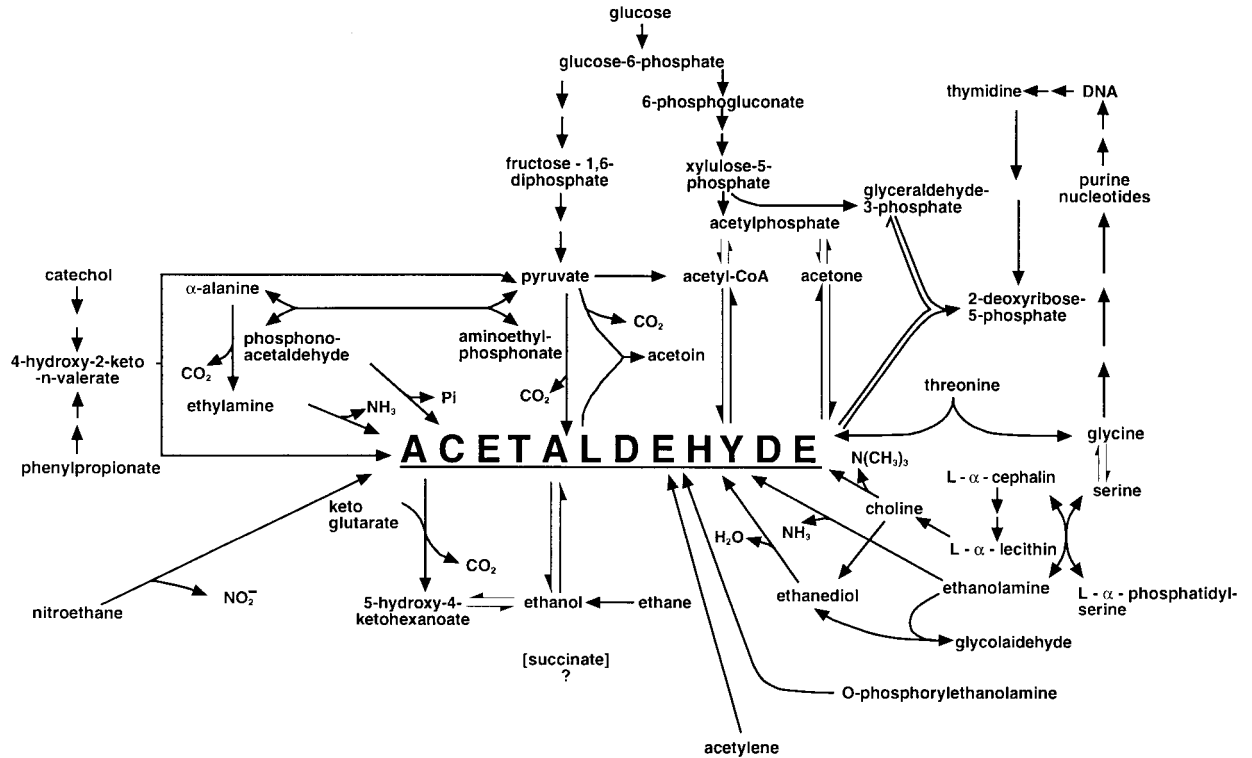
Fig. 7.4 Diagrammatic representation of known reactions involving acetaldehyde

After Lees and Jago (1978a).

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as Cu^{2+}, Zu^{2+}, Fe^{2+} and Co^{2+} (Schmidt *et al.*, 1983, 1989; Raya *et al.*, 1986a, b; Wilkins *et al.*, 1986a; Manca de Nadra *et al.*, 1987; Marranzini *et al.*, 1989).

Another amino acid, methionine, can also increase the level of acetaldehyde in a growth medium inoculated only with *S. thermophilus* (Shankar, 1977). He observed that by fortifying the growth medium with 100–400μg ml^{-1} methionine, the level of acetaldehyde after 20 hour of incubation had increased from 1μg g^{-1} in the control to 10 and 14μg g^{-1}, respectively, in the test media (see also Truffa-Bachi and Cohen, 1968; Rodwell, 1975). Another possible route for the production of acetaldehyde is the cleavage of threonine to glycine, reported by Sandine and Elliker (1970). Flavour production in mutant strains of lactobacilli has been reported by Bednarski and Hammond (1990), whilst glutathione and thiol group production in strains of *S. thermophilus* and *L. helveticus* have been studied by Fernandez and Steele (1993).

7.2.6.3   DNA components

Lees and Jago (1977, 1978a) detected deoxyriboaldolase activity in one of four strains of *S. thermophilus* tested, but this enzyme was not active in *L. delbrueckii* subsp. *bulgaricus* (see also Raya *et al.*, 1986a, b). This enzyme, along with thymidine phosphorylase and deoxyribomutase, degrades DNA to 2-deoxyribose-5-phosphate, which is further broken down to acetaldehyde and glyceraldehyde.

It can be observed, therefore, that the production of acetaldehyde by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* may involve a number of different metabolic pathways, and Fig. 7.4 illustrates the possible routes by which acetaldehyde may be formed from carbohydrates, proteins and/or nucleic acids.

7.3   Protein metabolism

Proteolysis in cheesemaking is an important factor in the selection of bacterial strains for starter cultures; however, proteolytic activity of strains used in the manufacture of fermented milks may be of a secondary importance. Nevertheless, although the yoghurt and bio starter cultures are considered to be only weakly proteolytic, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* may, during the fermentation, cause a significant degree of proteolysis and this activity may be important for the following reasons:

- The enzymatic hydrolysis of milk proteins results in the liberation of peptides of varying sizes and free amino acids, and these possible changes may be involved during the formation of the gel and can affect the physical structure of yoghurt.
- As discussed elsewhere (refer to Chapter 6), the liberation of amino acids into the milk is essential to the growth of *S. thermophilus*.
- Although amino acids and peptides may not contribute directly towards the flavour of yoghurt, they do act as precursors for the multitude of reactions which produce flavour compounds (see Groux, 1976; Viani and Horman, 1976).
- Important nutritional considerations apply through the release of so-called functional peptides (Tomé, 1998).

The range of products released by proteolysis is dependent on two main factors, first, the components of the milk protein fraction and second, the types of proteolytic enzyme that the yoghurt and bio organisms may possess.

7.3.1 Constituent compounds of the milk protein molecule

The protein fraction in milk is composed of casein and whey proteins and although the protein molecule is highly complex, it is important in the present context to describe briefly the structure of the protein molecule and show where hydrolysis may occur.

The basic constituents of a protein molecule are compounds known as amino acids. There are about 21 different types of amino acid which have been identified in milk proteins. Their basic structure is shown here

$$
\begin{array}{cc}
\overset{\displaystyle NH_2}{\underset{\displaystyle H}{R-C-COOH}} & \overset{\displaystyle NH_3^{(+)}}{\underset{\displaystyle H}{R-C-COO^{(-)}}} \\
& \text{"Zwitterion"}
\end{array}
$$

Each amino acid may consist of one or more amino group (NH_3^+) and one or more carboxyl group (COO^-). All the amino acids show asymmetry about the α-carbon atom – where the amino group is next to the carboxyl group – with the exception of glycine where $R = H$. The nomenclature of the amino acids is similar to that of the carbohydrates, that is, D and L indicate their configuration about the α-carbon atom. Some amino acids are cyclic (e.g. proline which is referred to as an imino acid) but their structure is similar to α-amino acids.

These amino acids are the basic units of the protein molecule and polypeptide chains are built up of sequences of amino acid residues (see Walstra and Jenness, 1984); the structure of the chain is shown here

$$
\left[\begin{array}{ccc}
N & CH & C \\
| & | & || \\
H & R & 0
\end{array} \right]_n
$$
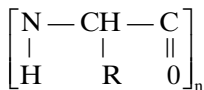
The buildup of a polypeptide chain results in a loss of water from the amino acids and the bonds between the adjacent units are known as peptide bonds (e.g. —NH.CO—). These polypeptide chains then link together due to the presence of various forces (e.g. hydrogen bonds, covalent and noncovalent bonds) and this aggregation leads to the formation of the protein molecule.

7.3.2 Proteolytic enzymes

These enzymes, as the name suggests, are specific in their action, and their main function is to catalyse the hydrolytic cleavage of the peptide bonds which form the backbone of the protein molecule. The action of the proteolytic enzymes on the peptide bond may be represented as follows:

$$
\cdots \overset{\displaystyle R}{\underset{\displaystyle HO}{-HN.CH.CO}} \overset{:}{\underset{:}{-}} \overset{:}{\underset{:}{HN.CH.CO.}} \cdots \cdots \overset{\displaystyle R}{-HN.CH.COOH} + H_2N.\overset{\displaystyle R^1}{CH.CO} \cdots
$$
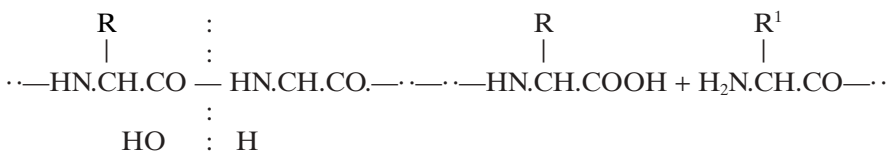
Table 7.4 Enzyme nomenclature of peptidases

Enzyme	Classification and general characteristics
Exopeptidases EC 3.4.11–19 These enzymes act only near the ends of polypeptide chains	Aminopeptidases EC 3.4.11.1–18 *EC 3.4.11.8*[a] now 3.4.19.3 and *EC 3.4.11.11*[a] deleted entry; these enzymes act at a free N-terminus liberating a single amino acid residue Peptidase EC 3.4.13.1–20 Six have been transferred to other EC numbers and two entries[a] deleted; these enzymes catalyse specifically dipeptides. Dipeptidyl-peptidases and tripeptidyl-peptidases EC 3.4.14.1–10 *EC 3.4.14.3*[a] now 3.4.19.1, *EC 3.4.14.7*[a] deleted entry and *EC 3.3.14.8*[a] now 3.4.14.9 & 10; these enzymes act at a free N- terminus liberating a di- or tripeptide. Peptidyl-dipeptidases EC 3.4.15.1–4 *EC 3.4.15.2*[a] now 3.4.19.2 and *EC 3.4.15.3*[a] now 3.4.15.1; these enzymes act at a free C-terminus liberating a dipeptide. Serine-type carboxypeptidases EC 3.4.16.1–4 *EC 3.4.16.3*[a] now 3.4.16.1; these enzymes act at a free C-terminus liberating a single residue. Metallocarboxypeptidases EC 3.4.17.1–17 *EC 3.4.17.5*[a] deleted entry; *EC 3.4.17.7*[a] now 3.4.19.10 and *EC 3.4.17.9*[a] now 3.4.17.4; these enzymes require divalent cations for activity. Cysteine-type carboxypeptidases EC 3.4.18.1 These enzymes act at a free C-terminus liberating a single residue, and require thiol dependence for activity. Omega peptidases EC 3.4.19.1–10 *EC 3.4.19.4*[a] deleted entry; these enzymes remove terminal residues that are substituted, cyclised or linked by isopeptide bonds, i.e. other than those of α-carboxyl or α-amino groups.
Endopeptidases EC 3.4.21–24 & 3.4.99 These enzymes are classified on the basis of catalytic mechanism and specificity	Serine endopeptidases EC 3.4.21.1–74 Ten have been transferred to other EC[a] numbers and eleven deleted entries[a]; these enzymes have an active centre serine of involved in the catalytic process. Cysteine endopeptidases EC 3.4.22.1–35 Ten have been transferred to other EC numbers and two deleted entries[a]; these enzymes have a cystein in the centre. Aspartic endopeptidase EC 3.4.23.1–34 Five have been transferred to other EC numbers and three deleted entries[a]; these enzymes depend on an aspartic acid residue for their catalytic activity. Metalloendopeptidases EC 3.4.24.1–54 Two have been transferred to other EC numbers and four deleted entries[a]; these enzymes use a metal ion (e.g. Zn^{2+}) in the catalytic mechanism. Endopeptidases of unknown catalytic mechanism EC 3.4.99.35–46 Major changes occurred in this section (see Anon., 1992).

[a] Indicate changes that occurred since the last publication of *Enzyme Nomenclature*.

Data compiled from Anon. (1992).

Enzymes acting on peptide bonds are known as peptide hydrolases and to date (1998), a large number of such enzymes have been identified. In the past the name given to an enzyme was derived from the substrate involved, but this approach has created such confusion in the field of enzymology, that the Nomenclature Committee of the International Union of Biochemistry amd Molecular Biology was established to consider a classification of universal application to enzymes and coenzymes. The latest communication of this committee was published (Anon., 1992) and the scheme for classifying and numbering the enzymes is as follows:

• The first number after EC (enzyme classification) indicates to which of the six main classes the enzyme belongs.
• The second figure indicates the subclass.
• The third figure gives the sub-subclass.
• The fourth figure is the serial number of the enzyme in its sub-subclass.

It is not acceptable (Anon., 1992) for the term peptidases to be used as synonymous with peptide hydrolases for the entire group of enzymes that hydrolyse peptide bonds. This is a change from the restriction of peptidases to the enzymes included in the sub-subclasses the exopeptidases and the term proteinase has been replaced by endopeptidases; for consistency, the sub-subclasses of peptidases are recognised as:

• Exopeptidases (EC 3.4.11–19)
• Endopeptidases (EC 3.4.21–24 and EC 3.4.99)

and their overall classification/characteristics is summarised in Table 7.4.

It is probable that this system will be widely adopted in due course, and hence in the present text, the terms endopeptidases and exopeptideses are used in accordance with the new scheme. The hydrolysis of protein to yield amino acids can, therefore, be accomplished in two major stages:

$$\text{Protein} \xrightarrow[\text{Endopeptidases}]{\text{1st Stage}} \text{Polypeptides} \xrightarrow[\text{Exopeptidases}]{\text{2nd Stage}} \text{Amino acids}$$
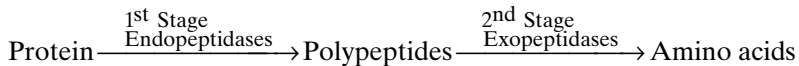
7.3.3 Proteolysis by the yoghurt and bio organisms

The data compiled by Tamime and Deeth (1980) on the proteolytic activity of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* indicate that both organisms possess different exopeptidases and peptidases. Since 1980 the proteolytic systems of lactic acid bacteria have been studied in detail using genetic, biochemical and ultrastructural methods. Reviews by Thomas and Pritchard (1987), Kok (1990), Zourari *et al*. (1992a), Pritchard and Coolbear (1993), Vescovo *et al*. (1995), Klaenhammer (1995), Kunji *et al*. (1996) and Law and Haandrikman (1997) describe the properties, regulations and cellular localisation of such enzymes of lactic acid bacteria. However, Bianchi-Salvadori *et al*. (1995) have profiled a wide range of enzymatic activities of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* which were isolated from commercial yoghurts and Italian cheeses; the former organism is considered to have more exopeptidase activity than *L. delbrueckii* subsp. *bulgaricus*, and only limited endopeptidase activity. The ability of *L. delbrueckii* subsp. *bulgaricus* to hydrolyse casein confirms that endopeptidase activity is much higher in

Table 7.5 Proteolysis of individual caseins by different starter cultures

Organism	Sequence of hydrolysis	References
S. thermophilus	β- > α-casein	Shidlovskaya and Dyachenko (1968)
	β- and κ- but not α_{s1}-casein	Desmazeaud and Juge (1976)
	κ- > α_s- and β-casein	Singh and Sharma (1983)
	κ- and α_s- > β-casein	Hegazi (1987)
L. delbrueckii subsp. *bulgaricus*	α_s- > κ- but not β-casein	Ohmiya and Sato (1968, 1969, 1978)
	β- > α_s-casein	Dyachenko and Shidlovskaya (1971)
	κ- > α_s- and β-casein	Chebbi *et al.* (1974)
	κ- and α_s- but not β-casein	Chebbi *et al.* (1977)
	β- > α_s- and κ-casein (whole)	
	α_s- > β- and κ-casein (purified)	Singh and Ranganthan (1977a, b, 1979)
	α_s- and κ- > β-casein	
	β- > α_s- > whole > κ-casein	Shankar and Davies (1978)
	β- > α_s- > κ-casein	Argyle *et al.* (1976), Chandan *et al.* (1982)
	κ- > α_s- and β-casein	Singh and Sharma (1983)
	κ- and α_s- > β-casein	Hegazi (1987)
	β- > α_s- and κ-casein	Moon and Kim (1986, 1990a, b)
	α_s- > κ- and β-casein	Moon *et al.* (1989a, b)
	α_s- and β-casein	Laloi *et al.* (1991), Abraham *et al.* (1993), Gilbert *et al.* (1997)
L. acidophilus	κ- > α_s- and β-casein	Singh and Sharma (1983)
	α_s- and β- but not κ-casein	Hebert *et al.* (1998)
L. helveticus	κ- and α_s- > β-casein	Hegazi (1987)
	α_s- and β- but not κ-casein	Zevaco and Gripon (1988), Yamamot *et al.* (1993, 1994), Martin-Herandez *et al.* (1994)
L. paracasei subsp. *paracasei*	β-casein	Kojic *et al.* (1991), Naes and Nissen-Meyer (1992)

the lactobacilli. This pattern of peptide hydrolysis in the yoghurt organisms provides further evidence of the associative growth relationship which exists between *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. Thus, the endopeptidase activity of *L. delbrueckii* subsp. *bulgaricus* hydrolyses the casein to yield polypeptides, which in turn are broken down by the exopeptidases of *S. thermophilus* with the liberation of amino acids.

The endopeptidases from *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus* and bio cultures that are capable of hydrolysing the casein fractions are shown in Table 7.5 (see also Poznanski *et al*., 1965). The pattern of casein catabolism by the yoghurt organisms, which is predominantly attributed to endopeptidase activity, may vary. With the limited data available on casein hydrolysis it is difficult to generalise, but the increased proteolytic activity of *L. delbrueckii* subsp. *bulgaricus* mutant strains (see Table 7.5) developed after exposure to γ-ray radiation, X-ray radiation, UV radiation or chemical mutagens, suggests that differences between ordinary strains may have resulted in the observed variations in casein hydrolysis (see also Dilanian *et al*., 1970, 1971; Krsev, 1976; Singh and Ranganathan, 1974a, b, 1978; Singh *et al*., 1978; Singh and Kaul, 1982a, b). Although mutant strains of *L. delbrueckii* subsp. *bulgaricus* with increased proteolytic activity were not specifically selected for the yoghurt industry, such activity is desired during the early maturation stages of some varieties of Swiss type cheese.

Laloi *et al*. (1991) observed that the endopeptidase present in the cell wall extract of *L. delbrueckii* subsp. *bulgaricus* was active on caseins (see Table 7.5), displayed the same hydrolytic patterns as whole cells, was strongly activated by dithiothreitol and partially inhibited by E-64 (i.e. a specific inhibitor of cysteine endopeptidase); the purified enzyme was not able to hydrolyse di- or tripeptides. However, Oberg *et al*. (1991) used amino acids analysis and the *o*-phthaldialdehyde test to characterise the proteolytic activity of 35 strains of *L. delbrueckii* subsp. *bulgaricus*, and the amino acid profiles provided a cluster analysis to differentiate the strains which was not available from the results of the other test. Furthermore, the caseinolytic activity of endopeptidase from *L. delbrueckii* subsp. *bulgaricus* had the following characteristics: the enzyme was zinc dependent, it degraded intact caseins with a significant preference for β-casein, and the caseinolytic activity increased as the pH was lowered (<5.0) which suggests that the enzyme could be involved in the later stages of the fermentation period (Stefanitsi and Garel, 1997) (see also Stefanitsi *et al*., 1995).

Metalloendopeptidase activity in *S. thermophilus* has been reported by many authors (Sato and Nakashima, 1965; Desmazeaud and Hermier, 1968; Rabier and Desmazeaud, 1973; Desmazeaud, 1974, 1978; Desmazeaud and Zevaco, 1976; El-Soda *et al*., 1978a, b; Shankar and Davies, 1978). More recently, Shahbal *et al*. (1991) reported that the endopeptidase activity of two dairy strains of *S. thermophilus*, CNRZ 385 and 703, was cell wall-associated and not released in the absence of $CaCl_2$, as is the case with *Lactococcus lactis* subsp. *lactis*. Also the high acidification rate of the two strains was correlated with the presence of a 10- and sevenfold increase in endopeptidase activity, respectively, compared with other *S. thermophilus* strains; however, the endopeptidase-negative mutants did not produce higher than average levels of acid.

The cell wall-associated endopeptidase in *L. paracasei* subsp. *paracasei*, *L. helveticus* and *L. delbrueckii* subsp. *bulgaricus* has been biochemically characterised and reported by Ezzat *et al.* (1985, 1987), El-Soda *et al*. (1986b, c), Laloi *et al*. (1991)

and Martin-Hernández *et al.* (1994). The proteolytic activity of some of these bacterial species is chromosome linked (El-Soda *et al.*, 1989), and the gene encoding the cell surface endopeptidase from *L. delbrueckii* subsp. *bulgaricus* has been recently sequenced by Gilbert *et al.* (1996); no plasmids have been detected in most of the strains. Furthermore, a comparison of DNA sequences for the cell surface endopeptidases of *L. delbrueckii* subsp. *bulgaricus* and lactococci showed little genetic homology (Gilbert *et al.*, 1996). The endopeptidase, which was purified from *L. delbrueckii* subsp. *bulgaricus*, was a monomer of ~70 kDa, and it was inhibited by EDTA and serine enzymes (Bockelmann *et al.*, 1996). Heating cells of yoghurt lactobacilli at 67–68°C for 15.5–16 s reduced endopeptidase activity, but retained aminopeptidase activity (Lopez-Fandino and Ardö, 1991). Endopeptidases from *L. paracasei* subsp. *paracasei* and *S. thermophilus* showed greatest activity in phosphate buffer followed by tris-HCl, but very low activity in phthalate buffer, whilst similar enzymes from *L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus* had greatest activity in tris-HCl and lowest in citrate buffer (Akuzawa *et al.*, 1983, 1984). However, when the cell surface caseinolytic activities of *L. paracasei* subsp. *paracasei*, *Lactobacillus delbrueckii* subsp. *lactis* and *L. helveticus* were compared, the characteristics of these endopeptidases of the former organisms were similar; *L. helveticus* displayed two endopeptidases with different cleavage specificities (Gilbert *et al.*, 1997).

The proteolytic system of *L. paracasei* subsp. *paracasei* strains has been investigated (Kojic *et al.*, 1991; Holck and Naes, 1991; Naes and Nissen-Meyer, 1992), and similar PrtP and PrtM genes were identified on the chromosomes; when sequenced, the PrtP gene appeared similar to lactococcal PrtP.

Following the hydrolysis of, for example, the casein in milk, the derived peptides need to be hydrolysed further by the exopeptidases that are present in the yoghurt and bio organisms. Until the 1970s, many authors made reference to exopeptidase activity of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (see the review by Tamime and Deeth, 1980). Currently, the general characteristics of exopeptidases of lactic acid bacteria are given below.

7.3.3.1   Aminopeptidase N (PepN)

In all the organisms studied (see Table 7.6), this enzyme has a molecular weight of ~95 kDa, is a monomeric metallopeptidase, and in most, if not all, it is located intracellularly. The PepN, which was purified from *L. delbrueckii* subsp. *bulgaricus* (Bockelmann *et al.*, 1992), was inhibited completely by 0.1 mM EDTA, and its activity increased by 1 mM Mn^{2+} and 0.1 mM Hg^{2+}; suitable substrates for the assay of enzyme activity were L-Lys-Na and L-Ala-L-Arg-NA. A similar enzyme from *L. helveticus* had a primary sequence PepN identical to the enzymes of *L. delbrueckii* subsp. *lactis* and *Lac. lactis* subsp. *cremoris* (Christensen *et al.*, 1995; Kunji *et al.*, 1996). However, PepN is capable of cleaving N-terminal amino acids, but the enzyme from *L. paracasei* subsp. *paracasei* is only capable of hydrolysing tripeptides containing proline in either the first or second position (Arora and Lee, 1990, 1992; see also Arora *et al.*, 1990).

Characterisation of aminopeptidases N of *L. acidophilus* and other lactobacilli has been reported by El-Soda and Desmazeaud (1982), Ezzat *et al.* (1982, 1986), Hickey *et al.* (1983a, b), Atlan *et al.* (1989), Machuga and Ives (1984) and Khalid *et al.* (1991). Whilst the PepN from *S. thermophilus* was inhibited by $CuCl_2$, $ZnCl_2$ and EDTA, the enzyme showed activity towards *p*-nitroanilide derivatives or di- and

Table 7.6 Some characteristics of exopeptides of selected starter cultures

Enzyme	Micro-organism	Type of Enzyme[a]	Mw[b] (kDa)	Optimum pH activity	References
Aminopeptidase N (PepN)	*L. delbrueckii* subsp. *bulgaricus* B 14	M	95	7.0	Bockelmann *et al.* (1992)
	L. paracasei subsp. *paracasei* LGG	M	87	7.0	Arora and Lee (1992)
	L. helveticus CNRZ 32	NR	97	6.5	Khalid and Marth (1990a, b), Varmanen *et al.* (1994), Christensen *et al.* (1995)
	LHE 511	M	92	7.0	Miyakawa *et al.* (1992)
	ITGL 1	M	97	6.5	Blanc *et al.* (1993)
	S. thermophilus ACA-DC 114	NR	89	6.5	Tsakalidou and Kalantzopoulos (1992)
	CNRZ 302	M	97	7.0	Rul *et al.* (1994), Rul and Monnet (1997)
	NCDO 537	NR	96	NR	Midwinter and Pritchard (1994)
Aminopeptidase C (PepC)	*L. delbrueckii* subsp. *bulgaricus* B 14	T	54	7.0	Wohlrab and Bockelmann (1993)
	L. helveticus CNRZ 32	T	50	NR	Fernández *et al.* (1994), Vesanto *et al.* (1994)
Aminopeptidase X[d] (PepX)	*L. delbrueckii* subsp. *bulgaricus* B 14	S	95	6.5	Bockelmann *et al.* (1991)
	CNRZ 397	S	82	7.0	Atlan *et al.* (1990)
	LBU 47	S	90	6.5	Miyakawa *et al.* (1991)
	L. paracasei subsp. *paracasei* LLG	S	79	8.0	Habibi-Najafi and Lee (1994a)
	L. helveticus CNRZ 32	S	95[c]	7.0	Khalid and Marth (1990b), Kunji *et al.* (1996)
	L. acidophilus	S	95	6.5	Bockelmann *et al.* (1991)
	S. thermophilus	S	165	>6.5	Meyer and Jordi (1987)
Prolinase (PepR)	*L. helveticus* CNRZ 32	NR	35	7.5	Kunji *et al.* (1996)
Proline iminopeptidase	*L. delbrueckii* subsp. *bulgaricus* CNRZ 397	S	3	6.5	Atlan *et al.* (1994), Gilbert *et al.* (1994)
Dipeptidase (PepV) (PepD)	*L. delbrueckii* subsp. *bulgaricus* B 14	M	51	7.5	Wohlrab and Bockelmann (1992)
	L. helveticus SBT 217 & CNRZ 32	M	50	NR	Tan *et al.* (1995), Kunji *et al.* (1996)
	53/7 & CNRZ 32	T	54	6.0	Kunji *et al.* (1996)

[a] M, metallopeptidase; T, thiolpeptidase; S, serine-protease. [b] Molecular weight. [c] Refer to text.
NR Not reported.

tripeptides (Rul *et al.*, 1994; Rul and Monnet, 1997); the gene sequence for the enzyme showed high homology with the sequence for PepN isolated from *Lac. lactis* subsp. *cremoris*. A similar enzyme was studied by Tsakalidou and Kalantzopoulos (1992) which was capable of degrading substrates by hydrolysis of N-terminal amino acids and it had very low endopeptidase and no carboxypeptidase activity (see also Kalantzopoulos *et al.*, 1990a, b; Tsakalidou *et al.*, 1992, 1993).

7.3.3.2   Aminopeptidase C (PepC)
This enzyme is similar to PepN and is capable of removing a broad range of N-terminal residues of peptides; it is a thiol peptidase ~50kDa. According to Law and Haandrikman (1997), the amino sequence of PepC revealed significant homology with the active site regions of cysteine endopeptidases including papain and mammalian belomycin hydrolase. Recently, Wohlrab and Bockelmann (1992, 1993, 1994) characterised an aminopeptidase from *L. delbrueckii* subsp. *bulgaricus* as similar to the lactococcal PepC; reducing agents such as dithiothreitol and β-mercaptoethanol increased enzyme activity, whilst chelating agents had an inhibitory effect. The site specificity of such enzymes is limited to dipeptides containing N-terminal hydrophobic amino acids, such as Leu-Leu and Ley-Gly (see also Table 7.6).

7.3.3.3   X-prolyl-dipeptidy-laminopeptidase (PepX)
The release of dipeptides from oligopeptides can be accomplished by PepX even when proline is in the penultimate position. Also, PepX is capable of releasing N-terminal prolyl-proline dipeptides from oligopeptides (see the reviews by Mulholland, 1994; Kunji *et al.*, 1996; Law and Haandrikman, 1997). The name of this enzyme has, however, been abbreviated to aminopeptidase X and has been extracted and purified from a wide range of lactic acid bacteria (see Table 7.6).

The PepX isolated from *L. delbrueckii* subsp. *bulgaricus* strains and *L. acidophilus* were ~90kDa, serine-proteases, and were severely inhibited by diisopropyl fluorophosphate (1mM) and divalent metal ions (1mM Cu^{2+}, Zn^{2+}, Fe^{2+} or Hg^{2+}) (Miyakawa *et al.*, 1991; Bockelmann *et al.*, 1991). In mutant strains, PepX was totally deficient and this absence caused a decrease in growth rate, an increase in cell wall endopeptidase activity and a loss of three cell wall proteins (Atlan *et al.*, 1990).

The molecular weight of PepX isolated from *L. helveticus* ranged from 72 to 95kDa, and the spread is possibly due to strain variation or to the method used to calculate the molecular weight (e.g. derived amino acid sequence of cloned gene, by gel filtration or sodium dodecyl sulphate-polyacrylamide gel electophoresis (SDS-PAGE); see also Ardö and Jönsson, 1994; Gatti *et al.*, 1997). The same enzyme was found in *L. delbrueckii* subsp. *lactis* and *S. thermophilus* (Meyer and Jordi, 1987), and the molecular weight was 165kDa in both species; below pH 5, both PepX isolates were unstable and the specificities towards various substrates, including the effect of metals, chelator and other inhibitors, varied with the microbial species.

7.3.3.4   Miscellaneous exopeptidases and endopeptidases
Tripeptidase from *L. delbrueckii* subsp. *bulgaricus* B14 of 85kDa has been purified and characterised. The enzyme consists of three subunits and a metal-dependent enzyme with an optimum temperature (40°C) and pH (6.0) (Bockelmann *et al.*,

1995, 1997). The characteristics of the prolidase gene (PepQ) and related cryptic gene (OrfZ) from *L. delbrueckii* subsp. *bulgaricus* have been reported by Rantanen and Palva (1997); the properties of enzymes, such as prolinase (PepR) (see also Varmanen *et al.*, 1998), proline iminopeptidase and dipeptidases (PepV and PepD), found in lactic lactobacilli are shown in Table 7.6 (see also Habibi-Najafi and Lee, 1994b, 1995; Kim *et al.*, 1996).

Little data are available on the proteolytic activity of bio cultures. It could be argued, however, that such microfloras do not grow to any extent during the manufacture of fermented milks and hence the proteolytic activity of the bio starter may be of secondary importance. Nevertheless, Goh *et al.* (1989) reported that, in full fat milk cultured with *B. bifidum* or *L. acidophilus*, soluble nitrogen compounds and free amino acids increased, suggesting that these organisms possess proteolytic enzymes. This view was confirmed by Abu-Taraboush *et al.* (1998) who observed that certain strains of bifidobacteria showed higher proteolytic activity in cultured camel's milk than in cow's milk. The proteolytic activity of *Bifidobacterium longum*, *infantis* and *adolescentis* is attributed to the presence of one aminopeptidase and two dipeptidases in each strain (El-Soda *et al.*, 1992; see also Desjardins *et al.*, 1990); the properties of aminopeptidase and proline iminopeptidase from *Bifidobacterium breve* have been studied by Cheng and Nagasawa (1985a, b). Nevertheless, the proteolytic activity of the yoghurt organisms appears to be at a maximum under the following conditions:

- Most intense activity is during the log phase.
- The rate of proteolysis decreases during storage or after the stationary phase has been reached.
- The ratio of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in the starter culture and the storage period can affect the level of amino acids in yoghurt, and for example, $70 \, \text{mg} \, 100 \, \text{g}^{-1}$ is liberated at a ratio of 1:1 after 1 day, followed by $50 \, \text{mg} \, 100 \, \text{g}^{-1}$ after 2 days and $41 \, \text{mg} \, 100 \, \text{g}^{-1}$ after 5 days. However, the acidity of these yoghurts was rather high, i.e. $1.9 \, \text{g} \, 100 \, \text{g}^{-1}$ lactic acid for the 1:1 ratio, and it is possible that the high level of liberated amino acids in the product was associated with the proteolytic activity of *L. delbrueckii* subsp. *bulgaricus* which becomes the predominant organism in such an acidic environment (refer later for further discussion).
- In yoghurt (24 hours old) the spectrum of amino acids changes in relation to the ratio of cocci:rods (i.e. at a ratio of 1:1, tryrosine, phenylalanine and leucine formed 56% of the amino acid pool but, at a ratio of 3:1, proline accounted for 71% of the free amino acids).
- The hydrolysis of whey proteins in milk yields lower levels of non-protein nitrogen as the ratio of *L. delbrueckii* subsp. *bulgaricus* to *S. thermophilus* is decreased.
- Free fatty acids, e.g. capric and, to a lesser degree, oleic, can reduce the proteolytic activity of the starter cultures and can affect the texture of the coagulum.
- Enhanced proteolytic activity in yoghurt is observed during the manufacture of lactose-hydrolysed yoghurt, due perhaps to protease residues present in the β-D-galactosidase preparations (Hemme *et al.*, 1979).
- Milk which was precultured with psychotrophic bacteria prior to the manufacture of yoghurt had enhanced proteolytic activity; however, the product developed unacceptable flavours (see Chapter 2).

Table 7.7 Soluble nitrogenous fractions from milk and milk cultured with the yoghurt micro-organisms

	Dialysable N		Ammonia N		Amino acid N		Urea N		Peptide N	
	$mg\,l^{-1}$	%	$mg\,l^{-1}$	%	$mg\,l^{-1}$	%	$mg\,l^{-1}$	%	$mg\,l^{-1}$	%
Milk	249	4.7	30	0.6	20	0.4	62	1.2	137	2.6
L. delbrueckii subsp. *bulgaricus*										
Av (6)	490	9.3	73	1.4	166	3.1	96	1.8	155	2.9
Range	438–545	8.3–10.3	63–89	1.2–7.9	56–314	1–5.7	51–146	1–2.8	71–270	1.3–5.4
S. thermophilus										
Av (5)	302	5.7	144	2.7	21	0.4	10	0.6	127	2.4
Range	222–406	4.2–7.7	88–190	1.7–3.6	16–26	0.3–0.5	3–30	0.1–0.9	117–197	2.0–3.7

Data compiled from Miller and Kandler (1967a, b). After Tamime and Deeth (1980). Reprinted with permission of *Journal of Food Protection*.

- Bitterness in yoghurt is usually attributed to the production of bitter peptides by the proteolytic activity of *L. delbrueckii* subsp. *bulgaricus*; however, fermentation of the milk at 44°C yields yoghurt which is less likely to be bitter than yoghurt produced at 38°C.

7.3.4 Products of proteolysis

The profile of nitrogenous compounds in yoghurt, compared with milk, changes due to the proteolytic activity of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, both during the fermentation period and, to a lesser degree, during the cold storage of the product. Basically, the change amounts to an increase in the level of soluble nitrogenous compounds, which also includes the liberation of amino acids and the release of peptides from the milk proteins.

7.3.4.1 Soluble nitrogenous compounds

The most comprehensive study in this field was conducted by Miller and Kandler (1967a, b) and a summary of their results is given in Table 7.7. These figures confirm that different strains of yoghurt organisms vary in their proteolytic activity and further, that the amounts of dialysable nitrogen released by *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* (490 and 302 $mg\,l^{-1}$, respectively) are compliant with the view that the former organism is more proteolytic than *S. thermophilus*. The same trend can be observed in relation to the amounts of amino acid nitrogen, urea nitrogen and peptide nitrogen (see Table 7.7), but the especial capacity of *S. thermophilus* to increase the level of ammonia nitrogen in cultured milks is due to the ability of the lactic streptococci/lactococci to split urea.

7.3.4.2 Liberation of amino acids

The spectrum of free amino acids in milk and yoghurt (see Table 7.8) is dependent on several variables such as:

- *Type of milk:* milks from different species (cow's, sheep's and goat's) have different contents of amino acids, i.e. ≤10, 3.78 and 20.6 mg 100 ml^{-1}, respectively, and in addition, goat's milk has, relative to the others, much higher levels of alanine, glycine, glutamic acid, serine and threonine.

Table 7.8 Free amino acid content (mg 100 ml^{-1}) of milk and yoghurt

Amino acid	Cow's		Goat's		Sheep's	
	Milk	Yoghurt	Milk	Yoghurt	Milk	Yoghurt
Alanine	0.16–0.64	1.17–3.80	1.33	3.83	0.56	1.30
Arginine	0.16–0.96	0.70–1.39	0.40	0.67	0.26	0.85
Aspartic acid	0.23–0.52	0.70–1.20	0.22	1.37	0.18	1.75
Glycine	0.30–0.53	0.28–0.45	5.91	6.06	0.15	0.25
Glutamic acid	1.48–3.90	4.80–7.06	3.54	3.78	1.08	4.10
Histidine	0.11	0.80–1.70	0.45	1.28	0.10	0.50
Isoleucine	0.06–0.15	0.15–0.40	0.18	0.43	0.06	0.25
Leucine	0.06–0.26	0.70–1.82	0.21	1.25	0.23	0.45
Lysine	0.22–0.94	0.80–1.11	0.60	2.35	0.19	0.72
Methionine	0.05	0.08–0.20	0.10	0.35	0.05	0.15
Phenylalanine	0.05–0.13	0.17–0.61	0.11	0.35	0.08	0.15
Proline	0.12	5.40–7.05	0.65	4.35	0.11	4.30
Serine	0.08–1.35	1.50–2.90	3.05	3.51	0.20	2.00
Threonine	0.05–0.26	0.24–0.70	3.34	2.80	0.13	0.55
Tryptophan	Tr	0.2	NR	NR	NR	NR
Tyrosine	0.06–0.14	0.18–0.61	0.30	0.60	0.16	0.24
Valine	0.10–0.25	0.90–1.86	0.30	0.50	0.24	0.90
Total	3.29–10.31	18.77–33.06	20.60	33.48	3.78	18.46

Tr; trace. NR; not reported.

Data compiled from Tamime and Deeth (1980).

- *Methods of manufacture:* slightly higher levels of amino acids are obtained when the fermentation is carried out at 42°C for 2–3 hours, rather than at 42°C for 1 hour followed by 5–6 hours at 30–32°C; the total amino acid contents of such yoghurts were 23.6 and 19.4 mg 100 ml^{-1} (Rasic *et al.*, 1971a, b; Stojslavljevic *et al.*, 1971).
- *Ratio of rods to cocci:* due to the fact that *L. debrueckii* subsp. *bulgaricus* is more proteolytic than *S. thermophilus*, the higher the ratio of rods to cocci in the starter culture, the higher the amino acid content is likely to be in the corresponding yoghurt. Nachev (1970) studied various strains of *L. delbrueckii* subsp. *bulgaricus* and classified them into three groups based on fermentation of sugars and types of amino acid released. The first group (118 strains) was characterised by releasing amino acids (leucine, glutamic acid, asparagine and proline) and an absence in the medium of β-alanine, tryptophan and aminobutyric acid. The second group (six strains) differed in that no glutamic acid was released, while the third group (one strain) was noted for the presence of tryptophan. Profiling of the amino acid content of Finnish fermented milk products has been reported by Kahala *et al.* (1993), who found a high content of proline compared to other amino acids. The glutamic acid content was also high.
- *Conditions during storage:* the temperature of storage of yoghurt can affect the level of free amino acids in the product, i.e. the higher the storage temperature, the greater the increase in free amino acids. Ottogalli *et al.* (1974) stored full and low fat natural yoghurts at 4°C and 20°C for a duration of 60 days and the

increases in the level of amino acids in these yoghurts were (at 4°C) 2.36 and 1.00, and (at 20°C) 7.57 and 14.65 mg 100 ml^{-1}, respectively. However, the same workers observed no increase in the level of amino acids in lemon and orange flavoured yoghurts stored under the same conditions for the same period of time, a difference that was attributed to the presence of natural metabolic inhibitors in the fruit, or the effect of some bacteriocidal agent added to the fruit concentrate, or the high acidity of the fruit preparation.

- *Level of lactic acid:* the amino acid content of yoghurt is dependent on the titratable acidity of the product. According to Luca (1974), yoghurts which contained 1.9 and 1.72–1.73 g 100 g^{-1} lactic acid had total amino acid contents of 70 and 41–50 mg 100 g^{-1}, respectively. Incidentally, the figure of 70 mg 100 g^{-1} in yoghurt is the highest level reported in the literature and it could be argued that such acidic yoghurt could be the result of prolonged incubation, and hence the amino acid content reflects directly the extent of the metabolic activity of the starter culture.

The final amino acid content of yoghurt made from cow's milk may range from 18.7 to 33 mg 100 ml^{-1} (see Table 7.8) and it is probable that the acidities of these yoghurts were 1.0–1.4 g 100 g^{-1} lactic acid. It is important, of course, that the total amino acid content of yoghurt reflects a balance between proteolysis and assimilation by the bacteria. Some amino acids, such as glutamic acid, proline and, to a lesser degree, alanine and serine, are presumably not required by the yoghurt organisms and thus accumulate in larger quantities in the product than the remaining amino acids which are utilised by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* during growth and/or fermentation.

7.3.4.3 Release of peptides

As mentioned earlier, some of the proteolytic enzymes which the yoghurt bacteria possess release peptides into the product. Some work carried out on this aspect of the fermentation has been reported from Bulgaria by Tanev and Zivkova (1977) and in this study, the behaviour of the short chain peptides in Bulgarian yoghurt during cold storage was monitored. The technique of peptide mapping, which included high voltage electrophoresis and finger printing by descending paper chromatography and differential staining of the peptides, was neatly demonstrated on both milk and yoghurt stored at 4°C for 1, 2, 3 and 65 days. The size and composition of these short chain peptides was not given, but the distribution of these peptides in yoghurt has been reported.

Kahala *et al*. (1993) reporting on the rate of proteolysis and peptide profiles of Finnish fermented milks (e.g. Bulgarian yoghurt, natural/plain yoghurt, biokefir and acidophilus milk) found that the rate of proteolysis increased during the storage period and the highest rate of proteolysis was found in fresh biokefir and after storage compared with other fermented milk products. However, the peptide profiles for Bulgarian and natural yoghurts were similar. The identified fractions were: Leu, Tyr, Phe, α_{s1}-casein 1–14, β-casein 47–57, β-casein 166–175 and β-casein 176–188 (see also Kyriakidis *et al*., 1993; Weimer *et al*., 1989); factors affecting the formation of amines in the growth medium by *L. delbrueckii* subsp. *bulgaricus* have been reported by Chander *et al*. (1989).

7.4 Lipid/fat metabolism

7.4.1 Introduction

Acyl glycerols constitute 96–98% of the total milk lipids/fats and the remaining fraction consists of phospholipids, sterols, fat-soluble vitamins (A, D, E and K), fatty acids, waxes and squalene. The lipids are found in the following phases of the milk: the fat globules, the membranes of the fat globules and the milk serum. The proportions of these fractions can vary in relation to such factors as species of mammal, breed, stage of lactation and type of feed (Walstra and Jenness, 1984; Weihrauch, 1988; Fox, 1991, 1994). The acyl glycerols present in milk are formed by the esterification of the alcohol radicals of the glycerol with one, two or three fatty acids residues to yield mono-, di- or triacylglycerides (triglycerides), respectively. Therefore, in broad terms, the enzymatic hydrolysis of milk lipids takes place at the ester linkages, eventually yielding free fatty acids and glycerol. The enzymes are known as triacylglycerol lipases EC 3.1.1.3 (Anon., 1992) and their mode of action may be specific to certain bonds on the glycerol molecules, that is, similar to the action of the peptidases (see Section 7.3.2). A simplified sequence of lipids hydrolysis is as follows:

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Di-} \xrightarrow{\text{Lipase}} \text{Mono-} \xrightarrow{\text{Lipase}} \text{Fatty acids + glycerol}$$

The triacylglycerol lipase enzymes in yoghurt may originate from the starter culture or from microbial contaminants that survived the heat treatment of the milk. Incidentally, the lipases, which occur naturally in milk, are inactivated at ordinary pasteurisation temperatures (Deeth and Fitz-Gerald, 1976). Therefore, any reduction in the percentage of fat, or increase in the level of fatty acids (free or esterified), or increase in the content of volatile fatty acids in yoghurt can be attributed to lipid metabolism by micro-organisms, including *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. However, before evaluating the role of the different lipases reported to be present in the latter organisms, it is pertinent to look at some factors which can affect the degree of lipolysis.

7.4.1.1 Fat content of yoghurt

The fat content ($g\,100\,g^{-1}$) of yoghurt differs from one country to another according to the existing or proposed standards for the chemical composition of the product, or alternatively in relation to the types of yoghurt produced. There are four broad categories of yoghurt and related products:

- fat free or <1%
- >1% and <3%
- >3% and <4%
- >4.5% and 10%

and the degree of lipolysis is likely to be greater in yoghurts with high fat contents.

7.4.1.2 Homogenisation

The process is carried out on the milk base and is widely practised in the yoghurt industry for two main reasons, first, to reduce the size of the fat globules and thus prevent "creaming" or fat separation in the milk during incubation, and second, to

Table 7.9 Triacylglycerol lipase activities of the yoghurt starter cultures

Enzyme	Substrate	*S. thermophilus*	*L. delbrueckii* subsp. *bulgaricus*
Tributyrase	Tributyrin	+++[a]	++
Trioleinase	Soy-milk and olive oil		
Glycerol ester hydrolase	Milk fat	++	
Enterases	Tween 40 and 60 and α-napthyl acetate or butyrate	+	+++
	Triacetin		Tr
Tricaproinase	Tricaproin		+

[a] Owing to different enzyme assay procedures employed, the enzyme activities are expressed as high (+++), medium (++) or low (+).

Tr = Trace.

Data compiled from Morichi *et al*. (1968), Otterholm *et al*. (1968), Angeles and Marth (1971), Formisano *et al*. (1972, 1973, 1974) and Umanskii *et al*. (1974).

improve the viscosity and texture of yoghurt. However, the extent of lipolysis in homogenised milk is much greater than in non-homogenised milk, due, in large measure, to the destruction of the protective layer of the fat globule, that is, the fat globule membrane (Mulder and Walstra, 1974).

Although the hydrolysis of fat by the yoghurt starter cultures occurs only to a limited degree, it may still be enough to contribute towards the flavour of the product. In fact, only Formisano *et al*. (1974) reported any appreciable loss of lipids, namely a decrease of 3.4% in the fat in yoghurt stored for 21 days at 4°C. This observation was not noted by other workers.

However, several authors in the 1960s and 1970s detected lipase activity in *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, and a list of these enzymes is shown in Table 7.9; the nomenclature of the enzymes is based on the substrate being hydrolysed, rather than on the systematic approach suggested by Anon. (1992). Nevertheless, all these triacylglycerol lipases in the yoghurt bacteria are reported to be located in the cytoplasm, since after cell disruption, very little activity is associated with the cell membrane (see also DeMoraes and Chandan, 1982); the fatty acid composition of dairy starter cultures has been reported by Rezanka *et al*. (1983) and Chand *et al*. (1992). Recently, Kalantzopoulos *et al*. (1990a, b) reported esterase activity in both yoghurt organisms and these enzymes were extracted from either the cell wall or the interior of the cell. The percentage of esterase activity was also high in *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (Bianchi-Salvadori *et al*., 1995).

The characterisation of esterase activities of lactobacilli species has been reported by El-Soda *et al*. (1986a, b) and Khalid *et al*. (1990) and could briefly be summarised as follows:

- Enzyme activities using nitrophenyl derivatives of fatty acids were recorded as positive towards derivatives up to 50°C.
- *P*-nitrophenyl derivatives were hydrolysed faster than the *O*-nitrophenyl derivatives.

Table 7.10 Changes in the free fatty acid contents of yoghurt made with milks from different mammals

Fatty acids	Cow	Sheep	Goat
Caproic	–	I	–
Caprylic	I	I	D
Capric	–	–	–
Lauric	I	I	D
Myristic	I	I	D
C-15	–	D	–
Palmitic	I	D	I
Palmitoleic	–	–	–
Stearic	D	D	–
Oleic	D	D	–
Linoleic	–	–	I

(I) Increase } by more than 1% compared with milk. (–) Signifies
(D) Decrease } no change.

Data compiled from Rasic and Vucurovic (1973) and Rasic *et al.* (1973).

- *L. helveticus* and *L. delbrueckii* subsp. *bulgaricus* strains had lower esterase activities than *L. acidophilus* and *L. delbreuckii* subsp. *lactis*.
- The enzymes activities were optimum at pH ∼7.0, and at temperatures in the range between 40 and 50°C.
- Freezing of cells, growth medium (e.g. MRS, sterile skimmed milk or whey-based medium) and stage of growth can influence esterase activities in *Lactobacillus* species (see also El-Sawah *et al.* 1995; Nadathur *et al.*, 1996).

7.4.2   Changes in the level of free and esterified fatty acids

The free and esterified fatty acids of yoghurts made from cow's, sheep's and goat's milk were studied by Rasic and Vucurovic (1973) and Rasic *et al.* (1973), and the changes which occurred are summarised in Table 7.10. From such data, it seems that the increase (or decrease) in the level of free fatty acids in the different types of yoghurt is inconsistent, and this variation probably reflects a difference in behaviour of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in cow's, sheep's and goat's milk (see also Boccignone *et al.*, 1983, 1985).

In another investigation from another laboratory (Formisano *et al.*, 1974), the reported change in the free fatty acids in yoghurt was somewhat simplified, in that there was a liberation of long chain fatty acids into the product and the final pattern did not change significantly during cold storage. However, fermentation of full fat milk with *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus* or *L. acidophilus* resulted in different effects on milk lipids, and according to Rao and Reddy (1984) the changes were as follows:

- Significant increase in saturated fatty acids and oleic acid.
- A concomitant decrease in linoleic and linolenic acids in the glyceride fraction.

Table 7.11 Changes in volatile fatty acids (VFA) in whole and skimmed milk fermented at 37° for different durations with yoghurt organisms

Fatty acids		Milk[a]	S. thermophilus		L. delbrueckii subsp. *bulgaricus*		Mixed	
			24 hs	72 hs	24 hs	72 hs	24 hs	72 hs
Total VFA (mg 100 g^{-1})	W	3.20	6.05	6.26	4.90	4.19	6.88	7.55
	S	2.97	5.89	6.32	4.88	3.79	6.80	7.20
C2	W	0.21	0.55	1.26	0.51	0.45	0.57	0.48
	S	0.20	1.95	1.36	0.45	0.37	0.12	0.20
C3	W	Tr	Tr	Tr	0.05	0.03	0.22	0.11
	S	–	0.05	0.05	0.03	0.03	Tr	Tr
i-C4	W	0.03	0.03	0.05	0.05	0.04	0.13	0.14
	S	0.03	0.04	0.61	0.05	0.05	0.03	0.06
n-C4	W	0.39	0.74	0.94	1.21	0.97	1.05	1.44
	S	0.38	0.50	0.96	1.20	0.90	0.66	1.08
i-C5	W	0.05	0.21	0.21	0.14	0.10	0.15	0.06
	S	0.03	0.13	0.18	0.11	0.09	0.07	0.17
n-C5	W	–	–	–	–	–		
	S	–	Tr	Tr	–	–		
n-C6	W	1.09	1.73	1.24	1.24	1.05	1.56	2.57
	S	1.13	1.72	1.35	1.25	1.07	2.40	2.04
C8	W	0.97	1.44	0.99	0.74	0.53	1.78	1.64
	S	0.96	1.30	1.18	0.87	0.56	2.26	2.36
C10	W	1.21	1.59	1.30	0.91	1.10	2.65	2.22
	S	1.10	1.81	1.74	1.06	0.68	3.11	2.92

[a] W: whole milk; S: skimmed milk.

Tr: Trace. (–): not detected. Empty space signifies test was not determined.

Data compiled from Yu *et al.* (1974) and Yu and Nakanishi (1975a–c).

After Tamime and Deeth (1980). Reprinted with permission of *Journal of Food Protection*.

- The increase in free fatty acids was moderate, but these were significant increases in stearic and oleic acids.
- The monoglyceride fraction disappeared completely upon fermentation.
- The changes in cholesterol content were not significant.
- A significant correlation ($r = 0.711$) was found between acid degree value and the level of free fatty acids.

7.4.3 Changes in the level of volatile fatty acids

During the manufacture and storage of yoghurt, there is an appreciable increase in the total level of volatile fatty acids in the product. Data on the release of these fatty acids by single strains of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* and by mixed cultures have been reported by many investigators, and of the two organisms, the *Lactobacillus* produces more of these acids than *S. thermophilus*. The increase in the level of volatile fatty acids in yoghurt is dependent on several variables, such as the strains of starter bacteria, type of milk (i.e. cow's, buffalo's or

goat's), duration and temperature of incubation, temperature of heat treatment of the milk and/or the age of yoghurt (Dutta *et al.*, 1971a, b, 1973; Singh *et al.*, 1980). However, a slight decrease in volatile fatty acids was observed in the presence of low concentrations of citric acids in milk (Dutta *et al.*, 1972).

Yu *et al.* (1974, 1985) and Yu and Nakanishi (1975a–c) have reported in detail on the levels of certain fatty acids in whole and skimmed milk cultured with yoghurt starter bacteria. Their data are shown in Table 7.11, and it can be observed that after 24 hours of incubation at 37°C, only a small degree of lipolysis has been exhibited by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. It could be argued, however, that the origin of volatile fatty acids in fermented milks, and in particular in those based on skimmed milk, may not be the result of lipid metabolism by the yoghurt organisms, but may arise from the breakdown of other milk constituents (e.g. the amino acid pool), as suggested by Nakai and Elliot (1965); in the course of oxidative deamination and decarboxylation, the amino acid is split into its corresponding volatile fatty acid. The lipid constituents of skimmed and full fat vita (i.e. Bulgarian fermented milk made with *L. delbrueckii* subsp. *bulgaricus*) have been reported by Ilinova and Naumova (1984).

Howover, Morichi *et al.* (1968) have pointed out that the presence of "true detected esterases" in the lactic acid bacteria (e.g. *L. delbrueckii* subsp. *bulgaricus*) is difficult to verify, since some of the proteolytic enzymes and other factors in milk may exhibit esterase activity. Consequently, it is safe to assume that the detected esterase activity of the yoghurt bacteria (see Table 7.9) is directly related to the action of proteolytic enzymes rather than lipases. Such a conclusion is in accord with the higher production of volatile fatty acids by *L. delbrueckii* subsp. *bulgaricus*, that is it is probably due to endopeptidases and/or exopeptidases rather than lipases.

7.5   Vitamin metabolism

7.5.1   General background

Milk contains both fat- and water-soluble vitamins. Table 7.12 (see also Chapter 9) indicates the levels of these vitamins in different milks (full fat and skimmed) and in the corresponding yoghurts (see also Ashoor *et al.*, 1983, 1985; Scott and Bishop, 1986; Rao and Shahani, 1987; Laukkanen *et al.*, 1988; Delgado Zamarreno *et al.*, 1996). The content of these vitamins changes during manufacture for the following reasons.

7.5.1.1   Decrease
- An excess of dissolved oxygen and/or a moderate heat treatment of milk can reduce significantly its vitamin content and the most susceptible vitamins are C, B_6, B_{12} and folic acid (see Chapter 2, Table 2.20).
- Excessive heat treatments of the milk, e.g. boiling for 5 min, cause even greater losses of the above vitamins; for example, vitamin B_{12} is reduced by $1.78 \mu g l^{-1}$ (Rasic and Panic, 1963).
- The yoghurt starter bacteria utilise some of the vitamins present in milk during the fermentation period to meet their growth requirements. This factor contributes, to some extent, to a reduction in the nutritional properties of the product. However, the quantities consumed are dependent on the rate of

Table 7.12 Vitamin contents of different milks and yoghurts[a]

Vitamin/ units	Cow		Cow		Goat		Sheep		Soya[f]	
	SS milk[b]	Yoghurt[c]	Milk[d]	Yoghurt[e]	Milk	Yoghurt	Milk	Yoghurt	Milk	Yoghurt
I. (μg 100 g^{-1})										
Retinol	1	8	52	28	44	N	88	86	0	23
Carotene	Tr	5	21	21	Tr	Tr	Tr	Tr	Tr	Tr
Vitamin D	Tr	0.01	0.03	0.04	0.11	N	0.18	0.24	0	0
Vitamin B$_{12}$	0.4	0.2	0.4	0.2	0.1	Tr	0.6	0.2	0	0
Folate	5	17	6	18	1	7	5	3	19	N
Biotin	1.9	2.9	1.9	2.6	3	0.5	2.5	N	N	N
II. (mg 100 g^{-1})										
Vitamin E	Tr	0.01	0.09	0.05	0.03	0.03	0.11	0.73	0.74	1.49
Thiamin	0.04	0.05	0.03	0.06	0.04	0.04	0.08	0.05	0.06	N
Riboflavin	0.17	0.25	0.17	0.27	0.13	0.17	0.32	0.33	0.27	N
Niacin	0.09	0.15	0.08	0.18	0.31	0.27	0.41	0.23	0.11	N
Tryptophan 60	0.78	1.20	0.75	1.33	0.73	0.83	1.27	1.03	0.52	0.88
Vitamin B$_6$	0.06	0.09	0.06	0.10	0.06	0.06	0.08	0.08	0.07	N
Pantothenate	0.32	0.45	0.35	0.50	0.41	0.23	0.45	N	N	N
Vitamin C	1	1	1	1	1	1	1	Tr	0	0

[a] Plain or natural yoghurt. [b] Semi-skimmed milk (1.6 g fat 100 g^{-1}). [c] Low fat yoghurt (0.8 g fat 100 g^{-1}). [d] Full fat milk (3.9 g fat 100 g^{-1}). [e] Full fat yoghurt (3.0 g fat 100 g^{-1}). [f] The product may be fortified with retinol and vitamin E.

Tr: Trace.

N: nutrient is present in significant quantities, but there is no reliable information on the amount.

Data compiled from Holland *et al.* (1989).

Table 7.13 Effect of incubation temperature upon vitamin synthesis in yoghurt

Vitamin (μg 100 g^{-1})	Milk + 2% SMP	Incubation temperature (°C) for 3 hours			
		37	40	42	45
Folic acid	0.37	3.74	4.04	4.32	3.94
Niacin	120	126	130	142	136

After Reddy *et al.* (1976).

inoculation, the strain of yoghurt starter and the conditions of fermentation (Shahani *et al.*, 1974; Friend *et al.*, 1983).

- Some vitamins decrease during the storage of yoghurt at 4°C, i.e. vitamin B$_{12}$ (Rasic and Panic, 1963; Cerna *et al.*, 1973). Reddy *et al.* (1976) observed losses of folic acid and vitamin B$_{12}$ of 28.6 and 59.9%, respectively, during the storage of yoghurt at 5°C for 16 days. The same workers also observed a decrease in the biotin, niacin and pantothenic acid contents. They attributed these losses to the combined effect of microbial catabolism during the incubation period and chemical decomposition of these vitamins during cold storage. This latter aspect was confirmed in yoghurt made by the direct acidification method rather than by microbial fermentation (see also Scott and Bishop, 1986; Saidi and Warthesen, 1993; Sharma *et al.*, 1996).
- A folic acid producing strain of *S. thermophilus* increased the folic acid content of yoghurt after 3.5 hours, and then the level decreased rapidly; this indicates that as the *L. delbrueckii* subsp. *bulgaricus* starts to grow, it utilises the vitamin produced by *S. thermophilus* (Kaneko *et al.*, 1987). Also, the same workers observed that a wide range of lactobacilli utilised vitamin B$_{12}$ when grown in reconstituted skimmed milk (see also Wachol-Drewek and Roczniak, 1982; Rao *et al.*, 1984).
- EPS-producing yoghurt starter organisms decreased the thiamin and biotin contents in the product, whilst non-EPS cultures increased the contents of biotin, folic acid and riboflavin (Erzinkyan *et al.*, 1987).
- *L. acidophilus* and *B. bifidum* utilised the folic acid present in milk (Drewek and Czarnocka-Roczniakowa, 1983).
- A long incubation of yoghurt (i.e. incubation at 30°C for 14–16 hours) decreased the synthesis of folic acid, but increased the content of thiamin and nicotinic acid in the product (Kneifel *et al.*, 1989).

7.5.1.2   Increase

Vitamins which increase during the actual manufacture of yoghurt are niacin and folic acid, because they are actively synthesised by the starter cultures. According to Reddy *et al.* (1976), the increases in folic acid and niacin in yoghurt (made from whole milk fortified with 2% SMP and incubated for 3 hours at 42°C) amounted to 3.95 and 22μg 100 g^{-1}, respectively (see also Table 7.13); losses in storage (see above) may exceed these gains in due course. Although there is a general agreement in the literature that vitamin B$_{12}$ decreases during yoghurt production, Mitic *et al.* (1974), Shahani *et al.* (1974) and Kilara and Shahani (1976, 1978) found that some species of *Lactobacillus* and strains of yoghurt starter culture synthesise vitamin B$_{12}$.

The reported folic acid contents in commercial yoghurt may range between 3.7 and 24.5 μg 100 g^{-1} (Kaneko *et al.*, 1987; Hoppner and Lampi, 1990; Wigertz *et al.*, 1997), and mutant strains of *S. thermophilus* increased the folic acid content in skimmed milk to 38.1 μg 100 g^{-1} (Kaneko *et al.*, 1987). Furthermore, as mentioned elsewhere, non-EPS yoghurt cultures increased the biotin, folic acid and riboflavin contents in the fermented product (Erzinkyan *et al.*, 1987). However, enhanced synthesis of vitamins in yoghurt can be achieved by using different combinations of starter cultures. Examples are the inclusion of *Propionibacterium* spp. in the yoghurt starter cultures increased the folic acid content in the product by 43% (Wachol-Drewek and Roczniak, 1983), yoghurt made with added *Saccharomyces cerevisiae* and preservatives had higher riboflavin and niacin contents during storage (Durga *et al.*, 1986), and a mixed culture of bifidobacteria, *L. delbrueckii* subsp. *bulgaricus* and kefir grains at a ratio of 1 : 0.5 : 0.5 increased the thiamin and riboflavin contents in the product by 27% and 18%, respectively (Khamagacheva *et al.*, 1988).

In the early 1990s, Austrian researchers studied a total of 47 commercially available starter cultures (e.g. yoghurt, bio-cultures and kefir), and the results (see below) suggested different patterns of synthesis and utilisation of water-soluble vitamins in fermented milks (Kneifel *et al.*, 1989, 1991).

In view of the existing evidence (see also Deeth and Tamime, 1981), it is safe to conclude that *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* synthesise niacin and folic acid and, to a lesser degree, vitamin B$_6$ during the production of yoghurt. Evidence of vitamin synthesis by *Bifidobacterium* species has been reported by Ballongue (1998) and there is evidence of biotin synthesis by different bifidobacterial strains (Noda *et al.*, 1994). Thus, taking into consideration that both the yoghurt micro-organisms and some bio starter cultures are capable of synthesising certain water-soluble vitamins, it is of some interest to consider the possible metabolic pathway(s) involved in the synthesis of these vitamins.

7.5.2   Biosynthesis of folic acid

The folic acid group (or folates) is a generic name given to around ten different compounds which share a basic structural unit connected to conjugates of different numbers of glutamic acid residues. These folates are, therefore, made from carbon, hydrogen, nitrogen and oxygen atoms, and their formulae range from C$_{15}$H$_{12}$N$_6$O$_4$ to C$_{49}$H$_{61}$N$_{13}$O$_{24}$. Thus, some or all of these compounds are active as folacin and a typical structure of one such compound (i.e. pteroylglutamic acid [*p*-(2-amino-4-oxodihydropteridyl-6)-methyl-aminobenzoyl-L-glutamic acid]), which may be synthesised by the bacteria is shown in Fig. 7.5.

Many organisms require folacin as a growth factor. It functions as a coenzyme in many different biochemical reactions (i.e. as an activator and carrier of carbon units during oxidation) and it participates in the metabolism of purines, pyrimidines and some amino acids. However, the synthetic pathways of folic acid in *S. thermophillus* and *L. delbrueckii* subsp. *bulgaricus* are not well established, and Lentner (1984, 1986) suggested that the synthesis of this compound in animals, plants and micro-organisms probably involves the biochemical reactions shown in Fig. 7.6.

It is worthwhile reporting that folate-binding proteins might be involved in folate absorption in the human intestine and that their concentration is important (Wigertz *et al.*, 1997), but since the milk base is heated to temperatures ≥90°C during

Folic acid
$C_{19} H_{19} N_7 O_6$
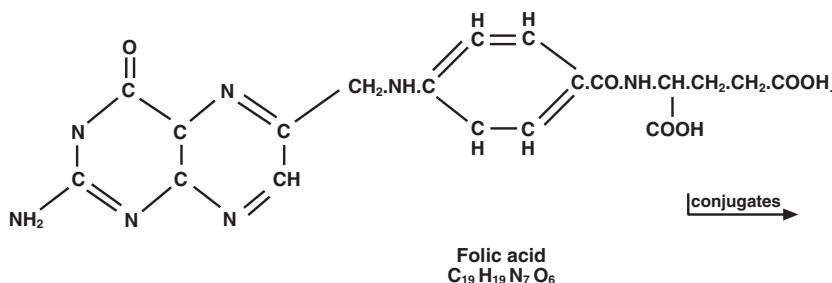
Fig. 7.5 Possible structure formation of folic acid by yoghurt micro-organisms
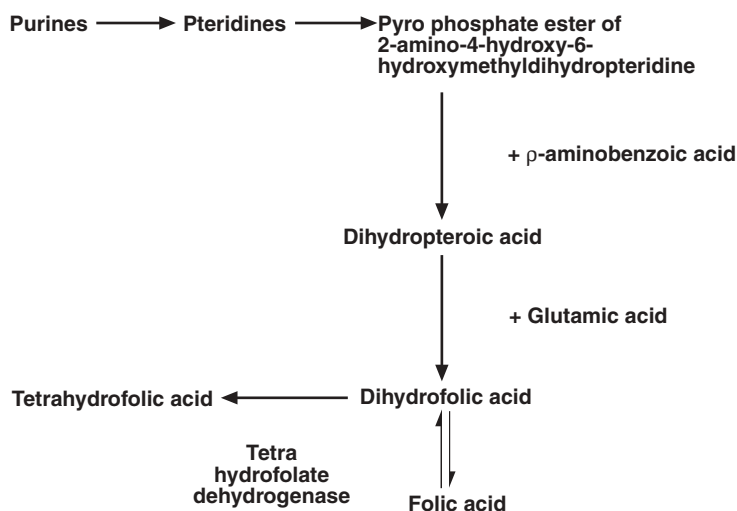
Fig. 7.6 Probable biochemical reactions during the synthesis of folic acid by micro-organisms

After Lentner (1984, 1986).

manufacture, yoghurt contains significantly lower concentrations of folate-binding proteins compared with other dairy products.

7.5.3 Biosynthesis of niacin

Niacin activity is exhibited by nicotinic acid and nicotinamide. The former compound constitutes part of the structure of two important coenzymes, that is, NAD and nicotinamide adenine dinucleotide phosphate (NADP). These two coenzymes are composed of adenylic acid and nicotinamide ribotide linked through their phosphate groups (see Fig. 7.7); however, NADP contains an additional phosphate group (Stanier *et al.*, 1987). As NAD and/or NADP are essential for many oxidative/ reductive biochemical reactions, the niacin synthesised by *S. thermophilus* and *L.*
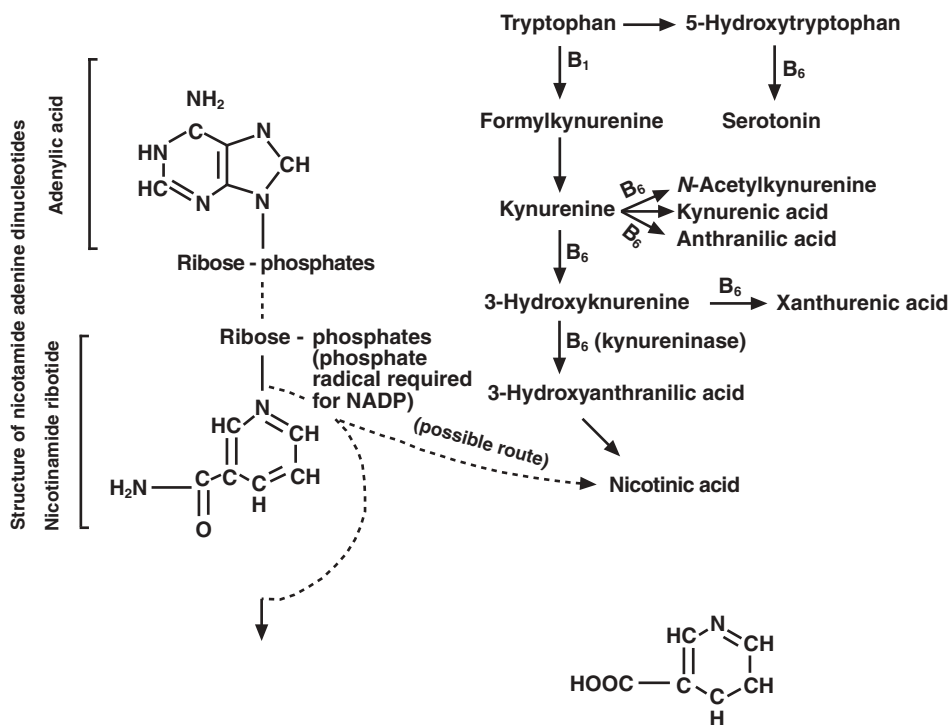
Fig. 7.7 Some possible schemes for the biosynthesis of niacin by yoghurt micro-organisms
Adapted from Lentner (1984, 1986) and Stanier *et al.* (1987).

delbrueckii subsp. *bulgaricus* may originate from the nicotinamide fraction arising during the formation of NAD and/or NADP. The biosynthesis of these nucleotides basically involves the following steps: first, the synthesis of a sugar moiety (possibly derived from the available milk sugar(s)) and second, the synthesis of the pyrimidine or purine base. Alternatively, after this formation of NAD and/or NADP, the nicotinamide fraction could be released as a result of the degradation of these nucleotides, but whether nicotinic acid could be derived from the released nicotinamide must be subject to further investigation.

However, nicotinic acid is derived by a few bacteria from the metabolism or breakdown of tryptophan, a pathway which is dependent on the availability of certain vitamins (e.g. thiamine, riboflavin and vitamin B_6), to activate the required enzymes (Lentner, 1984, 1986). As *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* utilise these vitamins and tryptophan does not accumulate during yoghurt production, it is possible that these organisms use the vitamins for the synthesis of niacin. In view of the limited information in this field, Fig. 7.7 can do no more than illustrate some possible schemes for the synthesis of niacin by the yoghurt microflora.

7.5.4 Biosynthesis of vitamin B_6

The activity of vitamin B_6 is exhibited equally by pyridoxine, pyridoxal and pyridoxamine. The basic structure of these compounds is similar in that it consists

Table 7.14 Levels of water-soluble vitamins in fermented milks

Vitamin	Yoghurt ($n = 30$)	Bio-yoghurt ($n = 14$)	Kefir ($n = 3$)
Units ($mg \, 10 \, ml^{-1}$)			
Thiamin	0.034	0.030	0.011
Riboflavin	0.121	0.135	0.145
Pyrodoxine	0.023	0.015	0.011
Nicotinic acid	0.088	0.109	0.076
Units ($\mu g \, 100 \, ml^{-1}$)			
Cobalamin	0.19	0.23	0.27
Folic acid	3.22	3.62	1.38

After Kneifel et al. (1989, 1991).

Pyridoxine ($C_8N_{11}NO_3$)   R = CH_2OH
Pyridoxal ($C_8H_9NO_3$)   R = CHO
Pyridoxamine ($C_8H_{12}N_2O_2$)   R = CH_2NH_2.
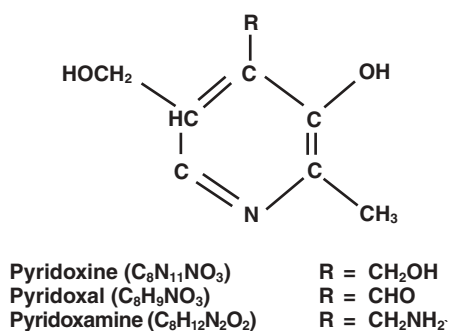
Fig. 7.8 Basic structure of vitamin B_6 including the different radical components

of a pyridine ring, but they differ in respect of the radical components (see Fig. 7.8).

According to Lentner (1984, 1986), no information is available on the biosynthesis of the pyridine ring in micro-organisms, plants or animals; however, the different forms of vitamin B_6 are interconvertible by micro-organisms in accordance with the scheme illustrated in Fig. 7.9. In view of the limited knowledge of the synthesis of vitamin B_6 in general, it is difficult to suggest any possible metabolic pathway by which *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* might synthesise this vitamin.

7.6 Miscellaneous changes

The biological activity of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus* and bio starter cultures during the manufacture of yoghurt and related products is highly complex. Current scientific work has elucidated some general information about the metabolic pathways employed by these organisms. Nevertheless, numerous changes do occur in the milk and some of the additional minor changes in the milk constituents are: (a) a reduction in the level of citric acid, (b) the content of hippuric
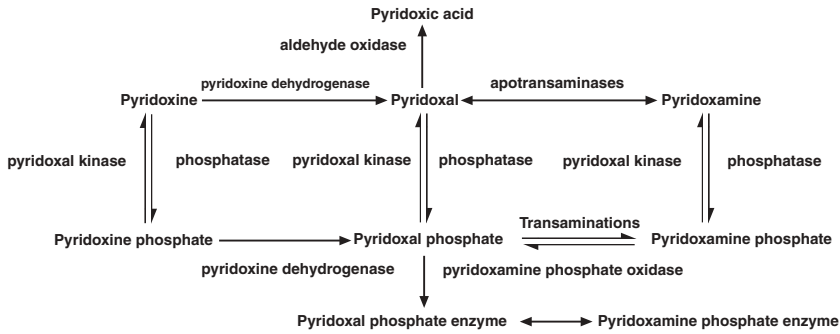
Fig. 7.9 Scheme to illustrate that pyridoxine, pyridoxal, pyridoxamine and their phosphates are interconvertible by micro-organisms

After Lentner (1984, 1986).

acid is lost altogether, and (c) the levels of acetic and succinic acids are increased, especially when bio starter cultures are used to ferment the milk. Other changes that may occur involve the following:

- Uracyl-4-carboxylic acid – this compound is better known as orotic acid or orotate anion ($pk_a = 2.4$). It is metabolised by the yoghurt starter cultures, most probably by *L. delbrueckii* subsp. *bulgaricus* and its content in milk is reduced by up to 50% (i.e. from 8.3 to 3.4–4.2 mg 100 ml^{-1}), during the manufacture of yoghurt (Okonkwo and Kinsella, 1969a; see also Lavanchy and Steiger, 1984; Haggerty *et al*., 1984; Prakash and Sharma, 1986; Navder *et al*., 1990; Saidi and Warthesen, 1989). However, orotic acid possesses some significant therapeutic properties, since it plays an important role in the biosynthesis of nucleic acids. Furthermore, according to Larson and Hegarty (1979), the level of orotic acid in cultured dairy products is dependent on the degree of fermentation and the amount of soluble whey solids in the product (see also Okonkwo and Kinsella, 1969b). Suzuki *et al*. (1986) have reported that pyrimidine biosynthesis from orotic acid may be negatively regulated by the intracellular level of purine nucleotides and *L. delbrueckii* subsp. *bulgaricus* could not grow in milk depleted of orotate; this indicates that pyrimidine synthesis in this micro-organism is very low.
- Metal ions – little is known about the utilisation of minor nutrients, such as metal ions, by lactic acid bacteria. Boyaval (1989) has reviewed the available information on the transport and importance of metal ions. For example: (a) the inhibition of certain exopeptidases of *S. thermophilus* and *L. delbrueckii* subsp. *lactis* by chemicals can be nullified by Co^{2+}, Zn^{2+} or Mn^{2+}, (b) the presence of Mn^{2+} and Mg^{2+} in the growth medium stimulated the growth of *S. thermophilus* and *L. acidophilus*, (c) Fe^{2+} stimulated the growth of *L. delbrueckii* subsp. *bulgaricus* and *L. acidophilus*, and (d) *S. thermophilus* required Ca^{2+} for growth, whilst for *L. acidophilus*, Ca^{2+} caused morphological changes (i.e. from filamentous to bacilloid) and the transitioned cells were more freeze resistant.
- 7 α-dehydroxylase activity on bile acids – strains of *Lactobacillus* spp., *Bifidobacterium* spp. and *S. thermophilus* test negative for this enzyme, which suggests that

the intake of these starter cultures is safe because their presence in the human intestine does not produce secondary bile acids that can promote colon cancer (Takahashi and Morotomi, 1994).

- Angiotensin-I-converting enzymes (ACE) – these enzymes tend to release exopeptidases that are associated with the renin–angiotensin system which regulates peripheral blood pressure (Meisel *et al.*, 1997). The inhibitory activity of these enzymes has been found to be low in yoghurt, but high in cheeses.
- Enzymatic activities – some enzymatic activities of lactic acid bacteria which might be of interest in the present review are: (a) *S. thermophilus* was the best producer of superoxide dismatase compared with six other lactic starter cultures (Hosono *et al.*, 1991), (b) glutamic acid uptake by *S. thermophilus* was energy dependent and NaCl strongly inhibited the uptake (Bracquart *et al.*, 1989), and (c) a rapid screening method of the yoghurt microflora for restriction endonuclease activity was reported by Poch and Somkuti (1998).
- Immunostimulating agent – this component contained *N*-acetyl-muramyl peptides which were derived from *L. delbrueckii* subsp. *bulgaricus* (Link and Pahud, 1991); the method of processing was patented and the immunostimulating agent could be used during the manufacture of fermented milk products to promote an immune response against Gram-negative bacteria in the intestine.
- Health benefits – the presence of other metabolites, for example β-galactosidase (Kilara and Shahani, 1976; Rao and Dutta, 1977, 1978), and various antitumour and antimicrobial agents (Reddy *et al.*, 1973a; Pulusani *et al.*, 1979; Rao and Pulusani, 1981) must not be forgotten, for such agents might be of medical and therapeutic value to humans. However, for an update regarding the health benefits of fermented milks including the production of bacteriocin by the dairy lactic acid bacteria, the reader should refer to Chapters 5 (Section 5.10), 6 and 9.

7.7   References

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8

Preservation and production of starter cultures

8.1   Introduction

The manufacture of yoghurt is now more centralised than in the past and while successful production is directly related to the processing techniques employed, the correct selection, preservation, handling and propagation of the starter cultures helps to standardise and maintain uniformity in the quality of the end product.

Yoghurt cultures consist of two species (i.e. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) and as these organisms are mainly grown and propagated together, they are referred to as mixed strain starter cultures. The culture organisms are preserved in small quantities known as stock cultures. When these cultures are reactivated for use in the dairy, a scale-up system of propagation is employed to supply the required volume. For example, if the daily production of yoghurt is 25 000 l and rate of inoculation is 2 ml 100 ml^{-1}, then the amount of starter needed is 500 l. Therefore the various stages of propagation are:

$$\text{Stock} \xrightarrow[\text{1 g or ml}]{} \text{Mother} \xrightarrow[\text{20 ml}]{} \text{Feeder} \xrightarrow[\text{10 l}]{} \text{Bulk} \xrightarrow[\text{500 l}]{} \text{Processing vat}$$

The stock and mother cultures are propagated in the laboratory, while the feeder and bulk cultures are produced in the starter room of the dairy. The above stages of culture propagation are illustrated in Fig. 8.1.

An active bulk starter culture must have the following characteristics:

- It must contain the maximum number of viable cells.
- It must be free from any contaminants, e.g. coliforms or yeasts and moulds.
- It must be active under processing conditions in the dairy and hence maintenance of the intermediate and other cultures is extremely important.

The mother and feeder cultures are grown in sterile media, mainly milk, under aseptic conditions and the activity of such cultures can be maintained by applying one of the following approaches (Foster, 1962). First, reducing or controlling the metabolic activity of the organisms by ordinary refrigeration; this is for short-term storage of a starter culture and it can be kept viable for up to a week. Second, con-
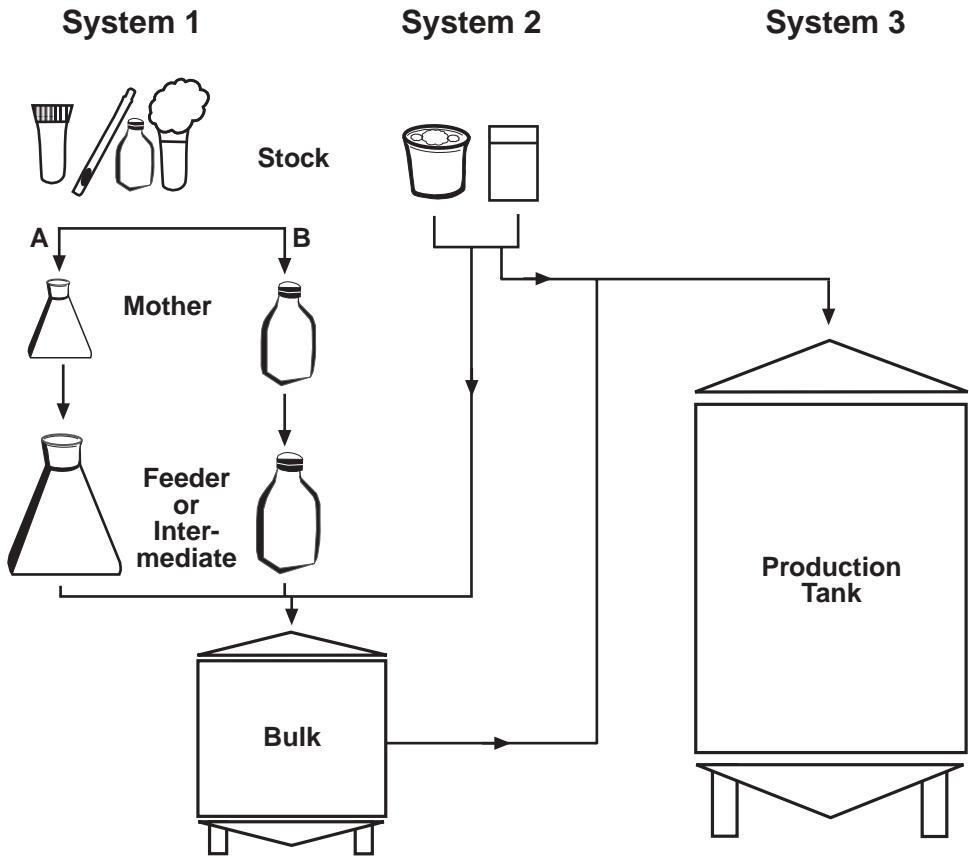
Fig. 8.1 Starter culture preparation

Note: System 1: stock culture may be liquid, freeze dried or frozen at −196°C for the production of bulk starter. Systems 2 & 3: stock culture may be concentrated freeze dried or frozen at −60°C to −196°C for the production of bulk starter and yoghurt, respectively.

centration and separation of the organisms from their wastes, followed by resuspension in a sterile medium and finally preservation by drying or freezing (Tamime and Robinson, 1976; Robinson, 1983; Tamime, 1990). The latter forms are used for extended storage of the starter bacteria and such cultures may be obtained from stock collections available in dairy research establishments, colleges or culture bank organisations, or from commercial starter manufacturers.

8.2   Methods of starter culture preservation

It is essential that starter cultures are preserved in order to maintain an available stock of these micro-organisms for the production of bulk starter and, in the case of a starter failure, some types of preserved cultures could be used for direct-to-vat inoculation (DVI). Also, successive culture transfers or subculturing can induce mutants which may alter the overall behaviour and general characteristics of the starter. Furthermore, in the case of mixed starter cultures, successive subculturing

could alter the balance or ratio of *S. thermophilus* : *L. delbrueckii* subsp. *bulgaricus*; in "bio" starters the counts of *Lactobacillus acidophilus* and *Bifidobacterium* spp. will be altered.

In general, dairy starter cultures may be preserved by one of the following methods:

- Liquid starter.
- Dried starter: (a) unconcentrated (spray dried or freeze dried/lyophilised; these methods are rather old and not used at the present time), and (b) concentrated freeze dried.
- Frozen starter: (a) frozen at −20°C (unconcentrated), (b) deep frozen at −40°C to −80°C (concentrated), and (c) ultra low temperature freezing at −196°C in liquid nitrogen (concentrated).

It can be observed that the main methods of starter culture preservation involved concentration of the bacteria, as well as various techniques of drying and freezing, and hence, the viability of a preserved culture may be dependent on:

- the basic growth medium,
- the presence of cryoprotective agents,
- rapid removal of metabolic compounds, e.g. lactic acid and carbonyl compounds,
- the nature of the suspending medium (if employed),
- conditions of freezing and/or drying,
- rate of thawing (deep frozen cultures),
- methods of concentration.

The latter aspect, sometimes referred to as cell biomass concentration, is of great importance; the number of bacterial cells per unit weight or volume is measured by counting the number of colonies produced after serial dilution, on an agar medium and the results are recorded by colony forming units (cfu) ml^{-1} or g^{-1}. However, the cell biomass can be concentrated using different systems. For further details refer to Section 8.3.2.

Nevertheless, the starter bacteria subjected to these physical conditions may die or be injured and, in view of the economic importance of starter cultures in the dairy industry, the general aim of scientists in this field has been to minimise the death rate of, or injury to, the preserved cultures. For further information about the factors affecting the survival of micro-organisms, including dairy starter cultures, reference may be made to Gray and Postgate (1976), Andrew and Russell (1984) and Hurst and Nasim (1984).

8.2.1 Liquid starters

Starter cultures can be preserved in a liquid form using one of two different growth media. The first type is reconstituted skimmed milk powder (SMP) (10–12 g 100 g^{-1} SNF (solids-not-fat)) which is free from antibiotics. The milk is sterilised by autoclaving at 69–103 kPa or 121°C for 10–15 min, and a sample is incubated for a week at 30°C to check its sterility. After inoculation (1 or 2 ml 100 ml^{-1}), the milk is incubated at 30°C for 16–18 hours or at 42°C for 3–4 hours. At the end of the incubation period, the clotted culture must be cooled immediately and it can then be stored for up to a week at ordinary refrigeration temperature (e.g. <10°C). Personal experience suggests that if the acidity of the cold culture is around 0.85 g 100 g^{-1} lactic

acid, both the activity of the starter and the ratio of *S. thermophilus* to *L. delbrueckii* subsp. *bulgaricus* (1:1) are easily maintained. This type of starter culture is referred to in the industry as a working stock culture. Alternatively, cool, autoclaved milk may be inoculated with a starter culture and then stored under refrigeration for incubation whenever it is required. It is worthwhile pointing out that successive sub-culturing is labour intensive, expensive and can induce mutant strains; furthermore, trained personnel are required to perform such duties in the laboratory. A maximum limit of 15–20 subcultures is recommended for the yoghurt starter bacteria to safeguard the proper ratio between cocci and the rods, and to reduce the effect of mutation.

A slightly extended preservation of liquid cultures (i.e. reserved stock culture) can be achieved using litmus milk [(g 100 g^{-1}) reconstituted SMP 10–12, litmus solution 2, yeast extract 0.3, dextrose/lactose 1; enough calcium carbonate to cover the bottom of the test tube; panmede 0.25 and lecithin 1, and both adjusted to pH 7]. The medium is autoclaved at 69 kPa for 10 min and incubated for a week to check sterility (Shankar, personal communication). The inoculated medium is incubated for a short period of time (42°C for 12 hours), and stored under ordinary refrigeration; it is only necessary to reactivate the culture once every 3 months. However, Kang *et al.* (1985) preserved liquid cultures in the presence of CaCO$_3$ and found, for example, *L. acidophilus* remained active for 150 day at 37°C when the growth medium was supplemented with 1.5 g 100 g^{-1} CaCO$_3$, whilst yoghurt cultures were preserved for 150 day at 0°C with added CaCO$_3$ (0.5 g 100 g^{-1}). Alternative methods for the preservation of liquid starter are given: (a) cultures can be preserved for 12 months at 4°C using Na-citrate or K-phosphate buffer solutions (Sultan *et al.*, 1987), (b) the activity and viability of liquid concentrated cultures of *L. delbrueckii* subsp. *bulgaricus* were improved when the cells were grown in a medium supplemented with Span 80 and then stored in 0.1 g 100 g^{-1} Na-ascorbate after bubbling nitrogen into the culture (Kaneko *et al.*, 1987), and (c) *L. delbrueckii* subsp. *bulgaricus* has been preserved successfully in gelatin spherules (20 g 100 g^{-1} gelatin) at 4°C or up to 3 months at room temperature without any significant loss of activity (Zlotkowska and Ilnicka-Olejniczak, 1993).

Starter culture activity is affected by the rate of cooling after incubation, level of acidity at the end of the incubation period and the temperature and duration of storage (see above the working or reserve stock culture). Cooling is important to control the metabolic activity of the starter. Goat's milk can also be used as a medium for the growth and maintenance of yogurt and lactococcal cultures (Abrahamsen *et al.*, 1982), but strongly flavoured milks either reduced or inhibited the growth of some cultures after 10 transfers; excess fatty acids in the strong goat's milk may have proved inhibitory.

8.2.2 Dried starters

An alternative method for the preservation of yoghurt starter cultures is drying. The different drying methods used are:

- Vacuum drying ⎫
- Spray drying ⎬ (old methods not used at present time)
- Freeze drying or lyophilisation (widely used in the laboratory)
- Freeze drying of concentrated cultures (widely used commercially).

The main objectives behind these developments are first, to reduce the workload which is involved in maintaining liquid cultures, second, to improve the shelf life of the preserved cultures, and third, to facilitate the dispatch of cultures by post without any appreciable loss in their activity.

According to Tofte-Jespersen (1974a,b, 1976), the drying process prior to 1950s was carried out under vacuum and the results were not encouraging (i.e. the preserved dried cultures contained only 1–2% viable bacteria). To regain maximum activity several subculturings were required. In essence, this method of preservation consisted of taking an active liquid starter culture, adding lactose as a protective agent and calcium carbonate to neutralise the excess acid, followed by partial concentration of the mixture (i.e. removal of whey). The concentrated starters, which were by then in a granular form, were dried under vacuum.

Owing to the poor results achieved by vacuum drying, alternative methods were sought, and one of these methods was spray drying which was first used in the Netherlands for the preservation of cheese starter cultures (Stadhouders *et al.*, 1969). Although the results proved promising, this technique has not been developed commercially. However, the Dutch process could be summarised as follows:

- Hydrolyse milk protein with trypsin for 4 hours at 37°C followed by steaming.
- Propagate the starter culture at 20°C with pH control using $Ca(OH)_2$ as a neutralising agent.
- Evaporate the starter at 27°C to $22\,g\,100\,g^{-1}\,TS$ (total solids) and spray dry (air temperature 70°C) to $9\,g\,100\,g^{-1}$ moisture with the powder temperature not exceeding 42°C.
- Vacuum dry at 27°C and 1–2 mm Hg; the dried culture has about $5\,g\,100\,g^{-1}$ moisture.

Work in the United States (Porubcan and Sellars, 1975a) showed that the addition of certain compounds, for example ascorbic acid and monosodium glutamate, helped to protect the bacterial cells during the drying process. Furthermore, Porubcan and Sellars (1975a) recommended that starter cultures must be propagated in a buffered medium. The objectives of buffering are firstly, to increase the number of viable organisms/volume of sample and secondly, to neutralise certain metabolites, mainly lactic acid, which can inhibit bacterial growth beyond a certain level. Cultures preserved by this process retained their activity after storage for 6 months at 21°C.

Another method of spray drying yoghurt cultures was developed in Sweden (Anderson, 1975a, b) for which the advantages of drying at high temperatures (75–80°C) without causing any bacterial damage and maintaining different ratios of *S. thermophilus* : *L. delbrueckii* subsp. *bulgaricus* in the preserved culture were claimed. For example, a ratio of 40 : 60 in a dried culture can be used for the production of a sharp flavoured yoghurt (due to high level of *L. delbrueckii* subsp. *bulgaricus*), while for a milder flavoured yoghurt a ratio of 60 : 40 can be used. The Swedish method of spray drying can be summarised as follows:

- propagate the starter culture in sterilised concentrated skimmed milk ($18–24\,g\,100\,g^{-1}\,TS$);
- fortify the growth medium with lysine, cystine and cyanocobalamine;
- dry at a temperature of 75–80°C.

Although this development claimed many advantages, the system is not widely used.

Recently, Teixeira *et al.* (1994, 1995a–c) reported that the death kinetics of *L. delbrueckii* subsp. *bulgaricus* using the spray drying process were influenced by many factors such as (a) to (f). (a) The logarithmic survival ratio decreased with increased outlet air temperature with first-order kinetics and the pseudo-z for the organism was about 17°C. (b) The calculated activation energy (E_a) values were $33.5 \, kJ \, mol^{-1}$ above 70°C and $86 \, kJ \, mol^{-1}$ at less than 70°C. (c) The relationship between the entropy and enthalpy of activation for spray drying and heating in liquid medium was linear; the data for drying, however, fell in the range of negative entropy. (d) High storage temperature and water activity reduced the survival of the dried cells. (e) The survival rate was higher in the presence of mono-Na-glutamate and ascorbic acid during storage at 4°C compared with 20°C. (f) The dried cells were sensitive to certain inhibitors (e.g. penicillin, pyronin Y, lysozyme and sodium chloride) due to damage of DNA, cell wall and cell membrane, respectively (see also Riis *et al.*, 1995; Teixeira *et al.*, 1996, 1997).

The addition of dextrin prior to drying, using a two-fluid atomiser and decreasing the air outlet temperature improved the survival rate of the yoghurt organisms (Metwally *et al.*, 1989; Abd-El-Gawad *et al.*, 1989). Other additives and/or methods of drying that improved the survival rate of yoghurt starter cultures and *L. acidophilus* may include the addition of betaine to an osmotically stressed medium protected some lactobacilli species, although not *L. delbrueckii* subsp. *bulgaricus* (Kets *et al.*, 1996; Kets, 1997), drying the cultures on porcelain beads (Magdoub *et al.*, 1987) or silica gel (de Silva *et al.*, 1983), the use of whey supplemented with yeast extract plus glucose as a cryoprotective agent (Gandhi and Shahani, 1994) and drying the cells in a fluidised-bed dryer (Rossi and Clementi, 1987).

Freeze-dried or lyophilised yoghurt cultures are produced when the starter culture is dried in the frozen state. This method of starter preservation enjoys widespread popularity and aims to increase the reliability of the preserved cultures, that is, the dried cultures should provide a high number of viable organisms and the maximum percentage survival during storage, compared with vacuum or spray-dried starter cultures (see Cattaneo *et al.*, 1986; Porubcan, 1990).

In lyophilised cultures, the survival rate is high and only a small quantity is needed to inoculate the mother culture. The number of viable bacteria/unit of addition is of the same order of magnitude as in the liquid starter culture (i.e. $2 \, ml \, 100 \, ml^{-1}$) (Tofte-Jespersen, 1974b, 1976). It can be observed, however, that freezing and drying can damage the preserved organisms and, in particular, the bacterial cell membrane. Thus, Porubcan and Sellars (1975b) filed a patent in the mid 1970s for the production of freeze-dried starter cultures with the growth medium fortified with certain additives to minimise the damage to the bacterial cell membrane. The growth medium consisted of the following components:

- milk base (pH adjusted to 6.0–6.5),
- additives (e.g. ascorbic acid, mono-Na-glutamate, aspartate compound),
- cryoprotective agents (e.g. inositol, sorbitol, mannitol, glucose, sucrose corn syrup, dimethyl sulphoxide (DMSO), PVO, maltose, mono- or disaccharides).

Another approach used by Morichi (1972, 1974) to minimise bacterial cell damage was the addition of certain cryogenic agents to the cell suspension. The protective solutes are of a hydrogen bonding and/or ionising group in nature. Hence, these

compounds stabilise the cell membrane and so prevent, to a certain degree, cellular injury during preservation procedures. The effect of such solutes on the survival of the yoghurt organisms and *L. acidophilus* is illustrated in Table 8.1. It can be concluded from the work of Morichi (1972) that the survival of *L. delbrueckii* subsp. *bulgaricus* was enhanced by L-glutamic acid, L-arginine and acetyl glycine, and that of *S. thermophilus* and *L. acidophilus* by the above mentioned compounds and DL-pyrolidone carboxylic acid and DL-malic acid; furthermore, *L. delbrueckii* subsp. *bulgaricus* is more vulnerable to cellular damage than *S. thermophilus* (see Table 8.1).

Thus, due to the low survival rate of starter cultures, the early commercial freeze-dried cultures were not suitable for DVI and it was necessary to propagate these cultures a few times to re-establish their activity prior to fermentation (Porubcan and Sellars, 1979). In the 1980s, Amen and Cabau (1984, 1986) patented a process in which cheese starter cultures were grown in a special medium containing a nutritive substrate, and the pH was maintained >5.5 by the addition of a neutralising agent such as ammonium hydroxide. The removal of the inhibitory ammonium lactate was carried out by UF and the addition of water (see Section 8.3.2). The concentrated culture was then freeze dried and made suitable for DVI application; it is safe to assume that a similar approach could be used for concentrating yoghurt starter cultures for use in DVI systems.

In view of the relative susceptibility of the yoghurt organisms to the freeze-drying process, many different protective compounds have been studied and some examples are shown in Table 8.2. Milk solids are widely accepted as very good cryogenic agents for the preservation of starter cultures and the use of levels up to 20–25 g $100\,g^{-1}$ TS has been reported (see Table 8.2); however, $16\,g\,100\,g^{-1}$ TS in the growth medium is a realistic level and a typical procedure for the preservation of a mixed strain yoghurt starter culture was reported by Tamime and Robinson (1976).

It is evident that the survival rate of lyophilised starter cultures is influenced by several factors.

8.2.2.1 Growth medium
Skimmed milk and/or whey supplemented with yeast extract or hydrolysed protein are good growth and suspension media for the preservation of freeze-dried cultures (Alaeddinoglu *et al*. 1989). The organisms should be propagated at their optimum

Table 8.1 Effect of certain cyrogenic agents (adjusted to pH 7 and 0.06 M) on the survival rate of freeze-dried yoghurt bacteria[a]

Cryogenic agent	*S. thermophilus*	*L. delbrueckii* subsp. *bulgaricus*	*L. acidophilus*
L-Glutamic acid	35–40	16–21	42–63
L-Arginine	21–40	20–35	39–57
L-Lysine	6–7	1–10	4–38
DL-Threonine	7–11	6–10	6–21
Acetyl glycine	29–44	7–33	3–35
DL-Malic acid	52–59	6–15	28–66
DL-Pyrolidine-carboxylic acid	24–48	9–11	24–56

[a] Figures as percentage of original numbers; the range of survival is due to different strains tested.

Adapted from Morichi (1972).

Table 8.2 Selection of different cryogenic compounds employed during the production of freeze-dried yoghurt starter cultures

Method of preparation	References
Skimmed milk + lactose or horse serum + glucose or Naylor and Smith (1946) reducing medium	Briggs *et al*. (1955)
Skimmed milk + peptonised milk + saccharose + Na-glutamate	Gavin (1968)
Suspend concentrated culture in sucrose solution and buffering salt + Na-glutamate	Bannkova and Lagoda (1970)
Suspend washed cells in skimmed milk + ascorbic acid + thiourea + ammonium chloride	Sinha *et al*. (1970, 1974)
Mix active culture + sucrose, gelatin and Na-glutamate + Na-citrate (for streptococci) or Na-acetate (for lactobacilli)	Lagoda and Bannikova (1974, 1975)
Suspend cell biomass in gelatin + Na-citrate + mono-Na-glutamate + sucrose or only malt extract + Tween 80 (see text)	Speckmann (1975) Porubcan and Sellars (1975b)
Suspend biomass in lactose or mono-Na-glutamate or arginine hydrochloride	Pettersson (1975a, b)
Grow culture in MRS broth or all purpose Tween + cryogenic agents[a]	Kilara *et al*. (1976)
Grow culture in low lactose medium + soya + casein and continuously buffer	Hup and Stadhouders (1977)
Mix culture with sugar solution or peptone or polymer 1500	Nikolova (1978)
Skimmed milk + yeast extract + Vit. E or Tween 80 + sheep's serum	Naghmoush *et al*. (1978)
Skimmed milk + Na-glutamate + sucrose	Ozlap and Ozlap (1979), Kim *et al*. (1988)
Suspend cell biomass in skimmed milk + sucrose + Na-glutamate + buffering salt	Lagoda *et al*. (1983)
Mix cell biomass with SMP + corn syrup or lactose	Ishibashi *et al*. (1985)
Mix cell biomass + sucrose + Na-citrate + gelatin + mono-Na-glutamate or skimmed milk + Na-glycerophosphate	Bozoglu *et al*. (1987)
Suspend culture in whey or skimmed milk + sucrose + adonitol + glycerol	Alaeddinoglu *et al*. (1989)
Suspend culture cells in skimmed milk + sucrose + Na-glutamate	Gupta and Ratnakar (1990)
Suspend cell biomass in skimed milk + glycerol + glucose + dimethyl sulphoxide + polyethelene glycol	Wolff *et al*. (1990)
Mix cell biomass with skimmed milk + whey base medium + Tween 80	Champagne *et al*. (1991a)
Mix cell biomass + milk + glucerol	Béal and Corrieu (1994)
Mix the cultures with sucrose + Mg-sulphate + ascorbic acid + Na-acetate + apilac[b] + Ca-hydroxide	Belov *et al*. (1995)

[a] Cryogenic agents for lactobacilli: casitone, lactose, malt extract, milk solids, mono-Na-glutamate, Myracet®, whey powder and/or peptonised milk; for streptococci: same as lactobacilli + dimethyl sulphoxide, glycerol and/or pectin. [b] Apilac® is a lyophilised preparation based on bee-collected pollen.

temperatures. However, improved survival rate during freezing and freeze drying of *L. delbrueckii* subsp. *bulgaricus* was achieved after growth in the presence of calcium (Wright and Klaenhammer, 1983). Also neutralisation of the growth medium to pH range 5–6 is recommended.

8.2.2.2   Cell biomass and suspension medium
Providing a culture >10^{10} cfu ml^{-1} (see Section 8.3.2) including neutralisation of the suspension medium improves the survival rate of the starter culture in the presence of cryoprotective agents (see Table 8.2; Font de Valdez *et al.*, 1983a, b, 1985a). Removal of carbonyl compounds from the growth medium is also recommended as they can react with amino groups in the bacterial cells and can accelerate their death (see the review by Champagne *et al.*, 1991b). Growth of *L. delbrueckii* subsp. *bulgaricus* in a medium at constant pH 5.7 and fortified with Na-citrate and Tween 80 improved the survival rate by a factor of ten (Champagne *et al.*, 1991a). However, the addition of certain polymers (gelatin, xanthan gum and maltodextrins) had a detrimental effect of the stability of *S. thermophilus* during storage at 20°C, whilst α- and β-galactosidase activity losses in *Bifidobacterium longum* during storage at 20°C were greater than parallel cultures stored at 4°C or –20°C (Champagne *et al.*, 1996).

8.2.2.3   Freeze-drying, packaging and storage
Wolff *et al.* (1990) reported that vacuum freeze drying was more suitable for *S. thermophilus* compared with atmospheric pressure freeze drying; suspension of the cells in reconstituted skimmed milk provided good protection for the cells. Whilst storage of the dried culture under vacuum or nitrogen provided better survival of the yoghurt cultures, *S. thermophilus* was found to preserve well, while *L. delbrueckii* subsp. *bulgaricus* was more sensitive to freezing and drying (Bozoglu *et al.*, 1987). In recent studies reported by Castro *et al.* (1995, 1996, 1997), the survival of lactobacilli was greatest when the dried culture was stored at 11% relative humidity and 5°C.

8.2.2.4   Reactivation
It is recommended that users follow the instructions of the starter culture manufacturer. However, rehydration medium and temperature (i.e. 20°C) can affect the leakage of cellular ribonucleotides from damaged cells. Detailed studies on both mespohilic and thermophilic lactic acid bacteria have been reported by Morichi *et al.* (1967) and Font de Valdez *et al.* (1985b–e, 1986) (see also the review by Tamime, 1990).

Lyophilised cultures tend to have a long lag phase and need to be subcultured at least twice to obtain an active liquid culture. Hence, for the production of bulk starter, System 1 is used (see Fig. 8.1), otherwise large quantities of dried culture are needed for direct inoculation of the bulk starter and a long incubation period is required. This approach is not advisable for two main reasons, first, the bulk starter may not be active when used for the manufacture of yoghurt and second, from an economic point of view, the approach can be very costly. More recently, concentrated freeze-dried cultures have appeared on the market and it is feasible to use such cultures for direction inoculation of bulk starter (see Fig. 8.1, System 2) or alternatively, for DVI of milk for the manufacture of yoghurt (see Fig. 8.1, System 3; Gatto *et al.*, 1993; Kreuder *et al.*, 1994; Riis *et al.*, 1995). In both cases, although the

production time may be extended by 2–3 hour, considerable savings can be achieved by eliminating the need for trained personnel to handle the starter cultures.

8.2.3 Frozen starters

Yoghurt starter cultures can be also be preserved in the frozen form and such cultures are produced by two different routes:

- Deep or subzero freezing (–30 to –80°C)
- Ultra low temperature freezing (–196°C) in liquid nitrogen

Sterile liquid milk freshly inoculated with an active starter culture is deep frozen at –30 to –40°C to preserve the mother or feeder culture. Such frozen cultures can retain their activity for several months when stored at –40°C and this method of culture preservation became popular in the dairy industry because deep frozen cultures produced in centralised laboratories could be dispatched to a dairy in dry ice whenever required. These cultures are mainly packed in plastic containers and a typical example is the Astell-type plastic bottle (see Fig. 8.6). The reactivation procedure for these deep frozen cultures is as follows:

- Remove starter from freezer, i.e. at –40°C,
- Thaw the starter very quickly in water bath at 20°C,
- Incubate at 42°C until the desired acidity is reached,
- Cool and store overnight in the refrigerator,
- Subculture for the propagation of feeder for bulk starter (see Fig. 8.1, System 1).

An alternative type of deep frozen culture involves freezing an active liquid starter at –40°C. The process consists of propagating the culture in a continuously neutralised growth medium in order to optimise the bacterial cell number per millilitre. The bacterial mass is then separated using a Sharples separator (see Section 8.3.2) and the cells are resuspended in a sterile growth medium and/or protective agent prior to packaging and freezing. As mentioned earlier, the preserved cultures must be stored at –30°C to –40°C and be dispatched to dairies in insulated boxes filled with dry ice.

   The freezing process can cause damage to the bacterial cells in particular to *L. delbrueckii* subsp. *bulgaricus*, and hence the activity of deep frozen cultures may tend to deteriorate after a certain time of storage due to several factors.

8.2.3.1 *Growth medium plus cryogenic compounds*

Imai and Kato (1975) reported that an improved medium for frozen cultures at –30°C contains skimmed milk supplemented with sucrose, fresh cream and $CaCl_2$ or gelatin. The same workers also observed that the presence of sodium acetate caused the starters to become sensitive to injury. In addition, concentrated cells (10^{10}–10^{12} cfu ml^{-1}) frozen at –30°C in the presence of certain mixtures of cryogenic compounds (Na-citrate, glycerol, Na-β-glycerophosphate, yeast extract, calcium sucrose, cream, sterile skimmed milk, peptone or lactose) have retained the activity of dairy starter cultures (Wright and Klaenhammer, 1983; Fayed *et al.*, 1985; Abraham *et al.*, 1990; Tamime, 1990; see also de Antoni *et al.*, 1989; Zlotkowska *et al.*, 1993). Other factors which affect the survival rate of *S. thermophilus* during freezing are growth phase and strain variation (Morice *et al.*, 1992); the

factors affecting the survival of lactobacilli have been reviewed by de Antoni *et al*. (1989).

8.2.3.2 Methods of concentration
Refer to Section 8.3.2.

8.2.3.3 Temperature of freezing and storage
Although freezing and storage at −40°C has proved to be a successful process for preserving dairy starter cultures, storage at −80°C to −100°C in liquid nitrogen vapour improves the survival rate of the organisms; also the rate of freezing should not be overlooked (Tsvetkov and Shishkova, 1982; Kim and Yu, 1990; Foschino *et al*., 1992; Béal *et al*., 1994).

8.2.3.4 Effect of thawing
Freezing and thawing can damage the cell membrane of *L. delbrueckii* subsp. *bulgaricus* and can induce sensitivity to NaCl and liver extract. The amino acid transport system of cells can also be damaged, but such cell injury is reversible if the cells are suspended in a solution of pyruvate, KH_2PO_4 and $MgSO_4$ (Font de Valdez *et al*., 1993; Libudzisz and Mokrosinska, 1995; Oberman *et al*., 1995; Piatkiewicz and Mokrosinska, 1995).

8.2.3.5 Miscellaneous factors
The destruction of bacterial cells during freezing is mainly due to an increased concentration of electrolytes and other solutes both inside the cell and in the suspending medium, rather than to mechanical damage as the result of ice crystal formation (Keogh, 1970). The former situation results in the denaturation of protein components and enzymes of the bacterial cell, while the concentration of electrolytes outside the cell results in the dehydration of the protoplasm due to the diffusion of water through the cell wall membrane. The kinetics of freezing and thawing processes of concentrated cell biomasses of lactic acid bacteria have been reported by Walczak *et al*. (1995).

Ultra low temperature freezing at −196°C in liquid nitrogen is by far the most successful method of preserving starter cultures. The reviews by Gilliland and Speck (1974) and Hurst (1977) illustrate the earlier research work which has been carried out in this field. The advantages of this technique of starter preservation have been summarised by Keogh (1970): at such low temperatures, the water molecules do not form large size crystals and the biochemical processes inside the cells cease to function, so that in biological terms, the bacterial cell is at a standstill.

Based on published reviews carried out in this field, the freezing and thawing cycle is still regarded as the most important factor in the successful use of frozen cultures in the dairy industry. An organism which is highly susceptible to damage during freezing is *L. delbrueckii* subsp. *bulgaricus*, but it was found that the presence of Tween 80 and Na-oleate improved cell stability (Smittle *et al*., 1972, 1974; Smittle, 1973). *L. acidophilus* is also susceptible to freezing and thawing, and the injury is associated with cell wall components other than peptidoglycan; such injury is reversible by natural repair of the cell wall components (Johnson *et al*., 1984). However, the type of growth medium, neutraliser used and/or cryoprotective compound(s) can play a major role in the activity of the preserved culture and the reviews by Gilliland (1977, 1985) highlight these factors in relation to different

species of lactic acid bacteria. However, Bulgarian workers reported that good results were obtained when yoghurt starter cultures were frozen at $0.36\,ml\,100\,ml^{-1}$ of lactic acid (Tamime and Robinson, 1976; Tamime, 1990).

Mitchell and Gilliland (1983) managed to grow *L. acidophilus* in a medium of 2.5 $ml\,100\,ml^{-1}$ pepsinised whey, maintained at pH 6.0 using a neutraliser consisting of sodium carbonate in ammonium hydroxide. The cell count was about $10^9\,cfu\,ml^{-1}$ and after freezing in liquid nitrogen, the stability of the culture was excellent during storage for 28 days.

In order to maintain the activity of the preserved starter at ultra low temperatures, the cultures are neutralised, concentrated, packaged and finally frozen. The various stages in the production of such cultures have been reported by Tofte-Jespersen (1974b) and Porubcan and Sellars (1979). Normally the concentrated starter is packed in an aluminium can of 70 ml capacity (i.e. the recommended volume to inoculate 1000 l of milk). The cans are fitted with a pull-ring type closure, which is convenient for easy opening. However, for smaller quantities (e.g. 5 ml) the culture can be packed in a screw-capped polypropylene ampoule which resists cracking in liquid nitrogen. Another type of packaging material which may be used is the laminated carton. While the aluminium can and the polypropylene ampoule are stored in liquid nitrogen, the laminated carton is stored in a special container in an atmosphere of nitrogen vapour. Incidentally, the latter type of packaging material is used to pack pelleted concentrated frozen starter cultures (see Fig. 8.2).

Fig. 8.2 Preservation and packaging systems used for stock and DVI starter cultures
A, Liquid (incubate and store under refrigeration); B, litmus milk (partially incubated followed by refrigeration); C, D, E, frozen at −30°C (inoculate, incubate and then freeze, or inoculate and freeze); F, concentrated low temperature frozen cultures (starter in granular form); G, frozen in liquid nitrogen (polyproplyene ampoule); H, frozen in liquid nitrogen (pull-ring can); I, freeze dried; J, concentrated freeze dried.

These developments in the liquid nitrogen freezing of yoghurt starter cultures are primarily aimed at preserving the feeder/intermediate culture for the preparation of bulk starter (see Fig. 8.1, System 2). However, the ultimate objective is to employ such cultures for DVI of milk for the production of yoghurt (see Fig. 8.1, System 3), for although their use can lead to a slightly prolonged manufacturing time (longer lag phase – see later), the advantages can be summarised as follows:

- convenience
- culture reliability
- improved daily performance
- improved strain balance
- greater flexibility
- better control of bacteriophages
- improvement in quality of product.

In practice, certain drawbacks may be encountered, such as: (a) too great a dependence of the dairy on the starter manufacturer, (b) non-availability of liquid nitrogen facilities; at present, special containers are supplied by the starter manufacturer to customers for the transportation and storage of cultures in liquid nitrogen, (c) apportioning of responsibility in case of starter failure, (d) a natural reluctance within the dairy industry to introduce new technology in place of one that is well established as satisfactory, and (e) the longer time required for manufacture of yoghurt.

It is of the utmost importance that the thawing and handling of frozen cultures is carried out according to the supplier's recommendations (see also Tamime, 1990). A typical procedure is as follows:

- Remove can from liquid nitrogen storage.
- Thaw in water containing 100–200 μg g^{-1} hypochlorite solution at 20°C for 10 min.
- When culture is partially thawed (i.e. contents are just loosened), remove can from water, open lid and add directly to bulk starter milk or milk for processing.

Yoghurt starter cultures are available in a number of forms (see Fig. 8.2) and depending on the method of preservation, the viable cell counts can vary slightly; Table 8.3 illustrates some typical differences and the blends of organism between the available types of commercially produced yoghurt and related starter cultures.

8.3 Technology of cell biomass production

It can be observed from the information on starter culture preservation that the survival rate is dependent on the processing conditions (growth medium, presence of cryogenic compounds, freezing and drying) and on the method of harvesting the cells. One of the main criteria of success during the preparation of the starter is the production of an active culture, that is a starter which consists of very large number of viable cells, so that when it is added to milk the fermentation process is initiated as quickly as possible.

Table 8.3 Enumeration of lactic acid bacteria in commercial DVI starter cultures ($cfu\,g^{-1}$) used for the manufacture of bio fermented milks and yoghurt

Starter culture	Bifidobacterim spp.[a]	L. acidophilus	S. thermophilus	L. delbrueckii subsp. bulgaricus
Freeze dried	4.6×10^{11}	1.4×10^{11}		
	1.1×10^{10}	5.6×10^{9}		
	4.8×10^{9}	7.1×10^{9}	7.5×10^{9}	
	3.6×10^{9}	1.2×10^{10}	4.2×10^{10}	
	5.4×10^{9}	2.1×10^{10}	1.7×10^{11}	
	3.4×10^{8}	4.8×10^{8}	8.7×10^{7}	
			6.6×10^{10}	2.5×10^{8}
Frozen	4.6×10^{7}	6.9×10^{9}	4.3×10^{7}	

[a] *B. bifidum*, *lactis*, *longum* and/or *infantis*.

Data compiled from La Torre (1997).

8.3.1 Growth characteristics

During the growth of any dairy starter culture, the cells divide and increase in number up to a certain level and then start to die. This behaviour gives rise to the characteristic "growth curve" illustrated in Fig. 8.3, where it can be seen that the rate of cell division is divided into four different sections:

- Lag phase – this is the phase which follows immediately after inoculation of the milk. The delayed bacterial activity could be due to adjustment or adaptation of the organism to a new medium.
- Log phase – during this phase the cells display maximum activity, i.e. shortest generation time, as long as optimum conditions (nutrients and temperature) are available.
- Stationary phase – at a certain point, the cell viable number remains constant due to a lack of nutrients and an accumulation of waste metabolites (e.g. lactic acid in milk); the death of old cells and the production of new cells is in balance.
- Death phase – the number of viable cells starts to diminish, mainly due to unfavourable growth conditions.

It is safe to assume that there is a direct relationship between the activity of the starter and its age, and that an active culture falls somewhere on the growth curve between the upper middle region of the log phase and the beginning of the stationary phase (see Fig. 8.3). Therefore, the most active type of starter is the liquid culture, which is characterised by having a short lag period followed by a rapid rate of acid development; on average the inoculation rate may vary between 2 and 3 ml $100\,ml^{-1}$ and the starter may contain in excess of $10^{8}\,cfu\,ml^{-1}$ or g^{-1}. Nevertheless, if such a culture is used for preservation, undoubtedly the survival rate will be low and may not be suitable for DVI of the milk. Therefore, cell biomass production becomes an important criterion in culture preservation.

8.3.2 Concentration of the cell biomass

Batch and continuous fermentation are used for the production of dairy starter cultures. The fermentation kinetics of *L. delbrueckii* subsp. *bulgaricus* have been
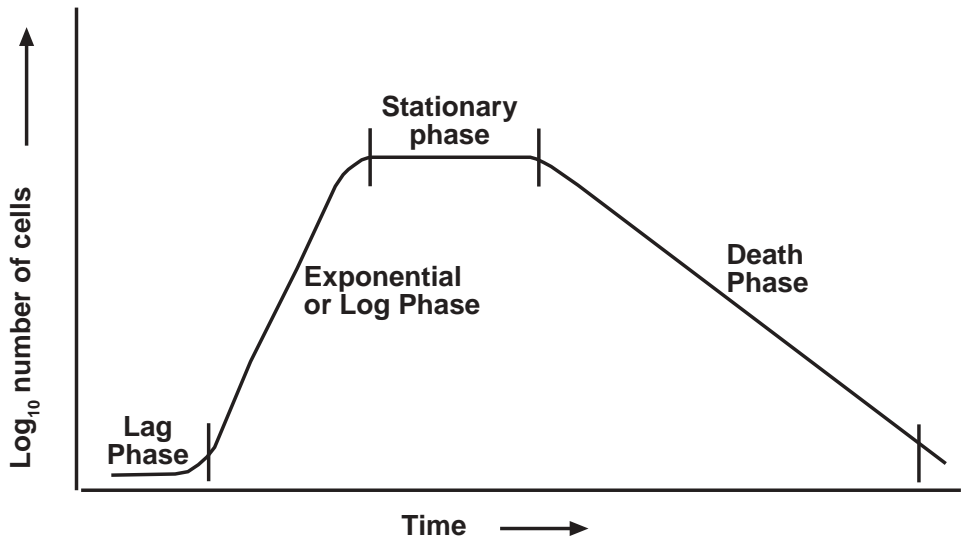
Fig. 8.3 Growth curve characteristics of a population of viable unicellular micro-organisms

examined by Venkatesh *et al*. (1993) who concluded that the relationship between adenosine biphosphate (ATP) concentration and cell biomass in a batch fermentation could be approximated by a Leudeking–Piret relationship and that cell productivity using continuous fermentation was three times higher than using a batch process. Thus, the systems which are used for the concentration of cell biomass are as follows.

8.3.2.1 Mechanical means

The equipment most widely available in the dairy industry (e.g. Sharples separator, desludging separator, clarifiers or bactofuge) may cause some physical damage to the bacterial cells, thus reducing the rate of survival during the preservation stage. Alternatively, ultracentrifuges ($20\,000\,g$) can be used and it is possible that these cause least physical damage to the cells. Béal and Corrieu (1994) concentrated yoghurt cultures (i.e. single and mixed strains) by centrifugation at $11\,000\,g$ for 15 min at 4°C at the end of the log phase following growth in a batch system with the pH controlled by adding $10\,\mathrm{mol}\,l^{-1}$ of NaOH. The cultures were either frozen at −75°C or freeze dried. They concluded that: (a) during concentration and preservation, culture activity was not altered significantly, (b) during storage for 24 weeks, the viability of the cultures decreased continuously and the decrease was greater in mixed cultures, and (c) the frozen cultures showed greater resistance during storage than the freeze-dried type (see also Lelieveld, 1984; Béal *et al*., 1989).

Shear stress during culture growth of *L. delbrueckii* subsp. *bulgaricus* affected the characteristics of the organism, for example, cell elongation, membrane permeability and resistance to freezing at −80°C. Arnaud *et al*. (1993) observed that, at a shear

stress level of 36 Pa, biomass concentration was higher and the lag time shorter compared to the same culture grown at 72 Pa.

8.3.2.2 Chemical neutralisation

The two different organisms in the yoghurt starter cultures can tolerate different levels of acidity in the growth medium, with *S. thermophilus* being more sensitive to lactic acid than *L. delbrueckii* subsp. *bulgaricus*. Thus, while lactobacilli can survive beyond 2 g 100 g^{-1} lactic acid, the streptococci can tolerate up to 1.2–1.5 g 100 g^{-1} lactic acid, and hence it is essential that the lactic acid is either removed or neutralised in order to protect *S. thermophilus* (see also Benthin and Villadsen, 1995). Sodium or ammonium hydroxide is widely used, but the latter compound is usually recommended. The reaction between lactic acid and the neutralising compounds results in the formation of sodium or ammonium lactate. However, at a certain level, lactate starts to inhibit the starter organisms also, and as a result the cell biomass concentration is limited to 10^{10} cfu ml^{-1} or g^{-1}. Therefore, to achieve a high concentration of about 10^{11}–10^{12} cfu g^{-1}, for example, in a freeze-dried starter culture, the cell biomass grown in a chemically neutralised system requires further concentration, possibly using a mechanical separator (see also Amen and Cabau, 1984; Barach and Kamara, 1986; Parente and Zottola, 1991; Borzani *et al.*, 1993).

Since the 1980s, there have been great technological developments by starter culture manufacturers in the production and preservation of DVI freeze-dried and frozen cultures. Martin (1983) described the production of the freeze-dried type by Rhodia Texel in France (formerly known as Eurozyme). An illustration of a fermentor used to produce high numbers of cells is shown in Fig. 8.4.

8.3.2.3 Diffusion culture

This technique involves the use of selected semipermeable membranes to concentrate micro-organisms and, in principle, this process consists of the following steps: (a) growth of the starter culture in a restricted volume of medium, (b) provision of a system that allows fresh growth nutrients to permeate in through the membrane, and (c) allows the metabolic waste materials to diffuse out.

This constant replenishment of the medium allows the concentration of bacterial cells to build up beyond normal levels and, using the diffusion culture technique with cheese starters, Osborne (1977) and Osborne and Brown (1980) have reported achieving >10^{11} cfu ml^{-1}. Although the waste metabolites diffuse out from the growth medium, some of the lactate is retained and this does tend to limit the cell biomass concentration. No work has been reported on yoghurt organisms, but it is possible that the principle of this technique could be applied to concentrate *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*.

An alternative diffusion method used to concentrate starter cultures uses UF and electrodialysis. Boyaval *et al.* (1987, 1988) used the continuous fermentation of sweet whey permeate to produce lactic acid and cell biomass in a membrane bioreactor. The organisms were a mixed culture of *L. helveticus* and *S. thermophilus*. Steiber and Gerhardt (1980) used dialysis to concentrate *L. delbrueckii* subsp. *bulgaricus* in a continuous fermentor using deproteinised whey; the cell biomass was more than double that from an ordinary fermentor. A UF method was also used to concentrate *S. thermophilus* with cellular productivity nine times higher than that obtained by conventional methods (Prigent *et al.*, 1988).

Fig. 8.4 Fermentor for the production of starter culture concentrate prior to freeze drying

Note: On-site view of a fermentation tank for the production of starter culture; ammonium hydroxide is used to neutralise the acid produced by the lactic acid bacteria.

Reproduced by courtesy of Rhodia Texel Ltd., Stockport, U.K.

8.3.2.4 *Cell immobilisation in gels*

This method of cell biomass concentration involves culture immobilisation in gum gels or porous foam-glass beads and this technique has reached commercial scale production. The published data available on the yoghurt cultures have been reported by Audet *et al*. (1989, 1990, 1991a, b), Buyukgungor and Caglar (1990), Buyukgungor (1992), Champagne *et al*. (1993), Ragout *et al*. (1996) and Turkur and Hamamci (1998). In essence, this technique involves entrapping the cell biomass in small beads (0.5–1.0 mm diameter) of κ-carrageenan/locust bean gum or Ca-alginate to give a concentration of around 10^9 cfu g^{-1}. The material can then be used for the continuous fermentation of milk, with the breakdown of lactose being achieved both by cells held in the solid matrix and cells released into the milk. As cells within the matrix are actively growing, such bioreactors can operate for long periods with selected, single cultures but, for yoghurt, maintaining the balance of cocci : rods could prove more difficult.

It has been suggested also that if the beads could be suspended in a physiologically neutral medium and perhaps deep frozen, this approach could provide an alternative means of preservation, with the supporting matrix acting as a protective agent. However, at the present time, the technology appears to be used for experimental purposes only.

8.4 Production systems for starter cultures

8.4.1 Introductory remarks

It is evident from the above information that the preserved cultures are relatively lower in activity compared with liquid culture. As a consequence, DVI starters (e.g. concentrated freeze-dried or frozen cultures) tend to show slightly longer lag phases.

Although the cell concentration is in the region of 10^9–10^{12} cfu ml^{-1}, the inoculation rate is relatively small. The use of higher inoculation rates is not recommended for two main reasons. First, it increases cost of production and second, it leads to excessive metabolic activity by the starter which can mean difficulties in controlling the fermentation process and the yoghurt can be of an inferior quality (i.e. bitter). In addition, the larger the inoculum of the starter culture (including liquid cultures), the greater the tendency for whey syneresis to occur in the retail yoghurt. Furthermore, the longer lag phase needed by these cultures is an indication that their metabolism at the time of inoculation is at a very low level, and hence more time is required for the essential adaptation. Incidentally the quality of the milk must be very good, because the presence of any inhibitory agents (e.g. antibiotics or detergent residues) can ultimately reduce the activity of the starter culture.

Currently, yoghurt starter cultures constitute mixed strains of different microorganisms. According to Stenby (1998) some of the criteria used to select strains for starter culture blends are:

- Acidity: Mild to medium or sharp taste in end product
  Post-acidification during storage (i.e. ability of strains to produce acid at low temperatures)
  Level of acetic acid (i.e. only for bio cultures)
- Flavour: Low, medium or high content of acetaldehyde
- Viscosity: Low, medium, high or very high
- Fermentation: Short (about 6 hours) or long (up to 16 hours) incubation
- Bacteriophages: Blend of bacteriophage unrelated strains

In some countries the statutory regulations may stipulate that there be a ratio of 1:1 between *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, a minimum number of cfu ml^{-1} in the final product and a pH level <4.4. Such constraints will limit the options of yoghurt manufacturers to diversify and provide consumers with a wide range of products. In some countries for instance, *L. delbrueckii* subsp. *lactis* and *L. helveticus* are blended with yoghurt organisms or in bio cultures other lactobacilli species and *Bifidobacterim* spp. are used. Thus, in order to maintain the starter culture characteristics mentioned above and to maintain the desirable counts of probiotic organisms (e.g. *L. acidophilus, L. paracasei* subsp. *paracasei, Lactobacillus rhamnosus* and/or *Bifidobacterium* spp.), the use of DVI starter added directly into the milk base (see Fig. 8.1, System 3) has become a popular practice in the industry. However, there is still a demand, especially in large factories, for traditional mixed starter cultures consisting only of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* for the manufacture of yoghurt.

Therefore, as illustrated earlier in Fig. 8.1, there are two main methods for the production of bulk starters. The first method (System 1) is a simple scale-up system

of starter propagation (stock → mother → feeder or intermediate → bulk); however, the second method (System 2) is DVI inoculation of the bulk starter. In either system the aim is to produce a pure active culture free from contaminants, mainly bacteriophages, and the different methods which have been devised may be divided into three main categories, simple microbiological techniques, employment of mechanically protected equipment and tanks and propagation of starters in bacteriophage resistant/inhibitory medium (BRM/BIM).

8.4.2 Simple microbiological techniques

In this system the equipment/materials are basically laboratory utensils and a starter tank and may consist of glass test tubes, McCartney bottles, 250 ml flasks (for propagation of mother culture), 2–5 l flasks (for production of feeder culture) and graduated and Pasteur pipettes.

Reconstituted skimmed milk powder ($10–12 \, g \, 100 \, g^{-1} \, TS$) is used as the growth medium and the glassware containing the milk is plugged with non-absorbent cotton wool. The whole is sterilised in an autoclave at 121°C for 10 min for small volumes (up to 250 ml) or for 15 min for larger quantities (2–3 l). However, the milk for the feeder culture is normally only steamed for 1 hour. It is recommended that a sample of sterilised milk for the mother cultures should be incubated at 30°C or 50°C for 2 days prior to use in order to check its sterility. Pipettes are sterilised in an oven at 160°C for a minimum of 2 hours.

The reactivation and subculturing procedures must be carried out under extremely hygienic conditions. For example, the freeze-drying ampoule is wiped with alcohol before breaking the glass, or alternatively, if a liquid stock culture is used, the lip of the test tube or McCartney bottle must be "flamed" over a bunsen burner when the cotton wool or the screw cap is removed, and again immediately before replacing it. It is also recommended that the starter working area and atmosphere must be clean (i.e. the air must be filtered) and, if possible, the whole starter laboratory should be under positive pressure so that unfiltered air does not enter the room whenever the door is opened. Alternatively, subculturing can be carried out under a laminar-flow hood to reduce the possibility of airborne contamination.

The production of a bulk starter using this system requires a simple tank design (i.e. batch pasteuriser/starter incubator). The tank is not pressurised and at the point of subculturing the lid is opened and the starter is poured into the milk. Very small quantities (45 l) of bulk starter can be produced in an ordinary milk churn or similar stainless steel container. A water bath or thermostatically controlled cabinet may be used as a combined pasteuriser, cooler and incubator for the production of limited volumes of bulk starter.

It is worthwhile mentioning at this stage that this method can also be used for the production of the feeder culture used for inoculating the mechanically protected Jones tank (refer to the review of Tamime, 1990).

8.4.3 Mechanically protected systems

Two aspects of starter production in mechanically protected systems are important: first, the growth medium is heat treated and cooled to incubation temperature in a completely enclosed vat and second, the inoculation of the starter takes place through a barrier which prevents the entry of air. Since 1950 there has been a great

improvement in starter culture equipment, mainly due to the centralisation of fermented dairy products manufacture and hence the demand for large quantities of bulk starter. As a result, different types of mechanically protected system have been developed; however, since the publication of the first edition of this book, few technical developments have occurred with respect to the design and structure of these tanks. The topic has been extensively reviewed elsewhere (see below for further information). As a consequence and in view of the wider application of DVI of milk for manufacture of yoghurt, only the main systems are described. Some examples of mechanically protected systems are given below.

8.4.3.1 The Lewis system

The development of this technique is well documented by Lewis (1956, 1987) and Cox and Lewis (1972) and involves the use of two-way hypodermic needles to carry out the transfer of stock to mother culture, mother culture to feeder culture to bulk starter; all inoculations take place through a barrier of chlorinated water. In order to facilitate easy transfer of the cultures during each stage of propagation, re-usable, collapsible polythene bottles are used (115 ml and 850 ml capacity) for the mother and feeder cultures, respectively. The polythene bottles are fitted with Astell rubber seals and a screw cap. These bottles are filled with the growth medium (10–12 g 100 g^{-1} reconstituted skimmed milk free from antiobiotics), sealed and capped; the contents are thus isolated from aerial contamination throughout the sterilisation, inoculation and incubation stages. At the point of intermediate transfers, the annular space of the Astell rubber seal is flooded with 100–200 mg l^{-1} hypochlorite solution, and finally the bottle containing the established culture is squeezed to discharge the inoculum. The overall technique is illustrated schematically in Fig. 8.5.
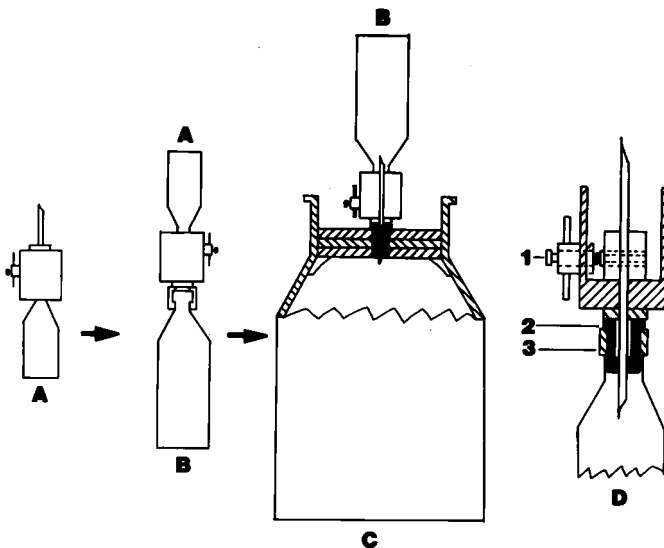
Fig. 8.5 Schematic illustration of the Lewis system for culture transfer

A, Mother culture; B, feed/intermediate culture; C, bulk culture; D, needle assembly (1, tap; 2, Astell seal; 3, hypochlorite solution).

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Fig. 8.6 On-site view of the Lewis system for culture transfer through a sterile water barrier

For the Lewis system, the milk is heated in a tightly sealed vessel and for safety reasons the tank is fitted with a pressure relief valve. During the heating stage some air may escape, but when the milk is cooled, no air enters the tank. The stainless steel pressure vessel is totally submerged within an insulated water tank, which provides maximum protection from aerial contamination as well as maintaining a constant temperature during incubation. The agitator shaft is fitted with a double mechanical seal, and water under pressure is fed to the seal housing to ensure efficient protection against contamination, cooling and lubrication. The transfer of the feeder culture to the bulk tank is carried out through a sterile barrier (i.e. water containing sodium hypochlorite solution). Figure 8.6 illustrates an on-site view.

8.4.3.2 The Jones system
The Jones tank is not a pressurised starter culture vessel, since air in the head space of the tank is forced out during heat treatment of the milk and enters again during the cooling stages. However, a slight positive pressure inside the tank can be achieved by incorporating a fan unit in the air filtration/sterilisation system. Detailed specifications of this bulk starter system have been reported by Tamime (1990) including a combined Lewis/Jones system.

8.4.3.3 Sterile and filtered air systems
Different types of bulk starter tank using filtered, sterile air (under positive pressure) have been made available to dairy processors in many countries by the major dairy equipment suppliers. One typical example using high efficiency particulate air (HEPA) filtration systems on bulk starter tanks was studied at NIZO (in the Nether-
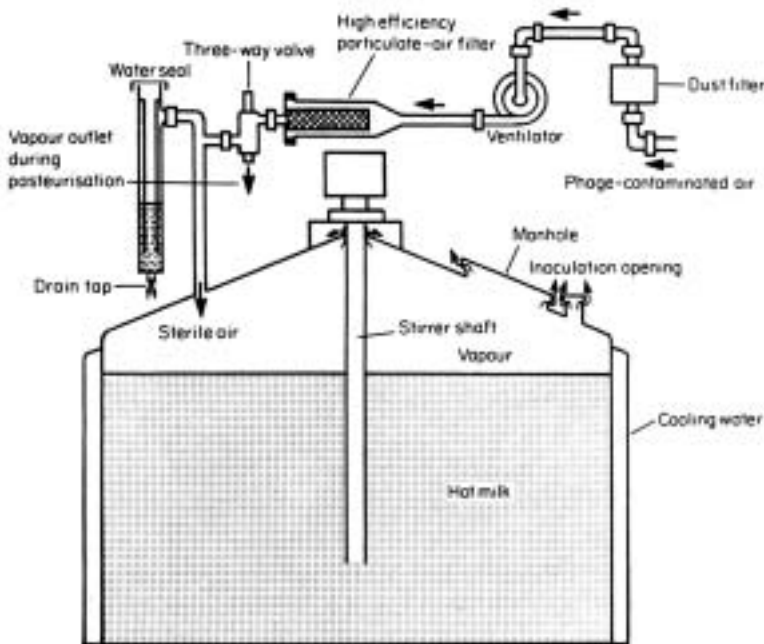
Fig. 8.7 Cultivation tank for production of bulk starter with over-pressure of sterile air

After Stadhouders *et al*. (1976) and Stadhouders (1986). Reproduced by permission of *North European Dairy Journal* and *Netherlands Milk and Dairy Journal*.

lands) in the 1970s and an illustration of the tanks is shown in Fig. 8.7 (see also Stadhouders *et al*., 1976; Tofte-Jesperson, 1979; Stadhouders, 1986). Leenders *et al*. (1984) evaluated the effect of the HEPA filter [ultrapolymembrane PF-PP 30/3 (0.2 μm HF)] on air entering bulk starter tanks in factories and observed that less than one phage out of 1.9×10^8 phage passed through – this is a priority requirement.

8.4.3.4 The Tetra Pak system

This system is described in detail by Baudet (1983) and Bylund (1995) and in principle is somewhat similar to the Lewis method except in two respects. First, the tank is of a different design and is fitted with a special filter consisting of hydrophobic paper with prefilters on each side; the whole filter unit is enclosed in a protective casing. During the heat treatment of the milk, the air diffuses out through the filter from the tank, and vice versa during the cooling stages. It is critical that the filter sterilises the air to reduce the effect of airborne contamination. Second, in the Lewis system, starter transfer from one container to another relies entirely on squeezing the collapsible polythene bottle to eject the culture, while the Tetra Pak method uses sterilised air (Fig. 8.8).

Glass bottles are used for the propagation of the mother culture and stainless steel cannisters for the feeder/intermediate stage (see Fig. 8.9). The bottles are sealed with rubber stoppers and a metal screw cap with an annular space. During culture transfer two disposable sterile syringes are used. The first syringe, which is short, is connected to the air supply and is fitted with an aseptic filter to sterilise the air. The second syringe is long enough to reach the bottom of the glass bottle and
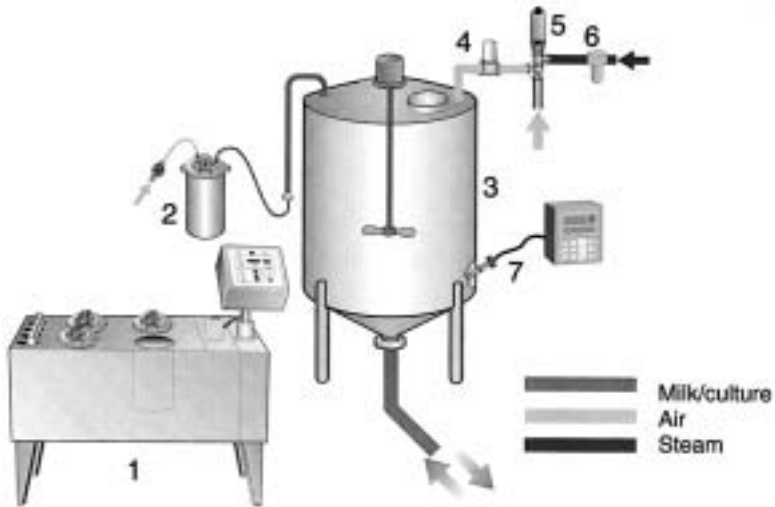
Fig. 8.8 Starter culture production using an aseptic transfer system

1, Incubator known as Viscubator; 2, feeder/intermediate culture container; 3, bulk starter tank;
4, HEPA filter; 5, air valve; 6, steam filter; 7, pH measurement unit.

Reproduced by courtesy of Tetra Pak (Processing Systems Division) A/B, Lund, Sweden.
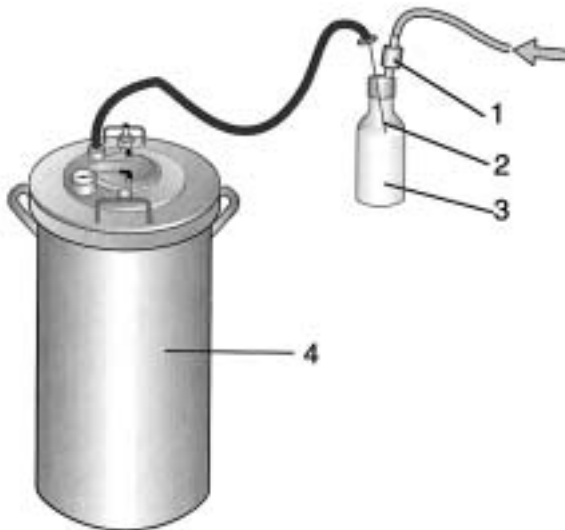
Fig. 8.9 Aseptic transfer of mother culture to feeder/intermediate culture container

1, Sterile filter; 2, aseptic needle; 3, mother culture glass bottle; 4, stainless steel feeder/intermediate
container.

Reproduced by courtesy of Tetra Pak (Processing Systems Division) A/B, Lund, Sweden.

is connected to the feeder container. Thus, when the supply is switched on, air is sterilised through the filter and enters the bottle via the short needle. This results in a buildup of pressure in the head space of the bottle, forcing the culture through the long needle into the feeder cannister. Incidentally, the bottles containing the skimmed milk are normally autoclaved and then cooled to the appropriate incubation temperature. The stock culture is injected into the bottle of the mother culture using a sterilised syringe inserted through the membrane or, alternatively, the freeze-dried stock culture is added into the bottle under aseptic conditions (i.e. the bottle cap is unscrewed in a laminar flow cabinet and the dried culture is added; another approach is to rehydrate the culture in sterilised milk and inject it into the bottle using a sterile syringe).

The feeder/intermediate culture is prepared using specially designed stainless steel containers as follows:

- Fill containers with skimmed milk and secure closure of the lid.
- Heat to 95°C for 30–45 min and cool to incubation temperature using the Viscubator (see Fig. 8.8).
- Transfer mother culture as described above, cool to <10°C; the culture is then ready to inoculate the bulk starter tank.

The feeder/intermediate containers have two special fittings, one for compressed air, and the other in the form of a tube made of stainless steel pipe which connects to the bulk starter tank during culture transfer. These fitments are equipped with special valves with quick release couplings. An on-site illustration of the feeder/intermediate culture container showing the pipe connections is shown in Fig. 8.10. A similar system of bulk starter production was reported by Rasic and Kurmann (1978) and an overall schematic illustration of culture transfer from feeder/intermediate container to the bulk starter tank is shown in Fig. 8.8 (see also Tamime, 1990; Bylund, 1995).

According to Bylund (1995), it is normally recommended that two tanks should be used in rotation; one contains ready-made starter for use and the other is for
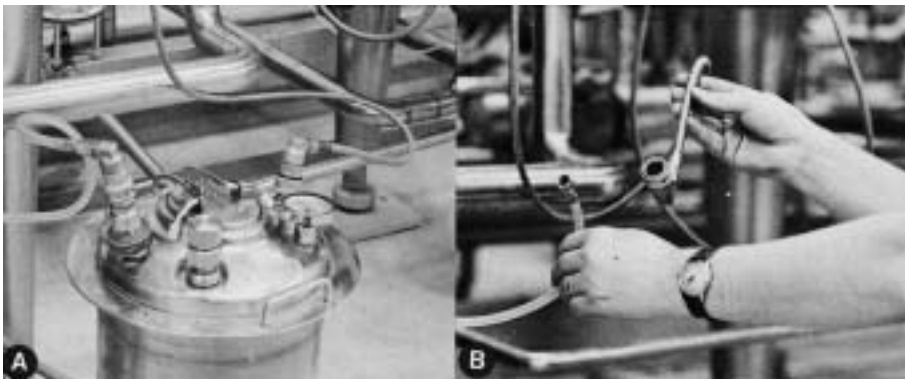
Fig. 8.10 Pipes, valves, connections and fittings for the feeder/intermediate culture container

A, Temperature dial, right hand pipe for air supply; the left hand plastic pipe is for outlet. B, Special valve fitments with quick release coupling; the pipe description is as (A).

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preparing starter for the following day. The specifications of the bulk starter tank could be summarised as follows:

- The tank is of an aseptic design (i.e. hermetically sealed and triple jacketed).
- It is capable of withstanding negative and positive pressures up to 30 and 100 kPa, respectively.
- The agitator is operated via a two-speed motor and the shaft of the agitator is double sealed.
- It is fitted with HEPA filters (see Fig. 8.8) which can be sterilised by steam at 140°C and a stationary pH meter designed to withstand the extreme temperature differences during the cleaning of the tank, preparation of the milk and production of the starter culture.

8.4.4   pH control systems

Bulk starter systems using pH control techniques were produced and developed for the following reasons:

- To overcome the drawbacks associated with BRM/BIM (see Section 8.4.5), including the cost of such media.
- To minimise daily fluctuations in acid development of the conventional cheese bulk starter (i.e. over-ripe or less active) that occur under commercial practice (see Pearce and Brice, 1973; Walker *et al.*, 1981).
- To produce concentrated starter cultures at high pH about 5 (i.e. reducing the cellular damage that may occur in certain starter cultures held for long duration at low pH) and, as a consequence, to require less culture for production.

Two methods are available for production of starter cultures using the pH control system: external pH control and internal pH control. To our knowledge these systems are not used for the production of yoghurt bulk starter cultures, but for further information see the reviews by Sinkoff and Bundus (1983), Thunell and Sandine (1985) and Tamime (1990).

8.4.5   Bacteriophage resistant/inhibitory medium (BRM/BIM)

BRM/BIM are also referred to as phage resistant or inhibitory medium (PRM/PIM) and the basic ingredients are milk solids, sugar, stimulatory compounds (yeast extracts, pancreatic extracts and/or hydrolysed cereal solids), phosphate–citrate buffer and chelating compounds (ammonium or sodium phosphates). The latter compounds are essential to bind the free calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions in the growth medium, and in particular Ca^{2+} which is required by bacteriophages during their proliferation and replication. Such growth media have been formulated mainly for cheese starter cultures and to a very limited degree for *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*; furthermore, the data compiled by Tamime (1990) on BRM/BIM indicate that, except under certain conditions, they are not very effective. It is important to note that phosphates in the bulk starter milk adversely affect the growth of *L. delbrueckii* subsp. *bulgaricus* (see Chapter 6, Section 6.3.10). At the present time, therefore, BRM/BIM are not widely employed in the yoghurt industry and it is safe to conclude that although such an approach may result in success with the mesophilic lactic starter cultures, its application for

the production of phage-free, yoghurt bulk starter cultures is limited. However, another approach to control the effect of bacteriophages on yoghurt organisms is the addition of formic acid to the culture (Lembke *et al*., 1987).

8.5   Conclusion

Since the 1950s, there have been many developments in the field of starter culture technology (i.e. preservation, maintenance and production). The ultimate objectives of this work were to secure the availability of different strains of yoghurt starter cultures for the dairy industry, to ensure the purity and activity of these culture(s), and to devise appropriate systems for their use in the production of bulk starters in a creamery. Mechanically protected starter tanks were developed primarily for the cheese industry in order to control the proliferation of phage during the production of bulk starter cultures, but in view of the fact that *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* are also vulnerable to bacteriophage attack (see Chapter 6), the same precautionary methods have been adopted in the yoghurt industry.

At present there is a growing tendency for yoghurt producers to use concentrated freeze-dried and frozen cultures for the production of bulk starters and/or yoghurt, especially when using bio starters or tailor-made blended starters to produce the desirable characteristics in yoghurt (e.g. mild or sharp taste, low or high in acetaldehyde and/or low or high viscosity). However, some of these cultures may pose a problem during culture transfer with some mechanically protected bulk starter systems. Rehydration of the freeze-dried culture in a sterile liquid or in the case of cultures packaged in cans (ring-pull type), the transfer of either the rehydrated or the thawed cultures to the bulk starter tanks, is carried out using a sterile hypodermic syringe, and hence it could be difficult to employ these cultures in conjunction with certain types of tank. Similar difficulties arise with the pelletted concentrated frozen cultures, where thawing prior to inoculation is not recommended, and with concentrated freeze-dried cultures, particularly when using the Lewis system. However, these difficulties will be readily overcome as starter culture technology progresses and the production of bulk starters within a creamery will no longer be required as DVI systems become more widely used.

8.6   References

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9

Nutritional value of yoghurt

9.1 Introduction

The chemical composition of a foodstuff provides a useful indication of its potential nutritional value and the data shown in Table 9.1 indicate the main components of some typical natural and fruit yoghurts. If these figures are accepted at face value, then it is evident that yoghurt could prove to be an important introduction to any diet, with the precise impact depending upon the type of yoghurt being consumed. At the same time, it must be accepted that numerical values reveal only part of the story, and even if the almost mystical properties ascribed to yoghurt are ignored for the moment, there are some aspects of the behaviour of yoghurt in the human body that are not revealed by chemical analysis (Robinson, 1977).

It is of some interest, therefore, to look at the constituents of yoghurt in a little more detail and, in particular, to assess the likely nutritional importance of the materials concerned. Earlier studies of the nutritional aspects of yoghurt have been reviewed by Deeth and Tamime (1981) and Alm (1982) and perodically the International Dairy Federation publishes monographs updating the nutritional properties of fermented milks including yoghurt (IDF, 1983a, b, 1988, 1990, 1991, 1992; see also the review by Gurr, 1982). In addition, the following are recommended for further reading on the nutritional properties of yoghurt (Amer and Lammerding, 1983; Renner, 1986; Rao *et al*., 1986; Rasic, 1987; Bourlioux and Pochart, 1988; Driessen and de Boer, 1989; Biacs and Beczner, 1990; Berner and Lofgren, 1991; Mann, 1993; Khedkar *et al*., 1993, 1994; Bronzetti, 1994; Gurr, 1987, 1994). Some textbooks (Renner, 1983, 1989; Chandan, 1989) detailing the importance of dairy products in human nutrition together with the proceedings on fermented milks and health which took place at the Netherlands Institute for Dairy Research (NIZO) in the late 1980s (Anon., 1989) deserve a mention.

9.2 Carbohydrates

9.2.1 Available carbohydrates

The expression "available carbohydrates" is intended to cover all those carbon compounds that can be assimilated by the human body and hence can act as a source of energy for metabolism. In the case of natural yoghurt (see Table 9.1), a number of mono- and disaccharides are present in trace amounts, but lactose remains the dominant sugar in natural yoghurt; even after fermentation, the product may contain some 4–5 g 100 g^{-1} lactose (Tamime, 1977; Scrimshaw and Murray, 1988; Barrantes *et al.*, 1994). The reason for this residue is that the process milk is often fortified to 14–16 g 100 g^{-1} TS (total solids) (i.e. up to about 8 g 100 g^{-1} lactose), so that the lactose content of the end product is little different from normal milk. What is different, however, is the effect that these apparently identical levels of lactose can have on people who are so-called lactose-intolerant or lactose maldigestors and the nature of this reaction is of considerable medical interest (Gilliland, 1991).

Lactose intolerance is the inability of humans to metabolise lactose (Rao *et al.*, 1985; Scrimshaw and Murray, 1988; Lerebours *et al.*, 1989; Fernandes and Shahani, 1989a; Dupont and Gendrel, 1992; Alm, 1993). However, most children possess, at birth, the ability to secrete the enzyme lactase (β-galactosidase), so that the lactose in mother's milk is readily broken down into glucose and galactose. These monosaccharides, and especially glucose, are readily metabolised, but as the energy demands of the child increase, so other foods become more important. In many communities this change means that milk plays an increasingly unimportant role in the diet, and as lactose intake falls, so the secretion of lactase declines. A point is then reached quite early in development when lactose can barely be assimilated at all and the free lactose produces a range of unpleasant symptoms, such as abdominal bloating, cramp and diarrhoea. These problems arise through the heterofermentative metabolism of lactose by the natural microflora of the colon, and the gas produced by the coliforms, for example, gives rise to extreme discomfort. This reaction

Table 9.1 Some typical values of the major constituents of milk and yoghurt (all units 100 g^{-1})

Constituent	Milk		Yoghurt[a]			
	Whole	Skim	Full fat	Low fat	Low fat/fruit	Greek-style
Water (g)	87.8	91.1	81.9	84.9	77.0	77.0
Energy value (kcal)	66	33	79	56	90	115
Protein (g)	3.2	3.3	5.7	5.1	4.1	6.4
Fat (g)	3.9	0.1	3.0	0.8	0.7	9.1
Carbohydrate (g)	4.8	5.0	7.8	7.5	17.9	NR
Calcium (mg)	115	120	200	190	150	150
Phosphorus (mg)	92	95	170	160	120	130
Sodium (mg)	55	55	80	83	64	NR
Potassium (mg)	140	150	280	250	210	NR
Zinc (mg)	0.4	0.4	0.7	0.6	0.5	0.5

[a] The nutrient levels in fruit yoghurt will vary with the type of fruit and stabiliser.
NR: Not reported.

Adapted from Holland *et al.* (1991) and Buttriss (1997).

to the ingestion of milk is usually referred to as primary lactose intolerance, and Garza and Scrimshaw (1976) have described a clinical test to confirm this form of deficiency. Similar reactions can, of course, be observed in patients suffering from a congenital absence of lactase, or from walls of the intestine that have become severely disfigured as a consequence of malnutrition but, in the present context, it is the widespread primary intolerance that is most relevant.

Thus, the occurrence of this primary reaction is observed extremely rarely among Europeans who consume milk or processed milk products throughout their lives, but is a common phenomenon in communities where supplies of liquid milk are scarce or erratic. Yet curiously enough, these latter groups may well rely on the production of various types of yoghurt to provide an outlet for any milk which is available, and the failure of lactose in yoghurt (as against lactose in liquid milk) to provoke an intolerance reaction is something of a curiosity.

The most obvious explanations are that either the micro-organisms in the yoghurt continue to metabolise the lactose even after ingestion or the organisms undergo lysis during digestion and the lactase so released ensures that the level of lactose reaching the colon is too low to cause an adverse reaction (Gallagher *et al.*, 1974; Desmaison *et al.*, 1990).

As some strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* are tolerant of low pH, it is feasible to suggest that some breakdown of lactose does continue in the stomach, particularly as the bacteria may be protected, to some extent, within the yoghurt coagulum which can act as a pH buffer. However, a more likely sequence of events is that the low pH of the stomach kills the yoghurt bacteria and that the cell walls of the bacteria then protect the β-galactosidase against the stomach acid. Thus, at pH < 3.0, lactase is rapidly destroyed *in vitro* but, within a bacterial cell, it could pass intact into the alkaline conditions of the intestine (Martini *et al.*, 1987b). Here the bile salts would be expected to lyse the cells (Gilliland and Kim, 1984), so releasing the enzyme into the intestine where it can act on the ingested lactose; some evidence to this effect was found by Goodenough and Kleyn (1976).

One further effect that may be relevant in this context is that yoghurt is already coagulated prior to entering the stomach, while liquid milk is clotted by the acid/enzymes in the body (Davis and Latto, 1957). This difference could mean that the yoghurt coagulum remains partially intact after ingestion, and hence that the lactose remains in the proximity of the disintegrating bacterial cells/escaping lactase (Shah and Jelen, 1991).

Most studies on humans who have been identified as lactose intolerant are in agreement with each other. Such subjects after ingesting live yoghurt had reduced levels of hydrogen secretion in their breath (see Fig. 9.1) and there were fewer reports of diarrhoea or flatulence (Kolars *et al.*, 1984; Savaiano, 1990; Mustapha *et al.*, 1997). Such results indicate that yoghurt, when compared with milk, facilitates the metabolism of lactose due to the intraintestinal digestion of lactose by β-galactosidase released from *Streptococcos thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (Rao *et al.*, 1991). Similar reduced breath hydrogen responses in adult lactose maldigestors were observed when tested against different types of yoghurt (i.e. low or full fat, lactose hydrolysed and frozen) (Martini *et al.*, 1987a; Rosado *et al.*, 1992). The same authors and Onwulata *et al.* (1989) concluded that endogenous lactase originating from the yoghurt micro-organisms is superior to exogenous commercial lactase preparations in alleviating lactose maldigestion. In addition, Pochart *et al.* (1989) have demonstrated that viable yoghurt organisms can, due to the
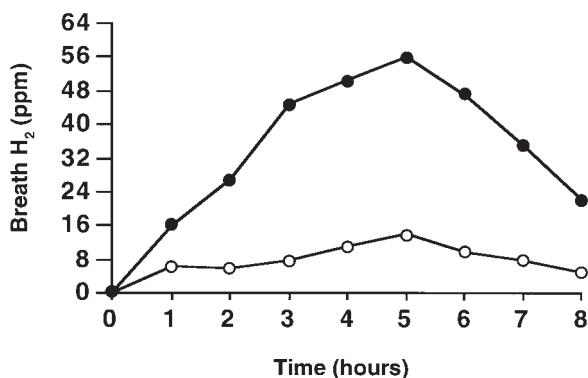
Fig. 9.1 Changes in breath hydrogen for humans ($n = 10$) after ingestion of milk (–●–) or yoghurt (–○–)

Data adapted from Kolars *et al.* (1984).

buffering capacity of the product, reach the duodenum and do have active β-galactosidase. Thus, the starter cultures are to some extent protected from gastric acid secretion and the retention of the microbial lactase inside the cells prevents it from hydrolysing the lactose in the duodenum; mixed yoghurt cultures displayed the greatest β-galactosidase activity, followed by *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (Ordonez and Jeon, 1995).

This degree of physiological acceptability means that yoghurt can provide a useful source of energy in the diet of many consumers, and it is important that while natural yoghurt contains around 6.4 g of carbohydrate $100 g^{-1}$, fruit yoghurts may contain up to $18–20 g 100 g^{-1}$ of sucrose and other available carbohydrates (Table 9.1). If each gram of sugar provides around 4 kilo-calories of usable energy, then the contribution of yoghurt towards combating a dietary deficit of carbohydrates can be appreciated, a feature that is enhanced in many brands by the use of modified starch as a stabiliser and at concentrations that may approach $1 g 100 g^{-1}$ of yoghurt.

Lactic acid is synthesised by the starter culture from lactose which is the principal substrate present in milk (Zourari *et al.*, 1992). Lactic acid occurs in two isomeric forms: L(+) and D(−). In yoghurt, *S. thermophilus* produces the L(+) form, while *L. delbrueckii* subsp. *bulgaricus* releases the D(−) isomer or a racemic mixture DL depending upon the strain. In nutritional terms, the L(+) isomer is the easily digested form and its contribution to the total concentration in yoghurt will vary with the ratio of *S. thermophilus* : *L. delbrueckii* subsp. *bulgaricus*; it is usually between 50 and 70% of the total (Kunath and Kandler, 1980). By contrast, the D(−) isomer is poorly metabolised and an excessive intake is reported to cause acidosis in some children.

9.2.2 Unavailable carbohydrates

Although natural yoghurt is based entirely on milk, stirred fruit yoghurts usually have stabilisers incorporated to reduce whey separation during distribution. The usage of these stabilisers has been considered in detail elsewhere (see Chapter 2),

but it is worth noting that many of them are complex carbohydrates. Thus, guar gum, locust bean gum, as well as the carrageenans and cellulose derivatives are long chain polysaccharides composed of regular arrangements of monosaccharide units and it is significant, in the present context, that the molecules cannot be attacked by digestive enzymes in the human body.

It is for this reason that these hydrocolloidal materials are often referred to as unavailable carbohydrates (Robinson and Khan, 1978) and as such they may contribute to human nutrition by:

- Providing a bulking agent for the contents of the intestine, so stimulating intestinal peristalsis and avoiding some of the risks of colonic malfunction.
- Absorbing some of the potentially toxic chemicals that may be formed in the large intestine as the result of bacterial action.
- Acting to delay the diffusion of sugars to the intestinal wall, a function that could help those prone to postprandial hyperglycaemia. Thus, the surge in insulin production that is required after each meal in order to stabilise the level of glucose in the blood, places an undesirable strain on the hormonal system of even normal subjects and for mild or incipient diabetics, the sudden demand poses particular problems. If the inclusion of unavailable carbohydrates in the meal reduces the rate of entry of glucose into the blood, then the stimulus for insulin production will also decline and this trend towards homeostasis can be regarded as biologically attractive.
- Lowering the cholesterol level in the blood (Jenkins *et al.,* 1975; Roberfroid, 1993).
- Acting in conjunction with the coagulated protein to slow the oro-caecal transit time of lactose, so allowing the microbial lactase to ensure that lactose-intolerant consumers do not suffer discomfort (Marteau *et al.*, 1990).

The level of stabiliser incorporation is, of course, rather low (about $0.5\,g$ in $100\,g^{-1}$), and there is tendency nowadays to avoid their use altogether because some of the plant gums have become expensive and because the less expensive forms often give the product an unacceptable mouthfeel. Nevertheless, some brands of yoghurt do contain gums (Anon., 1990), and Saldamli and Babacan (1996) incorporated sugar-beet fibre into yoghurt at levels of up to $2\,g\,100\,g^{-1}$ without any adverse effect on flavour.

9.3   Protein

The proteins in milk are of excellent quality biologically and both the caseins and whey proteins (α-La and β-Lg) are well endowed with essential amino acids. An indication of levels encountered is shown in Chapter 7 and it is clear that milk is a most valuable dietary component. The fact that the protein content of yoghurt is often elevated by concentration or addition of skimmed milk solids, means that it is an even more attractive source of protein than liquid milk (Table 9.1). The relevance of this point is highlighted by the number of protein-enriched yoghurts that are available in industrialised countries. Consumption of around 200–250 ml of yoghurt per day can easily provide an individual with the minimum daily requirement of animal protein (15 g) (Altschul, 1965; Cheeseman, 1991).

Obviously, such data are impressive in their own right, but two further points

about the protein in yoghurt should be borne in mind. In the first place, it is important that the proteins in yoghurt are totally digestible, a feature enhanced by the fact that some degree of initial proteolysis is caused by the starter organisms themselves. The extent of this breakdown will depend on the strains of bacteria being employed but, in general, at least some release of amino acids and peptides can be expected during incubation and storage (Breslaw and Kleyn, 1973; Butikofer *et al.*, 1995).

The other pertinent characteristic is that the milk proteins in yoghurt are already coagulated prior to ingestion and, in addition to the possible effect discussed earlier, the "soft clot" formed in the stomach may have other benefits. Thus, the contrast between the ingestion of yoghurt and liquid milk has some parallel with the comparative behaviour of warm milk and cold milk for, while the caseins in cold milk form a "hard clot" in the presence of acid in the stomach, the modified caseins (see Chapter 2) in warm milk coagulate more gently (Jay, 1975). The advantages of this latter type of coagulum are alleged to be that the softer structure does not give rise to any feeling of discomfort and that the more "open" nature of the casein aggregates allows the proteolytic enzymes of the alimentary canal freer access during digestion. It is, of course, impossible to quantify, or even assess with any degree of objectivity, effects of this type, but belief in their existence is sufficiently widespread to give some credence to the general hypothesis. What is beyond dispute is that yoghurt is an excellent source of protein and that this fact alone justifies its inclusion in a diet. However, Gaudichon *et al.* (1995) studied the exogenous and endogenous nitrogen flow rate and level of protein hydrolysis in the human ($n = 16$) jejunum after feeding with ^{15}N-labelled milk and yoghurt and they concluded that: (a) endogenous N secretion was significantly stimulated 20–60 min and 20–40 min after ingestion of yoghurt and milk, respectively, (b) the endogenous N flows over a 4 hour period were similar for milk and yoghurt, whilst the exogenous N flow rates indicated a delayed gastric emptying of yoghurt when compared with milk and (c) the non-protein nitrogen (NPN) flow rate in the jejunum increased significantly after milk and yoghurt due to an increase in the exogenous NPN flow rate, which ranged between 40 and 80%, whilst the net gastrojejunal absorption of exogenous N for milk and yoghurt were similar. It was concluded that the high level of exogenous N hydrolysis reflects the good digestibility of milk and yoghurt; however, fermentation of the milk modifies only the gastric emptying rate of N.

9.4   Lipids

Although much of the yoghurt sold in industrialised countries is produced from skimmed milk, traditional yoghurt has always contained some $3–4 \, g \, 100 \, g^{-1}$ milk fat (Table 9.1); indeed concentrated yoghurt (labneh) or Greek-style yoghurts will contain $9–10 \, g \, 100 \, g^{-1}$ fat (Anon., 1997b; Buttriss, 1997). The influence of these lipid materials on the consistency and mouthfeel of yoghurt has been discussed elsewhere, but it should not be forgotten that lipids are an integral part of a balanced diet. Thus, humans have a double requirement for lipids in that they possess:

- storage fat composed of saturated fatty acids and serving as a source of energy or as a protection for vital organs;
- structural fat which, with proteins, forms many of the essential membranes in animal cells, particularly in areas like the brain.

It is essential, therefore, that the human diet provides an adequate source of fats, a point that is of especial relevance for children. Thus, with each gram of fat providing around 9 kcal, fats are a most valuable source of energy. When this figure is viewed in relation to the fact that malnutrition in children is often associated with a lack of calories to metabolise available protein, then the potential relevance of this compact source of energy is evident. It is also important that yoghurt is widely accepted by children as a foodstuff and hence developing countries, in particular, would be well advised to look closely at the merits of yoghurt for school feeding programmes. In addition to this basic advantage of consuming full fat yoghurt, it must also be stressed that milk fat contains an extremely wide range of fatty acids. Most of these are present in the form of various glycerides, but over 400 individual fatty acids have been identified in cow's milk (Patton and Jensen, 1974). Obviously, it is impossible to assign a physiological role to all but a handful of these acids, but the fact that they are present in a normal mammalian secretion merely confirms that ignorance of function should not be equated with no function.

There is, of course, every incentive for a manufacturer to remove the fat from the process milk and sell it as cream, but it is clear that, both organoleptically and nutritionally, the interests of the consumer may be better served by leaving a reasonable level in the end product. Such a proposal would not find universal acceptance, for some authorities would be concerned at the additional intake of saturated fatty acids that would be involved. However, the evidence linking fats of dairy origin with coronary and similar problems is, to say the least, tenuous, and hence yoghurt manufacturers should be encouraged to base their judgements concerning fat content on the broader concept of quality (Gurr, 1992). Whether such an aim is feasible in light of the vociferous anti-cholesterol lobby remains to be seen and, certainly in some countries like the United States, challenging consumer groups could spell financial ruin.

The tragedy of this situation is that it is the consumer who loses out and, once again for no reason capable of objective assessment. The totally irrelevant demand for nutritional labelling of yoghurt and other foods falls into the same category, because it is more than evident that the nutritional value of yoghurt cannot be summarised by a few figures stamped on the side of a retail carton. In effect, therefore, the consumer will be paying for a quite useless set of data, in that the information implies that the designated nutrients will be absorbed into the human body, whereas in fact, chemical analyses should never be equated with nutrient availability and, in the case of yoghurt, any serious consideration of its nutritional value must include the question of whether the product possesses special therapeutic properties. Clearly no label could honestly convey to a consumer that yoghurt may be more than a mere carton of chemical compounds.

9.5 Vitamins and minerals

The increase in solids-not-fat (SNF) in yoghurt as compared with liquid milk carries with it the implication that the level of inorganic ions/unit weight is also going to be higher and this view is confirmed by the data in Table 9.1. In most cases, the figures speak for themselves, but the position of calcium is perhaps, rather special in relation to a typical recommended daily allowance (RDA) of 800 mg (Weaver

and Plawecki, 1994; Anon., 1997a). Thus, not only can yoghurt act as a source of calcium for sufferers of lactose intolerance but, in addition, calcium supplied by yoghurt may be better absorbed and utilised than calcium made available in other forms (Dupuis, 1964; Rasic, 1987); the role of dairy calcium in bone metabolism and prevention of osteoporosis has been recently reviewed by Renner (1994). Phosphorus, magnesium and zinc are also well represented and it is likely that the proportions of the total concentrations available for absorption and utilisation by the body is also high (Buttriss, 1997). However, Galan *et al.* (1991) reported that, under normal conditions, increasing the daily intake of dairy products probably has no effect upon iron absorption from meals already containing appreciable amounts of milk-based components.

Yoghurt contains appreciable quantities of sodium and potassium which may not be suitable for feeding babies less than 6 months (Doyle *et al.* 1981) but, as shown in , the mineral salts in milk can be reduced prior to the production of yoghurt.

The relative availability of vitamins in yoghurt is much more difficult to assess because, unlike minerals, many vitamins are sensitive to the conditions of processing. Thus, the method of fortification, for example, the addition of milk powder or membrane processing, the heat treatment of the milk base, the strains of starter bacteria used and the conditions of fermentation can all alter the concentrations of the more important vitamins (Noh *et al.*, 1994). For this reason, the figures quoted in Table 9.2 should be regarded merely as a guide to the vitamins available in yoghurt, and hence as an indication that, although regarded by many as a convenience food, it is certainly not a trivial item in terms of potential nutritional value. The fortification of yoghurt with vitamins, such as vitamins A or C, is possible (Anon., 1997a, b), and losses over two weeks in storage are unlikely to exceed 50%;

Table 9.2 Some typical vitamin contents of milk and yoghurt (all units $100\,g^{-1}$)

Vitamin	Milk		Yoghurt		
	Whole	Skimmed	Full fat	Low fat	Low fat/fruit
Retinol (µg)	52	1	28	8	10
Carotene (µg)	21	Tr	21	5	4
Thiamin (B_1) (µg)	30	40	60	50	50
Riboflavin (B_2) (µg)	170	170	270	250	210
Pyridoxine (B_6) (µg)	60	60	100	90	80
Cyanocobalamine (B_{12}) (µg)	0.4	0.4	0.2	0.2	0.2
Vitamin C (mg)	1	1	1	1	1
Vitamin D (µg)	0.03	Tr	0.04	0.01	0.01
Vitamin E (µg)	90	Tr	50	10	10
Folic acid (µg)	6	5	18	17	16
Nicotinic acid (µg)	100	100	200	100	100
Pantothenic acid (µg)	350	320	500	450	330
Biotin (µg)	1.9	1.9	2.6	2.9	2.3
Choline (mg)	12.1	4.8	–	0.6	–

Tr: Trace.

Adapted from Deeth and Tamime (1981) and Holland *et al.* (1991).

since low fat yoghurt is very popular in many countries, fortification with vitamin A should become mandatory in order to maintain the nutritive value of milk.

Some relevant aspects of the vitamin content of yoghurt have been reported by Rao *et al*. (1984) and Rao and Shahani (1987). It is of interest that certain B group vitamins are synthesised by the starter cultures. Kneifel *et al*. (1989) monitored these vitamins in yoghurt during fermentation using eight commercially available cultures and they concluded that using short time (i.e. 3–4 hours) incubation at 42°C, the starter cultures enriched the vitamins during fermentation by more than 20%, for example thiamin (two cultures), pyridoxine (four cultures), folic acid (one culture) and biotin (two cultures). Only two starter cultures were used to compare vitamin profiles at different incubation temperatures, but it was observed that fermenting the milk at 30°C for 14–16 hours led to a lower production of folic acid, but an increased concentration of thiamin and nicotinic acid. Therefore, it is important to use selected strains of the yoghurt starter cultures and processing conditions in order to maintain the nutritional properties of yoghurt.

9.6   Yoghurt and health

Although yoghurt and similar foods have long occupied a place in the diets of peoples from the Middle East and central Europe, the western world adopted a totally casual attitude to the product until rumours of its health giving properties became rife. In particular, the views of Metchnikoff (1910) linking longevity among the hill tribes of Bulgaria with their consumption of yoghurt caused a considerable flurry of interest.

In essence, it was suggested that one aspect of approaching senility in humans involved an undesirable passage of noxious compounds from the intestine to the blood stream and that these chemicals arose from the action of putrefactive bacteria in the lower ileum and colon. If the activity of these bacteria could be suppressed, then, so it was argued, the adverse effects of their metabolic products would no longer be manifest and the individual might anticipate a longer and healthier life. Such an hypothesis sounded perfectly reasonable, and the role of yoghurt in curtailing the putrefactive bacterial action was readily explained as follows. First, the lactic acid bacteria in yoghurt are tolerant of a low pH, whereas most bacteria show optimum growth and metabolism around neutrality. Therefore, as the acidic yoghurt passed along the intestine, the lactic acid in the food and, perhaps, that still being secreted by the bacteria, would kill the undesirable microflora. Second, it was further suggested that this effect of the yoghurt was enhanced by the ability of *L. delbrueckii* subsp. *bulgaricus* to become established in the intestine, and gradually to dominate the resident microflora. This latter change ensured the continued absence of the putrefactive organisms even during periods of reduced yoghurt availability and hence the vitality of the consumer would be maintained.

At present, the consensus among scientists is that the yoghurt bacterial cultures (*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) are unable to adhere to the mucosal surfaces of the intestinal tract although, in some cases, there are conflicting results which could be attributed to strain variation, differences in experimental design and/or the results of animal studies being wrongly applied to humans.

Over the years these original ideas have been the subject of intense discussion and investigation and it has become clear that the critical factor is the microflora of

the product (Tomar and Prasad, 1989; Lascar, 1995). Thus, while yoghurt should have a microflora consisting of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* alone (Bourlioux, 1986; FAO/WHO, 1990), the more recent entrants into the market may contain *S. thermophilus*, *L. acidophilus*, *Lactobacillus paracasei* subsp. *paracasei* and/or *Bifidobacterium* spp. These latter products, often referred to as bio-yoghurts, may be similar to yoghurt in terms of chemical composition, but the impact of the microflora on the digestive system of the consumer is totally different. For this reason, the health implications of consuming these products will be dealt with separately.

9.6.1 Therapeutic properties of yoghurt

There is no doubt that bacteria in the large intestine produce a range of phenolic compounds, such as sketol and indole, which could damage living tissue. Whether they could have any discernible effect on the intestinal wall, or even be absorbed, will depend on their concentration, the ability of other gut contents (e.g. hydrocolloids) to absorb them and their residence time, but, nevertheless, there is definite concern over their possible involvement in the initiation of cancer in the lower intestine (Aries *et al.,* 1969; Sellars, 1991). Any process that tended to suppress their production could, therefore, be advantageous, and the action of lactic acid in inhibiting the growth/metabolism of the putrefactive bacteria could be one such process.

Whether, in fact, any of the acid in yoghurt can survive the neutralising effect of the bile components is open to debate, but the prospect remains that yoghurt could change, albeit slightly, the pH gradient within the intestine. If this change does occur, then there could well be a basis of truth in Metchnikoff's proposal, and certainly the traditional products of Bulgaria would have been extremely acidic (see also Friend *et al*., 1983; Hitchins and McDonough, 1989; Fernandes and Shahani, 1989b, 1990; Reid *et al*., 1990; Kotz *et al*., 1990; Marteau *et al*., 1993; Lin, 1995).

A hypocholesterolaemic action has also been attributed to yoghurt (Mann and Spoerry, 1974; Mann, 1977; Hepner *et al.,* 1979). The exact reason for this effect is not clear (Richardson, 1978), but the fact that yoghurt is more active in this respect than unfermented milk implies that some enzyme system or biochemical compound of bacterial origin may well be involved. Hydroxymethyl glutarate has been proposed as one metabolite of starter cultures that could limit cholesterol synthesis but, for the present, both the reality of the phenomenon and its possible cause remain subjects for speculation (Anon., 1987).

It has been noted in studies with rats and mice that the consumption of yoghurt, live or pasteurised, inhibited the growth of certain types of tumour, and it has been suggested that some factor in the cell walls of the bacteria could be responsible for the effect (Gilliland, 1991). Whether such results can be interpreted as applicable to humans is another matter, but it is a possible benefit of yoghurt consumption that cannot be dismissed (Morissette *et al.*, 1991).

Similarly, stimulation of the normal microflora of the gut has been attributed to the regular consumption of yoghurt and it is proposed that the lysing cells of the starter bacteria release vitamins or other growth factors that encourage the development of *L. acidophilus*, for example, in the small intestine (Robinson, 1989). Some clinical evidence does exist to support this idea but, as with many human studies, it is difficult to establish how widespread the impact of regular consumption would be in a normal population of consumers from a given community.

Table 9.3 Update of current studies of health promoting aspects of yoghurt and related products[a]

Test model or subject	Comments	References
Humans ($n = 194$)	Elderly patients (males and females ~72 years old) fed a mixture of prune whip and yoghurt improved the bowel movement against constipation and only very few required laxative.	Ferrer and Boyd (1955)
Humans	Lactinex®, a pharmaceutical preparation of *L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus*, ingested for 1 week did not reduce the incidence of traveller's diarrhoea, whilst Gotz *et al*. (1979) reported that the same product was effective in preventing ampicillin-induced diarrhoea.	Pozo-Olano *et al*.(1978)
Humans	Yoghurt and Ca^{2+} supplementation of the diet altered cholesterol metabolism in females ($n = 16$), but not in males ($n = 5$).	Bazzarre *et al*. (1983)
Humans and *in vitro*	Survival of the yoghurt organisms in human stomachs and adhesion to intestinal cells was much lower when compared with *L. acidophilus*; by careful strain selection it is feasible to achieve elevated levels of *Lactobacillus* spp. in the intestine.	Conway *et al*. (1987)
Children ($n = 156$)	Ayran (a Turkish drinking yoghurt) was used successfully to dissolve oral rehydration salts in the treatment of diarrhoea in children aged 3–48 months.	Caglayan *et al*. (1989)
Men ($n = 18$)	Eating yoghurt had no effect on plasma cholesterol levels in normolipidemic in males.	McNamara *et al*. (1989)
Children ($n = 52$)	Children aged 3–36 months with persistent diarrhoea were fed yoghurt or milk, and the results suggest a clinical advantage of feeding yoghurt.	Boudraa *et al*. (1990)
Humans ($n = 68$)	Chronic high level consumption of live yoghurt ($450\,g\,day^{-1}$ for 4 months) showed the following results: (a) no negative side effects were found in many parameters studied including cholesterol, (b) significant and potential increase in serum ionised Ca^{2+} levels, and (c) increased production of γ-interferon isolated T cells.	Halpern *et al*. (1991)
Women ($n = 13$)	Daily ingestion of yoghurt (~230 g for 6 months) containing *L. acidophilus* decreased both candidal vaginitis colonisation and infection.	Hilton *et al*. (1991)
Women ($n = 32$)	Female patients with bacterial vaginosis were treated by intravaginal application with yoghurt and the results were favourable, because the continuous adjustment of the vagina pH and implantation of lactobacilli flora are crucial in normal vagina ecology.	Neri *et al*. (1993)
Boys ($n = 9$) & girls ($n = 11$)	Five out of six lactose maldigestors had decreased symptoms and significant reduction in breath H_2 excretion following eating yoghurt.	Montes *et al*. (1995)
Humans ($n = 259$)	Results of questionnaire survey do not support the hypothesis of an increased consumption of fermented milks or dietary calcium decreases the risk of colon cancer.	Kampman *et al*. (1994a)

Table 9.3 *Continued*

Test model or subject	Comments	References
Men (*n* = 331) & Women (*n* = 350)	Studies conducted in USA suggest that: (a) total milk and fermented dairy products consumption did not relate to colorectal adenoma risk and (b) vitamin D from supplements rather than diet was slightly and universely associated with such risk among women only.	Kampman *et al.* (1994b)
Children (*n* = 49)	Different preparations of lactic acid bacteria and *Lactobacillus* GG [currently known as *L. rhamnosus* (Tamime and Marshall, 1997)] which were fed to children, and not the yoghurt starter cultures have promoted serum and intestinal response to rotavirus.	Majamaa *et al.* (1995)
Mice	Animals implanted with Ehrlich ascites tumour and fed with yoghurt showed inhibition of these cells suggesting that the antitumour factor(s) is synthesised by the starter culture.	Friend *et al.* (1982), Friend and Shahani (1984)
Rats	Both starter cultures failed to colonise the gut of germ free rats maintained on stock diet and yoghurt was administered orally; feeding of yoghurt altered the lactobacilli flora of the gut from predominantly *Lactobacillus reuteri* to *Lactobacillus salivarius*.	Garvie *et al.* (1984)
Mice (*n* = 40)	In obese mice, the hepatic lipid was significantly greater in the yoghurt (Y) diet than in the same product supplemented with dietary chromium (Y + Cr), whilst the plasma immunoreative insulin level was lower in animals fed Y + Cr which was significantly correlated with hepatic lipid and plasma cholesterol.	Li and Stoecker (1986)
Rats (*n* = 10)	Apparent protein digestion (*in vivo*) in rats was higher in the yoghurt diet.	Lee *et al.* (1988)
Rats (*n* = 36 & 20)	Yoghurt fed rats showed a significant lower incidence of gastric tumour (50%) when compared with the controls.	Morishita and Shiromizu (1990)
Rats (*n* = 40) & humans (*n* = 133 & 289)	Results *may* provide protection against tumour development, possibly via stimulation of the immune system.	Schaafsma *et al.* (1990)
Mice (*n* = 20)	Milk fermented with *L. delbrueckii* subsp. *bulgaricus* showed no effect on the humoral immune response when fed to mice, but a significant increase in the broncho-alveolar IgA level after 8 days.	Moineau and Goulet (1991a)

Mice ($n = 24$)	No stimulatory effect on the phagocytic activity of pulmary alveolar macrophages (Amø) in mice was observed after administering fermented milk made with lactococci and *S. thermophilus* + *L. delbrueckii* subsp. *bulgaricus*; the results from this work and Moineau and Goulet (1991a) suggest that the proteolytic activity of fermented milks might be implicated in the stimulation of non-specific immune system in mice rather than the degree of proteolysis.	Moineau and Goulet (1991b)
Hamsters ($n = 10$)	Yoghurt did not exhibit any bactericidal activity in the prevention of *Clostridium difficile* infection in hamsters.	Kotz *et al.* (1992)
Mice ($n = 5$)	Groups of mice were fed Deodan® [cell wall product of *L. delbrueckii* subsp. *bulgaricus* (i.e. patented by I. Bogdanov, strain tumoronecroticance B51-ATCC 218165)], which is a primer and trigger of endogenous tumour necrosis factor-α (TNFα), is useful for the treatment of neoplastic disease in humans.	Davidkova *et al.* (1992)
Mice ($n = 10$)	Mice fed with yoghurt (unheated or heated post fermentation) or milk fermented with *L. paracasei* subsp. *paracasei* LcFM exhibited higher kinetics of specific antibody responses when compared with the control (i.e. milk fed mice); the IgG$_{2a}$ levels remained stable, but the results suggest that fermented milks stimulate the systematic immune system.	Portier *et al.* (1993)
Mice	Deodan® (see above) fed to mice activated the phagocytic secretory functions of mononuclear cells and increased the host resistance to bacterial infection.	Popova *et al.* (1993)
Rats ($n = 8$)	Feeding yoghurt or high Ca^{2+} milk enhanced the resistance to *Salmonella enteritidis* infection by lowering the luminal cytolytic activity or diminishing the Fe^{2+} availability for the pathogen to grow.	Bovee-Oudenhoven *et al.* (1996)
Rats ($n = 54$)	Ordinary yoghurt had no hypocholesterolaemic effect, but the same product made with lactose hydrolysed WPC[b] and fermented milk with *B. bifidum* lowered the serum cholesterol level in the blood.	Beena and Prasad (1997)

[a] Health studies on fermented milks made with *L. acidophilus* or *Bifidobacterium* spp. are not included. [b] Whey protein concentrate.

n is the number of subjects in the study.

Up to the late 1980s most of the nutritional studies using humans or animals have been reviewed extensively by the International Dairy Federation Group F20 (IDF, 1991), and they concluded the following:

- Some data reported in the literature are not based on well designed experiments and not all the interpretations given are based on differences that are statistically significant.
- *In vitro* results cannot be found always *in vivo* and observations found in animals cannot be translated directly to humans.
- There are problems in generalising the results given the large number of types of micro-organism used.

However, substantial progress in knowledge of this subject has been reported and in an effort to update the data published by IDF (1991), Table 9.3 summarises some of the nutritional studies since the late 1980s (see also Hargrove and Alford, 1978, 1980) relating to yoghurt and other fermented milks.

9.6.2 Therapeutic properties of bio-yoghurt

It is well known that *S. thermophilus* is intolerant of acidity and hence few cells of this species will survive passage through the stomach; even *L. delbrueckii* subsp. *bulgaricus*, which is able to resist acidity to a much greater degree, is unlikely to reach the intestine in a viable state (Accott and Labuza, 1972). The resistance of these same bacteria to bile salts, including sodium taurocholate and glycolate, is also poor (Lembke, 1963), even though certain strains of *L. delbrueckii* subsp. *bulgaricus* have been implanted in the intestines of laboratory rats (Mabbit, 1977). Consequently, the general consensus is that neither *S. thermophilus* nor *L. delbrueckii* subsp. *bulgaricus* survive the digestive process in humans.

However, the new generation of so-called bio-yoghurts has a very different microflora from the traditional product, and indeed the usual absence of *L. delbrueckii* subsp. *bulgaricus* from bio-products has led to some debate about whether it is appropriate to use the term yoghurt at all (see later). Thus, in bio-yoghurts the usual flora may include *L. acidophilus, L. paracasei subsp. paracasei* or *Lactobacillus paracasei* biovar *shirota*, *L. rhamnosus, L. reuteri, Lactobacillus gasseri*, *Bifidobacterium adolescentis, B. bifidum, Bifidobacterium breve*, *Bifidobacterium infantis* and *Bifidobacterium longum*, and more recently *Bifidobacterium lactis* (Mitsouka, 1990; Romond and Romond, 1990; Speck *et al.*, 1993; Pedrosa *et al.*, 1995; Anon., 1996; Marshall and Tamime, 1997). In addition, some products contain *Bifidobacterium animalis*; this latter species is attractive for the manufacturer in that it grows more rapidly in milk than the other species of *Bifidobacterium* mentioned above but, unlike the other species, it has never been isolated from the human intestine. Certain *in vitro* studies are reported to show that strains of *B. animalis* can attach to epithelial cells of human origin but, even so, the use of the species in products alleged to have health-promoting properties is the subject of some debate.

What is important about this group is that all the species are natural inhabitants of the human intestine, unlike *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, with the lactobacilli colonising the distal portion of the small intestine and the bifidobacteria forming one of the dominant groups in the colon. In the small intestine, the lactobacilli occupy both the lumen of the gut and physically attach to specific receptors on the epithelial cells (Salminen *et al.*, 1993). In this niche, the

microflora, which will be composed of a number of species of *Lactobacillus*, occupy the surface area of the intestine, absorb nutrients, secrete lactic acid and, perhaps, antimicrobial compounds (Shahani *et al.*, 1976; Barefoot and Klaenhammer, 1983; Tamime and Marshall, 1997). One prophylactic effect of this combination of activities is that bacteria capable of causing intestinal infections cannot compete, and hence an active population of lactobacilli can provide a degree of protection against *Salmonella* spp. and other causes of traveller's diarrhoea (Alm, 1991; Marteau and Rambaud, 1996). The same high population of lactobacilli will metabolise lactose, so ensuring that the concentration of any residual sugar reaching the colon is sufficiently low to avoid adverse symptoms.

These two results of colonisation of the small intestine by lactobacilli are well established, but Sellars (1989, 1991) has raised the possibility that the same population may also stimulate the immune system of the body, lower serum cholesterol levels and offer some protection against certain forms of cancer (for further information refer to Perdigon *et al.*, 1986, 1990, 1991, 1994, 1995a, b). Some evidence in favour of these ideas does exist, but it is important to note that the results suggest that variation between individuals is critical for success or failure. In other words, it is likely that the intestinal lactobacilli are able to protect most people from food-borne infections, but the other benefits may only be manifest in a small fraction of any given population.

Consequently, great care must be exercised in statements about the health-promoting effects of bio-yoghurts, and Table 9.4 attempts to highlight those influences of ingested cultures that are widely accepted as valid. Some of the other possible advantages mentioned in Table 9.3 may be apparent in some people but, at the present time, the evidence is not convincing, a point borne out by the health claims that are currently permitted on bio-products, for example: "This product contains a culture which may improve the health of the digestive tract". Any further claim(s) would almost certainly be deemed to be misleading by any responsible advertising standards authority.

The bifidobacteria, by contrast, occupy the lumen of the colon and, more specifically, colonise the walls in very high numbers. In this zone, the various species of *Bifidobacterium* occupy the surface area of the intestine, absorb nutrients, secrete lactic and acetic acid and, perhaps, antimicrobial compounds (Gibson and Wang, 1994). However, the dominance of bifidobacteria at the wall of the colon is enhanced by the ability of the genus to metabolise mucin, a complex polysaccharide that eases the passage of faeces (Robinson and Samona, 1992). The population is able, therefore, to prevent colonisation of the walls of the colon by undesirable bacteria (e.g. *Escherichia coli*) or yeasts (e.g. *Candida* spp.) and so protect the individual from diarrhoea associated with overgrowth by yeasts or coliforms. Suppression of the growth of putrefactive bacteria in the faeces is a further advantage deriving from the presence of an active population of bifidobacteria, and it is proposed that this restriction could lower the risk of carcinogenic compounds being liberated during fermentation in the colon (Gotti, 1977; Grill *et al.*, 1995; Rowland, 1996).

Whatever the final outcome of the various controversies surrounding the precise role of the major components of the intestinal microflora, there is no doubt that they are essential for the healthy functioning of the intestine and the population levels of both the lactobacilli and bifidobacteria can be reduced dramatically by outside influences. The administration of antibiotics by the oral route is one obvious adverse factor (Colombel *et al.*, 1987), but radiation and disease can prove equally

Table 9.4 Some of health-promoting activities attributed to cultures in yoghurt and bio-yoghurt and an indication of their likely validity for humans

Action/effect	Alleged health benefit	Established in humans[a,b]
In digestive tract	Active against *Helicobacter pylori*	
	Enhanced lactose digestion	✓
	Stimulation of intestinal immunity	
	Stabilisation of Crohn's disease	
	Stimulation of intestinal peristalsis	
On intestinal microflora	Improves balance between microbial populations	Increase in faecal bifidobacteria
	Decrease in faecal enzyme activity	✓
	Colonisation of intestinal tract	✓
	Reduced carrier time for *Salmonella* spp.	
On diarrhoea	Prevention/treatment of acute diarrhoea	✓
	Prevention/treatment of rotavirus diarrhoea	✓
	Prevention of antibiotic-induced diarrhoea	✓
Other effects	Improved immunity to disease	
	Suppression of some cancers	
	Reduction in serum cholesterol	
	Reduction in hypertension	

[a] More than one publication and no conflicting evidence. [b] A tick indicates confirmed treatments in humans.

After Sanders (1994) and Saloff-Coste (1997).

destructive (Robinson and Samona, 1992). Even alcohol or strong foods like onions or garlic can damage the microflora of some people and it is interesting to speculate whether individuals within specific populations acquire microflora that are tolerant of the major food items in a typical diet.

9.7 Conclusion

The general view is that citizens of western societies are at some risk of suffering damage to their intestinal microflora and hence the question has arisen; could a damage situation be alleviated by the consumption of bio-yoghurt? In general, it is now agreed that bio-yoghurts can have a positive therapeutic effect (Sellars, 1991; Tamime *et al.*, 1995), provided that:

- The product contains at least 1.0×10^6 viable cells of *Lactobacillus* and/or *Bifidobacterium* ml^{-1} as consumed. In the U.K., the majority of bio-yoghurts appear to meet this requirement by a considerable margin (Anon., 1997c), but reports from elsewhere have been more variable (Rybka and Kailasapathy, 1995; Shah *et al.*, 1995).
- The organism is of human origin, so that not only will it withstand transit through the stomach and upper digestive tract, but it will be able to colonise/become implanted upon the epithelial walls of the lower intestine.
- Consumption is on a regular basis – perhaps 200–300 ml per week, with the exact definition of regular depending upon the individual and his/her life style.

If these provisions are met, then there can be little doubt that the cultures in bio-yoghurts can be described as oral probiotics, that is, "living micro-organisms which, upon digestion in certain numbers, exert health benefits beyond inherent basic nutrition" (Buttriss, 1997). Whether the cultures act by replacing severely damaged native microflora or merely enhance recovery of a depleted population will depend upon the specific circumstance, but there is growing evidence that the effect is real enough.

If the consumer appeal of both natural and fruit yoghurts/bio-yoghurts is also placed on record, together with their excellent performance in respect of public health, then it is evident why the market for these products is an expanding one.

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10

Quality control in yoghurt manufacture

10.1   Introduction

The quality of any food product can be defined against a wide range of criteria, including, for example the chemical, physical, microbiological and nutritional characteristics, or simply in relation to its overall appeal to potential consumers. As a result, quality has to be judged by a range of tests with varying degrees of objectivity, and yet all of them can be useful in ensuring that a product:

- is safe for human consumption with respect to both chemical or microbial contamination;
- conforms to any regulations enshrined in law, or advisory/statutory requirements laid down by public health or other local authorities/agencies;
- is capable of achieving a specified shelf life without spoilage;
- has as high an organoleptic standard as can be achieved within the existing constraints of manufacture or marketing.

An examination of some of these points implies, naturally enough, a critical laboratory assessment of the retail product, but it is essential to bear in mind that the end product can only be as sound as the raw materials from which it is made and, in hygienic terms, as "clean" as the plant in which it was manufactured. This breadth of potential for conflict means that quality control must be regarded as an all embracing concept and, furthermore, one that demands constant attention. Thus, enthusiasm in response to a crisis is of little value in maintaining standards and the successful companies are those that rate quality appraisal as a high priority. Even small firms with minimal facilities can achieve a great deal by maintaining records of simple features like incubation times, product acidity and so on, and even though the services of a consultant may be required for more specialised examinations, the value of routine monitoring should never be underestimated.

Indeed, routine has become the lynchpin of successful manufacture and is enshrined in two compatible and, to some extent, overlapping concepts – good manufacturing practice (GMP) and the hazard appraisal (analysis) critical control points

(HACCP) system. The starting point has to be the current legislative controls in the country in question and, in England, Scotland and Wales, for example, a dairy product has to conform to the following:

- Food Safety Act (Anon., 1990)
- Dairy Products (Hygiene) Regulations (SI, 1995a)
- Dairy Products (Hygiene) (Scotland) Regulations (SI, 1995b)
- Miscellaneous Food Additives Regulations (SI, 1995c)
- Sweeteners in Foods Regulations (SI, 1995d)
- Colours in Foods Regulations (SI, 1995e)
- Food Labelling Regulations (SI, 1996)
- Weights and Measures Act (Anon., 1985)
- Weights and Measures Regulations (SI, 1987)

Specifically for yoghurt, there are codes of practice that may or not be observed according to views of the producer (MAFF, 1975; DTF, 1983). In all European Union (EU) countries, labelling is covered by Council Directive 79/112 (EU, 1979) and most producing regions will have similar patterns of legislation (Pappas, 1988; Anon., 1989; Glaeser, 1992).

Assuming that, in theory at least, neither the product nor the packaging contravenes any of these Regulations, then the manufacturer must be able to demonstrate that compliance with the Regulations is being achieved in actual practice. The key word is, of course, demonstrate, for while it is anticipated that any manufacturer can produce a faulty batch of produce, what the same manufacturer must be able to show is that the fault arose despite due diligence being shown by all concerned. It was this blanket responsibility that gave rise to the HACCP concept, and the basic principles of the system are now widely accepted as the basis for responsible operation of a factory.

10.2 Principles of HACCP

10.2.1 Brief introduction
In theory, the only way of ensuring that every carton of yoghurt from a given production line is safe, from a chemical or microbiological standpoint, is to test every carton! Clearly, such a suggestion is totally ludicrous, so that instead, a representative group of cartons is withdrawn against a sampling plan appropriate for the product and the history of the plant. However, whilst this approach is essential to confirm that preset standards of hygiene are being met and that potential contaminants are at a low level or absent, the procedure can never prevent some spoiled cartons from reaching the consumer. Consequently, the emphasis within quality assurance has turned to the avoidance of problems, a concept that forms the basis of HACCP. The HACCP system aims to identify specific hazards that, if they arose, could adversely affect the safety of a food and to put in place a procedure that will either prevent a hazard arising or will be able to control the situation in a manner that reduces the risk to the consumer (Vazquez, 1988; Pierson and Corlett, 1992; Corlett, 1992; WHO, 1993; Asperger, 1994; Mortimore and Wallace, 1994; IDF, 1994a; van Schothorst and Kleiss, 1994; Loken, 1995; FAO, 1995; Anon., 1997a, 1998a).

In particular, the system identifies seven aspects of production that merit constant attention and these aspects are enshrined in seven principles:

- First – any potential hazards associated with yoghurt production from the growth/collection of raw materials through to manufacture and distribution must be identified and an assessment made of: (a) the likelihood that a given hazard will arise, and (b) the preventative measures that are necessary to reduce any inherent risks.
- Second – the precise points in the above sequence that can be controlled in order to eliminate a hazard or minimise the risk of occurrence must also be identified. If failure to control a particular hazard is a risk to public health, then the step in the process is regarded as a critical control point (CCP); if no major risk is involved, the step may be identified as a control point (CP). For example, the filling machine is a CCP, because contamination with a pathogen could present a direct risk to the consumer, whereas the failure to empty a waste bin in the same area could be treated as a CP because, however undesirable with respect to the growth of potential spoilage organisms, the failure is not likely to result in a consumer health problem. Similarly, it is important that a manufacture has control over the chemical composition of a yoghurt and the details on the label, but again such points need only be graded as CPs.
- Third – there must an established set of targets which must be achieved in order for a Section to claim control over a CCP/CP, e.g. total colony counts on product contact surfaces (CCP) or the viscosity of stirred yoghurt with agreed tolerances (CP).
- Fourth – a monitoring system must be established to record that particular facets of production are under control.
- Fifth – if the monitoring procedure indicates that a CCP/CP is not under control, then an agreed programme of corrective action must be capable of immediate implementation.
- Sixth – there must be procedures for verification that the HACCP system is working throughout the factory, e.g. the introduction of supplementary checks to ensure that the principal components of the system are operating to the required standard.
- Seventh – a system of documentation must be in place that records accurately the details of all operations, e.g. times/temperatures and microbiological parameters, but also the responsibilities of the individual operators associated with that specific section of the process.

At first glance, this approach may appear daunting but, if each stage in a manufacturing process is identified and considered as a separate entity, then isolating the areas of risk can bring considerable benefits to a manufacturer. For example, retailers have confidence in a company that has proper control over its manufacturing procedures and, for this reason, the introduction of HACCP is fast becoming an essential of operation in the commercial world. It is important, however, that no two production plants are ever identical, and hence the personnel responsible for routine examinations must exercise their discretion as to which tests are both desirable and feasible in a given situation (see also Cullor, 1997; Gardner, 1997).

Although the systems employed to monitor the quality of yoghurt fall within the HACCP umbrella, each aspect of production has, by its very nature, to be assessed

in a different way, and hence it is appropriate to deal with the separate facets of quality on an individual basis. It is relevant in this context that, although quality control is a broad concept, hygiene is inevitably a dominant feature and excellent accounts of the principles and practice of microbiological quality control in the dairy industry have been published by Lück and Gavron (1990), Jervis (1992) and IDF (1992e); anyone likely to be concerned with the hygienic aspects of production would be well advised to consult these works.

10.2.2 Implementation of a HACCP system

The successful implementation of a HACCP system demands, perhaps above all, the whole-hearted commitment of top management and the willingness of that same management to support those charged with running the monitoring procedures on a day-to-day basis. In return, each operative must know exactly the nature and extent of his/her responsibilities and that any decisions made in the interests of the Company within the confines of that remit will be approved irrespective of any adverse financial implications. To build up the necessary personnel structure and confidence to ensure smooth operation is not an easy task but, once the essential framework is in place and functional, the anticipated freedom from unforeseen crises is reward enough for the effort.

The first stage is the easiest and involves little more than the production/quality control managers drafting a flow-diagram of the overall process and annotating it with indications of the likely control points. A typical example for set natural yoghurt is shown in Fig. 10.1 (Kalantzi, personal communication) and the relative importance of the identified CPs will need to be assessed. For example, both the heat treatment and inoculation steps might be considered as critical (i.e. CCP), for if the vegetative cells of pathogens survive the heating stage and starter activity is poor, a serious public health risk could arise. By contrast, dusty cartons could lead to an avalanche of product returns as moulds grow on the surface of the yoghurt, but the actual risk of illness for any given consumer would be negligible and constitutes a CP.

Once the overall scenario has been agreed, further details have to be added. Table 10.1 gives an example of the type of reception tests that might be applied to the raw milk arriving from a farm or collection centre (Kalantzi, personal communication). Some typical specifications for these attributes are given later (see Section 10.4.1) and the selection of tests to be completed may have to be adjusted according to the situation in the laboratory. For example, the measurement of pH may be sufficient for routine purposes, provided that calibration of the meter is carried out regularly, so that the measure of acidity or clot on boiling test might be omitted. However, the total colony count might be applied on a regular basis at least once weekly to gain a more accurate picture of microbial quality. Details of targets and tolerances will be a matter for local negotiation, but all manufacturers should be seeking zero tolerance for inhibitory substances, that is, below the level of detection by the best procedure available in the country concerned. Thus, not only can antibiotic residues lead to partial starter failure, but the passage of β-lactam antibiotics like penicillin into the food chain can cause allergic reactions and even death amongst susceptible consumers. For this latter reason alone, the reception of raw milk could be rated as a CCP.
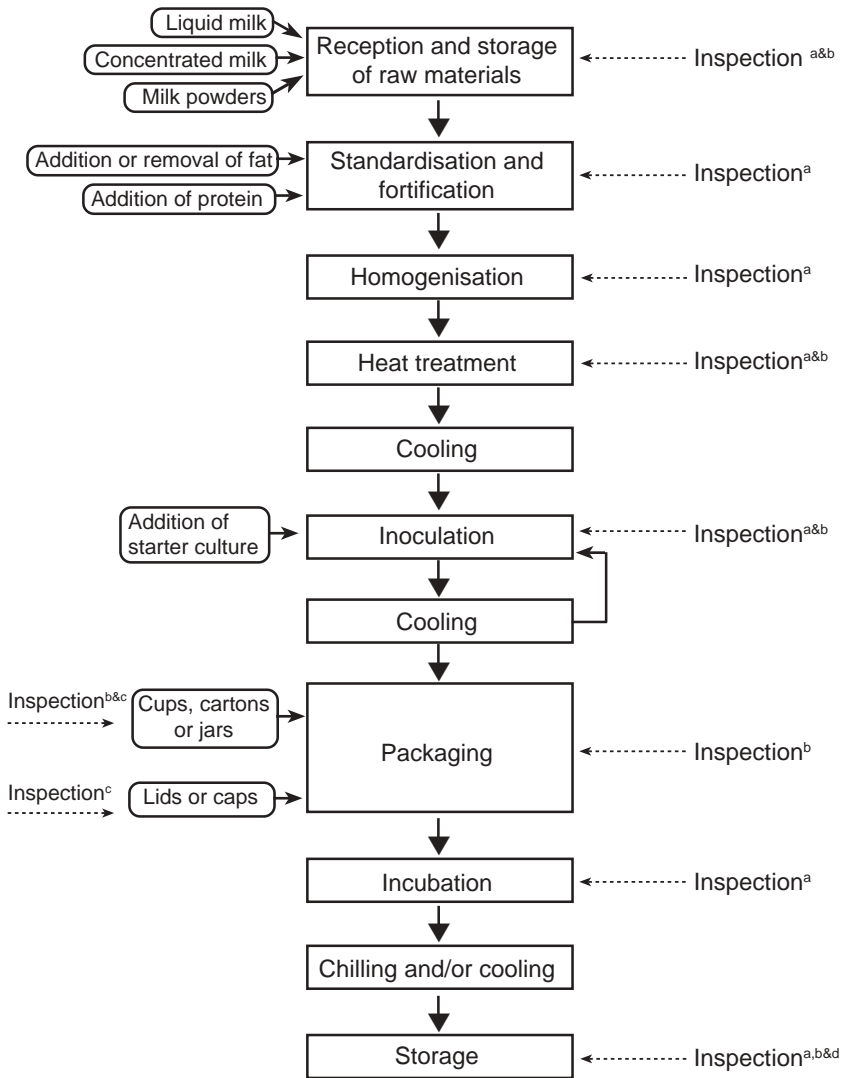
Fig. 10.1 Typical HACCP scheme for the production of set natural yoghurt
a, Physicochemical; b, microbiological; c, visual; d, organoleptic.

A similar chart can be drawn-up for other raw materials, for example, milk powder or fruit, or for a partly processed product. A case in point might be the yoghurt base prior to the addition of fruit for, if the retail product is to be acceptable to consumers, this yoghurt base must have certain defined properties with respect to acidity and viscosity; if the base is suspect, there may be little point in wasting large volumes of expensive fruit. However, these simple records are specific requirements that help to underpin the overall system, a point that is highlighted by the small section of interaction chart shown in Fig. 10.2 (Kalantzi, personal communication).

Table 10.1 Example worksheet for recording the quality of raw milk at reception

Description of the Product: Raw Milk

Type: Cow's, goat's, sheep's or buffalo's milk
Combination of Milks: % in final product (optional or if applicable)
Characteristics: see below

Analysis	Target	Tolerance	Reject
Physicochemical			
Moisture ⎫			
Fat ⎬ $(g\,100\,g^{-1})$			
Protein ⎭			
Titratable acidity (%)			
pH			
Specific gravity			
Temperature (°C)			
Antibiotics			
Clot on boiling			
Microbiological (cfu ml^{-1})			
Total viable count			
Thermoduric count ⎫			
Psychrotrophic count ⎬ Optional			
Organoleptic			
Colour			
Odour			
Foreign objects			
Origin:			
Duration at and Farm and/or collection centers:			
temperature: During transport:			
In silos:			
On-site storage temperature: <5°C			

Thus, assuming that the yoghurt base is moving along the central axis, the chart shows just some of the questions that need to be answered as the HACCP scheme evolves. In some cases, it may be agreed that the existing operation is satisfactory and only the following will need to be written in the HACCP manual:

• The identified point in the process and the required standards, e.g. the times and temperatures that must be achieved during heat treatment of the milk;
• The importance of loss of control with respect to the process, i.e. is it a CP or CCP and what are the implications of failure?;
• The designation of operatives/supervisors for each operation, and the procedures for reporting; and
• Corrective actions that may be necessary, with clear statements of responsibilities and expected outcomes.

However, at other points, new responsibilities may emerge and actions or procedures which were once taken for granted will have to be formalised in relation to questions, such as how often should samples be taken or instruments checked?; who should carry out the work?; what checks are essential?; and why, and to whom should the results be shown for analysis/action?
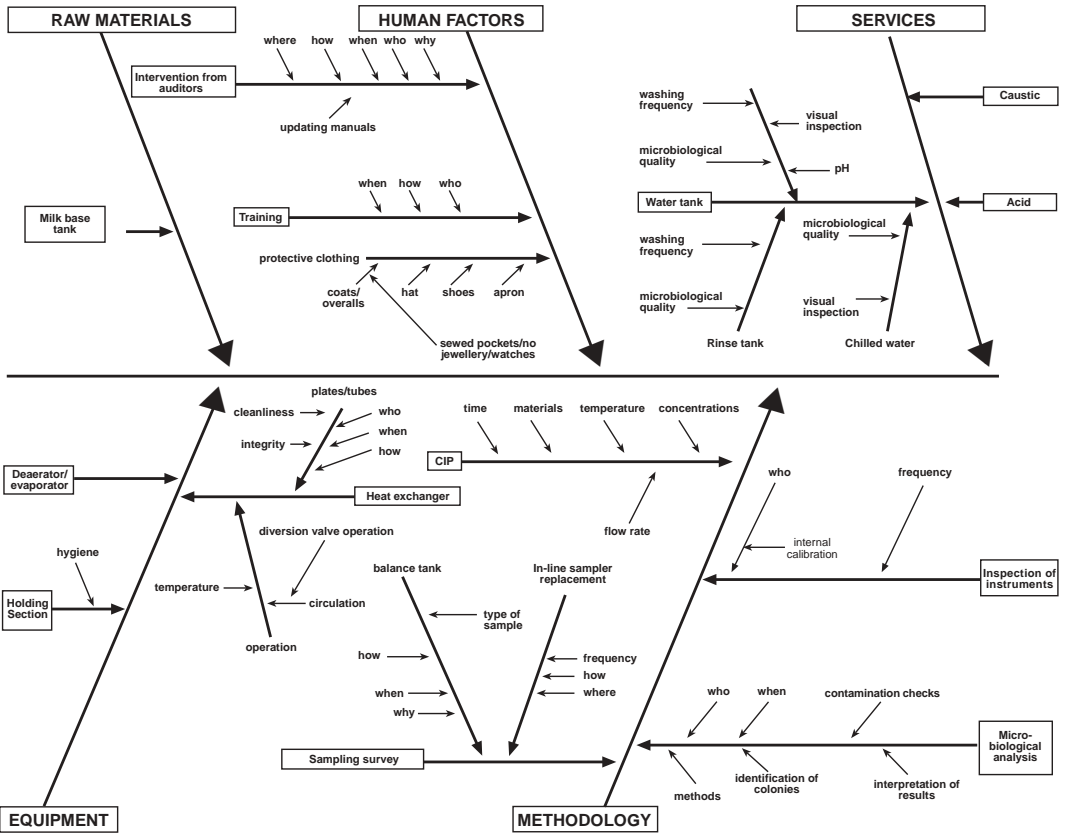
Fig. 10.2 Illustration of an interaction chart that highlights records that are required in a yoghurt factory

In addition, a protocol must be developed to audit the performance of the system, for whether the auditors are internal or from outside the company, regular inspections of the operation of the agreed HACCP procedures are essential.

Obviously, the initial establishment of a HACCP system will be both time consuming and demanding on the patience of the personnel involved, but most companies agree that the benefits more than compensate for the tedium of implementation. Whether or not HACCP should form part of a total quality management package is a matter for debate and the advantages and disadvantages of introducing systems like ISO 9000 are best debated within individual companies (see also BSI, 1991a, 1993, 1994; Lamprecht, 1993; Bolton, 1997).

10.3   Monitoring of process plant

The acidity of yoghurt means that spoilage is often associated with yeasts and moulds and the latter in particular often have their origin in the microbial flora of the air. The control of the atmosphere within the factory environment will depend on the level of air cleanliness that is essential for completion of a particular

operation (Bruderer and Schicht, 1987; Schicht, 1989, 1991; Fitzpatrick, 1990; Blümke, 1993). For example, laminar flow cabinets may be able to provide a local, high quality region for certain manual mixing operations (Audidier, 1996) and high efficiency particulate air (HEPA) filtration systems can reduce the overall microbial loading in the air by 90% (Hampson and Kaiser, 1995). It is important, however, that plant designed to induce air flow through a filling room or production area can also act as a source of contamination (Anon., 1988b) and some specifications for air quality have been published by the US Federal Standards – 209D (Anon., 1988c). Packaging materials stored adjacent to the filling line can also cause problems, as can the unnecessary movement of personnel and these aspects of plant operation deserve constant attention. If the problem of airborne contamination becomes really serious, then one of the air sampling methods described by the United States Public Health Service (USPHS, 1959) Ottaviani and Franceschetti (1983), Pfleger (1985) and APHA (American Public Health Association) (1992) could be employed to isolate the source(s) of the invading propagules.

Although yeasts and moulds of atmospheric origin can be important, especially at certain times of the year (Gregory, 1961), it is the contact surfaces of the plant that usually pose the greatest threat to product security. In small factories, strict attention to hygiene and visual inspections may be supplemented by a bioluminescence test for total adenosine-5-triphosphate (ATP). In this test, a small area of plant surface (perhaps $100 \, cm^2$) is carefully swabbed and any biological material collected (i.e. food and microbial contaminants) is transferred to a solution containing firefly luciferase and reduced luciferin (Anon., 1997a). In this situation, the ATP is reduced to adenosine monophosphate (AMP) and energy released is emitted as light. As the quantity of light recorded by a photometer is proportional to the initial level of ATP, the photometer reading will give an indication of the total level of biological material in the reaction fluid. If the swabbing procedure has been carried out correctly, then the photometer reading is, in effect, a measure of the state of hygiene of the plant surface (Pettipher, 1993). Obviously the readings are not intended to correlate with a microbial count, but there is an excellent correlation between clean surfaces and low levels of ATP. In large factories, the same approach can be used for regular monitoring of tanks, pipelines and other equipment, but it is often supplemented by specific tests for the general microflora and/or specific organisms.

However, whatever tests are employed, it is essential for the maintenance of hygienic conditions that they are applied routinely, for individual readings are in themselves meaningless; only when values for a typical, high standard of hygiene have been established for a given plant, along with acceptable tolerances, do the results of any microbiological/hygiene test become valuable.

For large items of equipment, one technique of almost universal application is the swab method (Harrigan and McCance, 1976; BSI, 1991b; APHA, 1992; IDF, 1993a, 1996a), in which a damp swab of cotton gauze (or some approved alternative) is rubbed over a designated area of the contact surface. The swab is then agitated in a known volume of a physiologically neutral solution and once the microorganisms are deemed to have been removed from the swab, samples of the solution, diluted if necessary, are examined by the plate count method (BSI, 1984; see also IDF, 1989). Milk agar is a most useful medium for dairy equipment and after incubation at 30°C for 72 hours, a colony count is obtained which can readily be

transformed into a figure for colony forming units (cfu) $100\,cm^{-2}$ of equipment surface.

The regular examination of selected or critical components of the production system can provide a useful indication of any decline in standards of cleaning and the rinse method (Harrigan and McCance, 1976; BSI, 1991b; APHA, 1992; IDF, 1993a, 1996a) can provide similar information for small items or containers. The performance of tests of this type on successive occasions (same operator and same conditions) is somewhat variable, hence the need for agreed tolerances, but it is trends away from the norm for any specific piece of equipment that are important. Some suggested standards for plant in contact with products prior to pasteurisation/heat treatment have been cited by Harrigan and McCance (1976):

cfu $100\,cm^{-2}$	Conclusion
500 (coliforms < 10)	Satisfactory
500–2500	Dubious
>2500 (coliforms > 100)	Unsatisfactory

With improved cleaning regimes, a total colony count of $200\,cfu\ 100\,cm^{-2}$ would be expected nowadays, and below $50\,cfu\ 100\,cm^{-2}$ for any plant containing pasteurised product (Lück and Gavron, 1990).

Different plants will achieve different levels of cleanliness even under ideal conditions and the manufacturer of yoghurt is perhaps fortunate that the product is fairly resistant to spoilage, at least of bacterial origin. Its reaction to yeasts and moulds is quite different, however, and if yeasts become the dominant contaminant, then numerous problems can be expected during retailing.

As an alternative to the procedures mentioned above, an agar contact method may be employed in which the sterile surface of a small Petri dish prefilled with an appropriate medium, or the exposed surface of an agar sausage (Cate, 1965), is placed in contact with the test surface. If the surface is not too heavily contaminated, then individual or clumps of micro-organisms adhere to the agar surface, and after incubation give rise to colonies that may be counted (Lück and Gavron, 1990). The results can again be related to a known area of plant surface, and as with data obtained in other ways, can provide an indication of the efficacy of the cleaning procedures.

It is clear, therefore, that examinations of this type are valuable as a means both of monitoring cleaning performance and of eliminating potential hazards, and the testing of raw materials has much the same function.

10.4 Examination of raw materials

10.4.1 Liquid milk
The basic ingredient of most yoghurt is whole milk or skimmed milk and hence the quality of the incoming milk is an important consideration. The methods of extracting representative samples will vary with the size of factory concerned, but it is essential that the portion examined truly reflects the quality of the bulk (IDF, 1990a, 1992a, 1995b).

The extent of any examination will depend on the scale of the operation, but may well include, as a minimum, some of the tests indicated in Table 10.2. If the milk is purchased in bulk, then the supplier can be expected to meet an agreed specification (see also Allen, 1995; SI, 1995b; Anon., 1994a; IDF, 1991d, 1995a, c, 1996b, d), and a typical set of figures might be:

Temperature on arrival	<10°C
Total colony count	≤100 000 cfu ml^{-1} (target)
	(<250 000 cfu ml^{-1} may well be acceptable in practice)
Inhibitory substances	≤0.007 IU ml^{-1} (0.004 μg ml^{-1})
Chemical composition	≥3.0 g fat 100 g^{-1}
	≥3.0 g protein 100 g^{-1}
Somatic cell count	≤4.0×10^5 ml^{-1}
Freezing point depression	≤0.520°C
Titratable acidity	≤0.2% lactic acid

Table 10.2 Some tests that might be applied to raw whole or skimmed milk to be used in the production of yoghurt

Examination	Reason	Method	Reference
Total solids	Allows accurate concentration or fortification of process milk	Hydrometer	BSI (1959, 1962, 1973), IDF (1993c)
		Drying	IDF (1991a)
		Infrared	Andersen et al. (1993)
Fat	Legal or sensory requirements	Gerber	BSI (1989a), IDF (1981a, 1997a)
		Röse Gottlieb	IDF (1996e)
		Light scatter	Anon. (1987a), IDF (1990c)
		Infrared	Andersen et al. (1993) IDF (1996c)
Protein	Fortification requirement	Infrared	IDF (1996c)
		Dye-binding	IDF (1985)
		Kjeldahl	IDF (1993b)
Antibiotics	Prevents inhibition of starter culture	Delvotest® P[a]	IDF (1970, 1991b) Anon. (1994b)
Taints	Chemical taints can be detected in individual supplies of milk	Odour/"sniff" test	
Organochlorine	Pesticides can affect flavour or inhibit growth of starter cultures	Many	IDF (1991c)
Organophosphorus	As above	Chromatography	IDF (1990b)
Dirt	Straw, hair or soil	Filtration	BSI (1982)

[a] The alternative Lac-Tek® test is legally acceptable in some countries (see also IDF 1995a, 1997b; Zeng et al., 1998).

While total colony counts are excellent for monitoring producer performance and, if required, making payment adjustments in-line with quality, the milk will have been processed long before the results of the count are known. In order to meet this criticism, a widely used alternative is the direct epifluorescent filter technique (DEFT), which gives a total viable count within 20 min (Sato *et al.*, 1986; Pettipher, 1993) or detection and enumeration of yeast in yoghurt (Rowe and McCann, 1990).

The availability of automated techniques means that the chemical composition of the incoming milk can be monitored as well (see later), but one essential test must be for inhibitory substances (IDF, 1986, 1991e, 1995a, 1997b). Thus, while minor variations in chemical composition may alter the quality of the end product and/or economics of the process, the presence of antibiotics in the milk can lead to total vat failure. The disc assay (IDF, 1970, 1991b; BSI, 1987) is able to detect 0.005 IU of penicillin G ml^{-1} of milk, while the more user friendly Delvotest® P (Anon., 1994b) can detect 0.004 IU of penicillin G ml^{-1} of milk in 2.5 hour; at a level of 0.006 IU of penicillin G ml^{-1} of milk, the Delvotest® is reported to be 100% accurate (Scannella *et al.*, 1997). More recently, the Lac-Tek® and Delvo-X-Press® βL-II tests have been introduced and these systems can identify a range of β-lactam antibiotics again at levels of 0.006 IU ml^{-1} of milk but, in this case, the detection time is around 7 min (Anon., 1997b). This rapid response means that all milk required for processing can be tested ahead of introduction into the production area. Alternatively, the Charm test(s) offers another alternative for checking for β-lactam residues (APHA, 1992), and standard methods are also cited for the high pressure liquid chromatography (HPLC) detection of sulphamethazine, the brilliant black reduction test for inhibitory substances, as well as various enzyme-linked immunosorbent assay (ELISA) techniques (Hands, 1989; Masolun *et al.*, 1992; Jacobs *et al.*, 1995).

The acid production test (see later) can also function as a simple, albeit slower, means of checking that a sample of milk will support a yoghurt fermentation and Hawronskyj *et al.* (1993) have proposed that the ATP bioluminescence procedure could be used as an alternative. However, it is unlikely that either of these latter approaches will replace the commercial systems that are available on the market.

10.4.2 Milk powder

Although process milk can be concentrated by evaporation or ultrafiltration (UF), raising the total solids of the milk base through the incorporation of a milk-based powder is still widely practised in small dairies. In some places, skimmed milk or full cream milk powder may be the only feasible raw material, but whatever the precise role of the powder, an examination of each consignment to ensure its adherence to agreed specifications can avoid problems at a later stage. Standard methods for monitoring the solubility of a milk powder and the production of sediments are well established, and the moisture and fat contents of a powder can likewise be recorded by the agreed procedures of the American Dairy Products Institute (ADPI, 1990) (previously the organisation was known as American Dairy Milk Institute (ADMI); see also Chapter 2 and IDF (1992b) for other specifications of milk powders.

Each consignment must also be tested for antibiotics and a microbiological

Table 10.3 Some suggested specifications for spray dried milk powders to be employed in the production of yoghurt

Standards	Satisfactory	Doubtful	Unsatisfactory
Microbiological			
Total colony count	<10 000	<100 000	>100 000
Coliforms	<10	<100	>100
Yeasts	<10	>100	<100
Moulds	<10	>100	<100
Staphylococci (coagulase positive)	<10	<100	>100

Chemical	
Acidity:	Acidity of reconstituted skimmed milk powder ($9\,g\,TS\,100\,g^{-1}$) should not exceed 0.15% lactic acid (see also IDF, 1981b).
Solubility:	Sediment in the solubility index tube (ADPI, 1990) produced by 10 g of skimmed milk powder should not exceed 0.5 ml (see also IDF, 1982, 1988a)
Scorched particles:	Employing the apparatus specified in BSI (1982), the filter disc should conform to Disc B of the ADPI photographic standards.
Moisture content:	Moisture content of skimmed milk powder should not exceed $4.5\,g\,100\,g^{-1}$ (see also IDF, 1993c).
Fat content:	Fat content of skimmed milk powder should not exceed $1.25\,g\,100\,g^{-1}$.
Inhibitory substances:	Powder should not contain above $0.006\,IU\,g^{-1}$ of inhibitory substances.

examination covering the groups of organisms suggested by Davis and Wilbey (1990) should be routine. Some proposed specifications are indicated in Table 10.3 and with good manufacturing practice, there is no reason to suppose that these standards cannot be attained. The yoghurt manufacturer is fortunate, however, in that the process milk does receive a severe heat treatment (e.g. 85°C for 30 min or equivalent) and hence some latitude with respect to the microbiological quality of the milk powder can be tolerated. The same margin of freedom applies to the stabilisers or other ingredients added prior to heating, but materials incorporated into the finished yoghurt (e.g. fruit and flavouring/colouring agents) need to be monitored with particular care. Unpasteurised fruit, in particular, can prove to be a troublesome source of yeasts or moulds and, in any yoghurt that contains sucrose, fungal infections can rapidly lead to spoilage and consumer rejection. The importance of this aspect can be judged from the standards proposed for some typical fruits (see Table 10.4) and any additional natural or artificial flavours should achieve at least the same specifications. Sucrose can also on occasion act as a source of yeasts and moulds and although rarely a source of infection, its presence should not be forgotten if spoilage problems should arise; osmophilic yeasts can even survive in some of the syrups employed for fruit yoghurts. Success or otherwise in this area can be judged in relation to the microbiological standards proposed for the end product (see Table 10.11), since failure at this latter point can often be traced to faulty ingredients. A further, and sometimes unexpected, source of contamination can be the bulk starter and an additional function of quality control centres on the provision of a viable, clean culture.

Table 10.4 Typical microbiological specifications that can be applied to some additives employed in the manufacture of yoghurt

Product/ organisms	Count ($cfu\,g^{-1}$)
Fruits	
Moulds	<10
Yeasts	<10
Total count	<1000
Coliforms	negative
Other ingredients including chocolate:	
Moulds	<10
Yeasts	<10
Total count	<2000
Coliforms	negative

After Spinks (personal communication).

10.4.3 Starter cultures for standard yoghurt

10.4.3.1 Microbiological examination

The type of starters available have been discussed earlier, but one popular material for inoculation of the production vessels is still a liquid culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in the ratio of 1:1 (chain:chain). In practice, this requirement means checking the balance by direct microscopic examination and, if the count is made quantitative as well (i.e. with a breed smear technique) then the total count for each species should confirm that the culture is suitable for use.

If the number of bacteria is too high to be counted directly, then a 10^{-1} dilution in quarter-strength Ringer's solution can be made prior to preparation of the slides (Robinson and Tamime, 1976). If the sample is agitated for 30s before the 0.01 ml aliquot is removed, then the areas of the slide ($1\,cm^2$) should contain a countable number of bacteria. Staining with Newman's stain or, after defatting, with methylene blue (Cooper and Broomfield, 1974) or Gram's stain (Davis *et al.*, 1971) is a useful aid to differentiation and for routine purposes, the number of fields to be examined can be reduced from the figure required, in theory, to give an accurate count (Wilson, 1935; Wang, 1941). Thus, Tamime (1977) found that counting ten fields in a five by five cross-pattern overcame uneven spreading and a reasonable estimate of the cell count ml^{-1} of a starter culture could be obtained. The only adjustment required was in relation to the expected ratio, because the chains of streptococci tend to breakdown into small units of two or three cells during dilution. If each one of these units is recorded as "one", then the ratio of streptococci:lactobacilli rises to around 2.7:1 and this ratio has been found to be repeatable with cultures incubated at 42°C.

An alternative technique for obtaining information about the ratio between the two organisms in a starter culture, or in the retail product for that matter, is the total colony count using a medium that selects for one or other species, or differentiates between them on the same plate. Obviously viable counts are more time

consuming than microscopic counts, but they do offer the advantage of recording only viable colony forming units and for the most part these units can be equated with individual cells. The fact that dilution and plating will have broken most of the chains necessitates a modification of the expected ratio, and figures of 5–10 *Streptococcus* : one *Lactobacillus* may well become the accepted norm; the chains of streptococci counted as one in the clump count tend to be longer than the chains of lactobacilli.

A selection of possible media is shown in Table 10.5 and the final choice will probably reflect the preference of the individual operator. However, it is important that different strains of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* will behave differently in the same medium and the performance of Lee's medium is a case in point (Ghoddusi and Robinson, 1996). Thus, while some strains of *L. delbrueckii* subsp. *bulgaricus* will give white colonies, others produce colonies that are identical to those of *S. thermophilus*. Lee *et al.* (1974) suggested that the acid-producing capacity of *L. delbrueckii* subsp. *bulgaricus* was the critical factor and hence that their medium should only be employed for monitoring a starter culture once its performance had been tested; as shown in Table 10.6, L-S differential medium and modified lactic agar (Matalon and Sandine, 1986) are other media

Table 10.5 Some of the differentiating media which can be employed to enumerate *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* from yoghurt or starter cultures

Culture medium	Micro-organism	
	S. thermophilus	*L. delbrueckii* subsp. *bulgaricus*
Hansen's yoghurt agar	High mass colonies, 1–3 mm	Low mass colonies, 2–10 mm
LAB	Smooth colonies	Irregular, hairy or rough colonies
Lee's medium	Yellow colonies	White colonies
L-S differential medium	Round red colonies with clear zone (<0.5 mm)	Irregular, red colonies with opaque zone (>1.0 mm)
Modified lactic agar	Small red colonies	Large, white colonies
Reinforced clostridial medium with Prussian blue	Pale blue colonies with thin, blue halo	Pale blue colonies with wide, royal blue balo
TYP-HGME agar	Small light blue colonies	Large dark blue colonies
YGLP-YL agar	Small brilliant white colonies	Large white colonies
Tryptose proteose peptone yeast agar with eriochrome dye	Oval colonies convex (1–3 mm) opaque white/violet often with a dark centre	Transparent, diffuse colonies (4–6 mm), unidentified shape with an irregular edge
Tryptose proteose peptone yeast agar with Prussian blue	Pale blue colonies with thin, blue halo	Small, shiny white colonies with wide royal blue halo

Note: these media may NOT be selective against other thermophilic lactic acid bacteria and not all strains of *S. thermophilus* or *L. delbrueckii* subsp. *bulgaricus* will give typical reactions.

Data compiled from Lee *et al.* (1974), Eloy and Lacrosse (1976), Robinson and Tamime (1976), Driessen *et al.* (1977), Johns *et al.* (1978), Bracquart (1981), Matalon and Sandine (1986), Millard *et al.* (1989), Bridson (1990), Sanches-Banuelos *et al.* (1992), Onggo and Fleet (1993), Ghoddusi and Robinson (1996) and Graciela Briceno and Martinez (1996).

Table 10.6 Relative performance of some media[a] employed under conditions specified by the original author(s) to enumerate the species in a standard yoghurt culture

| Medium | Differential count[b] | |
	Lactobacillus	*Streptococcus*
Lee's medium	1300	5000
TPPY agar	300	2700
TPPYPB agar	200	3000
Modified lactic agar	200	1200
L-S differential medium	(48)	
RCPB agar	500	3200
Elliker's agar	(900)	
M17	–	560
Acidified MRS	105	–

[a] Further details of the media are given in Table 10.5. [b] All figures as cfu $\times 10^6$ ml^{-1} of yoghurt, and are overall means from four separate trials (duplicate plates); there were no significant differences between the trials.

( ), Figures in brackets indicate that no differentiation was observed. –, no growth at dilutions used (10^{-6}–10^{-8}).
After Ghoddusi (1996).

that appear to give different responses according to the strains of bacteria under examination.

This problem of strain reaction is also evident in the data shown in Table 10.6, in that recovery from the same culture did on occasions differ by a factor of ten. However, tryptose proteose peptone yeast (TPPY) agar with eriochrome black gave good differentiation, as did reinforced clostridial prussian blue (RCPB) agar and, on both of these media, recovery (confirmed by Gram staining of selected colonies) was good; extremely clear definition was achieved by incoporating Prussian blue into TPPY (TPPYPB) agar in place of eriochrome black T (Ghoddusi and Robinson, 1996; Rybka and Kailasapathy, 1996).

While a single differentiating medium may be preferred for visual counts, the introduction of automatic colony counters may necessitate a change to the use of a medium selective for only one species (e.g. M17 agar for *S. thermophilus* (IDF 1988b, 1991f; Jordano *et al.*, 1992)), or one that gives a total colony count for all organisms of starter origin; a typical selection of such media is shown in Table 10.7. However, it should be noted that even laser counters are prone to error (e.g. there may be clusters of colonies close to the margin of the Petri dish) and that selective media are not always entirely inhibitory of other organisms. For example, acidified MRS agar can support the growth of yeasts and, although the difference in colony morphology is evident to the human eye, the electronic system will record just one total count, a point that could be important if the same medium is employed to monitor total viable counts of starter bacteria in a sample of commercial yoghurt (see also IDF, 1992c, 1997c).

10.4.3.2 Activity tests
The essential characteristic of a good starter (i.e. liquid type) for yoghurt is that it should produce the desired level of lactic acid within a given time. A simple test for this characteristic involves:

Table 10.7 Some of the media which can be employed to enumerate either *S. thermophilus* or *L. delbrueckii* subsp. *bulgaricus* as individual species from yoghurt or starter cultures

Culture medium	Micro-organism	
	S. thermophilus	*L. delbrueckii* subsp. *bulgaricus*
Eugon	No growth	Growth
Lactic agar (low pH)	No growth	Growth
M17	Growth at pH 6.8	No growth
Microassay	Growth	No growth
MRS medium (acidified)	No growth	Growth
Streptosel agar	Growth	No growth
Trypsin digest agar	No growth	Growth
Trypticase soy agar	Growth	No growth
TGV + Na-acetate	No growth	Growth
Elliker's agar[a]	Growth	Growth

[a] This medium is selective for both *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in the presence of casual contaminants and can be useful to check the performance of one of the selective media (see Table 10.5).

Note: these media may NOT be selective against other thermophilic lactic acid bacteria.

Data compiled from Elliker *et al.* (1956), de Man *et al.* (1960), Robinson and Tamime (1976), Shankar and Davies (1977), Driessen *et al.* (1977), Johns *et al.* (1978), Eloy and Lacrosse (1980, 1982), Hamann and Marth (1984a, b), Ogihara *et al.* (1985), Reuter (1985), Bridson (1990), Anon. (1991), IDF (1991f), Sinha (1991), Sanches-Banuelos *et al.* (1992), Onggo and Fleet (1993), Espinosa *et al.* (1994) and Graciela Briceno and Martinez (1996).

Note that other methods for differential enumeration of the yoghurt starter cultures have been reported by Coker and Martley (1982), Radke-Mitchell and Sandine (1984), Peral de Portillo *et al.* (1988), Bracquart and Sagnard (1989), Camaschella and Cislaghi (1989), Millard *et al.* (1989, 1990), Neviani *et al.* (1992), Ehrmann *et al.* (1994), Pirovano *et al.* (1995), Yamani and Ibrahim (1996), and Dave and Shah (1996).

- making a 1:10 dilution of the starter with 9 ml of Ringer's solution (1/4 strength) or peptone solution;
- placing 10 ml of process milk into a test tube and adding 1 ml of diluted starter;
- incubating the inoculated milk for 4 hours at 42°C.

At the end of this time, the acidity of the milk should be around 0.85–0.95% lactic acid and any cultures that fail to achieve these figures should be regarded with suspicion. This concern stems from the fact that, with a system of daily starter propagation, the balance between the organisms can change over a number of transfers. During manufacture, this swing may be manifest in a number of undesirable ways and an early warning of impending problems, gained through this simple activity test, can be helpful (IDF, 1991f, 1997c; Anon. 1995a).

10.4.3.3 Absence of contamination

The presence of gas bubbles in a liquid type starter culture or an unclean smell are clear indications of gross contamination and a useful confirmatory test is the catalase reaction. Thus, the starter organisms are catalase negative, so that if 5 ml of a culture are added to 1 ml of hydrogen peroxide (10 v), the formation of gas bubbles indicates a considerable infection by non-starter bacteria.

If the starter is being propagated on a daily basis, then a routine examination for coliforms may be worthwhile for, although the high acidity should restrict their survival, slow acid development can allow sufficient buildup to give taints or off-flavours to the retail product. The straightforward test for "acid plus gas" in single strength MacConkey broth is usually adequate for this purpose and if three tubes of broth are inoculated at three consecutive dilutions of the starter (e.g. 10^{-1} down to 10^{-3}) an indication of numbers of presumed coliforms can be obtained; "absent in 1 ml of starter" should be regarded as the minimum acceptable standard.

Although an examination for coliforms can be helpful, if only as an indicator of poor hygiene, the presence of yeasts or moulds at $>10\,\text{cfu}\,\text{ml}^{-1}$ of starter is likely to lead to spoilage during the shelf life of the retail product. Contamination of this magnitude can be readily monitored using malt extract agar acidified with lactic acid or chloramphenicol agar (IDF, 1990d) and a 10^{-1} dilution of the starter is convenient for incorporation into pour plates (1 ml per Petri dish). This approach should, at least, indicate if yeasts are present but, if the original counts are $<100\,\text{cfu}\,\text{ml}^{-1}$, it may be necessary to dispense 1 ml of undiluted culture into three standard Petri dishes (9.0 cm diameter) or one large dish (14 cm). Particular attention should be paid to any signs of infection by species capable of utilising lactose (e.g. *Kluyveromyces marxianus* var *marxianus* or var *lactis*) and their presence must be regarded as a stimulus for immediate action, namely improvements in the hygiene of the culture facility and the propagation of a fresh mother culture.

These routine examinations of bulk starters are essential where culture maintenance is carried out on-site and if the necessary laboratory facilities are not available, then consideration should be given to the use of freeze-dried or deep-frozen cultures for direct inoculation of the bulk starter milk. Thus, the cultures available from commercial manufacturers have an excellent record in respect of freedom from contamination and overall performance and the yoghurt manufacturer can normally be excused the rigours of a detailed starter examination (see also IDF, 1988b, 1991f, 1992c, 1997c).

10.4.4 Starter cultures for bio-yoghurts

While bulk starter cultures are still used for the production of normal yoghurt, the cultures for bio-yoghurts are usually of the concentrated freeze-dried or deep-frozen, direct-to-vat inoculation (DVI) type. The reason for this contast is that: (a) *Lactobacillus acidophilus*, *Lactobacillus paracasei* subsp. *paracasei, Lactobacillus reuteri*; *Lactobacillus rhamnosus*, *Lactobacillus paracasei* biovar *shirota* and *Bifidobacterium* spp. are difficult to grow in milk, and it is difficult to maintain the ratio(s) in a bulk starter if grown as a mixed culture, and (b) the end products must have viable cell counts above the agreed therapeutic minimum (Robinson, 1989; Marshall and Tamime, 1997). By using a DVI culture with a known cell count, the manufacturer is able to calculate with some accuracy the incubation time necessary to obtain the desired final counts and, equally important, can have confidence that those same counts will be achieved day after day. For this reason, the manufacturer of a bio-yoghurt will rely on the specification from the culture supplier and any microbiological checks are made on the end products instead.

10.5   Quality appraisal of retail products

However advisable it may be to monitor standards of plant hygiene or to insist that raw materials meet agreed specifications, it is the end product that must pass the final test – does it meet any legal requirements and is the quality acceptable to the consumer? In some countries, the imposition of compositional standards aims to encourage the maintenance of quality but, for the most part, the nature of the product in terms of consistency and related features ensures that the proposed standards are met with little difficulty. Nevertheless, analysis of the end product is an essential feature of quality control, because problems in manufacture are almost certain to manifest themselves as faults in the product. Consequently, examinations at this stage:

- protect the consumer from the purchase of poor quality product or, in extreme cases, product that might constitute a health hazard;
- protect the manufacturer from the inconvenience and expense of a barrage of returned goods;
- assist in the smooth operation of a plant by identifying variations in product quality at an early stage, so that any necessary corrective actions can be taken before the onset of serious problems.

The appraisal of product quality has become, therefore, a vital function of factory operation, and the gamut of examinations that may be performed can be considered under the headings that follow (see also Brant, 1988).

10.5.1   Analysis of chemical composition

Many countries have legal standards, or at least provisional regulations, for example MAFF (1975) and DTF (1983) in the UK, covering the composition of yoghurt and a selection of the existing proposals is given in Table 10.8 (see also IDF, 1984; Anon., 1986, 1987c, 1988a; Kirihara *et al.*, 1987; FAO/WHO, 1990). The requirement for a value for SNF is, in reality, more decorative than essential, because the texture or viscosity of a natural yoghurt with an SNF below the stipulated minimum would be barely acceptable. An overall measurement of total solids could, however, be valuable as a check that the concentration or fortification has been carried out correctly and a modification of the standard gravimetric method for milk has been proposed (Kirk and Sawyer, 1991) as suitable for yoghurt. The sample is neutralised before drying with 0.1 N strontium hydroxide and 0.0048 g ml^{-1} of alkali is deducted from the dry weight of the sample (see also Dordevic *et al.*, 1990). Davis and McLachlan (1974) suggested the use of vacuum drying with sodium hydroxide as the reagent. Either technique provides a convenient method of monitoring total solids; drying samples in a microwave oven did not appear to be satisfactory for yoghurt (Marquez *et al.*, 1995).

The routine measurement of protein is essential in large dairies because, over a typical year, the protein content of cow's milk may vary from 3.2 to 3.6 g 100 g^{-1}, and these differences are enough to alter the quality of the yoghurt. The cost of standardisation is, therefore, acceptable for a plant using several million litres of milk per week and the Kjeldahl method (IDF, 1985, 1990c, 1993b) remains the reference method for total nitrogen/protein in milk. Although the Kjeldahl remains the standard method, Karman and van Boekel (1986) have questioned whether 6.38 is the

Table 10.8 Some reported standards for the chemical composition (g 100g^{-1}) of yoghurt in terms of milk fat and solids-not-fat (SNF)

Country of origin	Types of yoghurt based on fat				SNF
	Strained	Normal	Medium	Low	
Argentina	–	2.8	0.5	0.5	–
Australia	–	3.0	0.5–1.5	0.5	–
Belgium	–	3.0	1.0–3.0	1.0	8.2
Denmark	–	3.5	1.5–1.8	0.3	–
FAO/WHO	–	3.0	0.5–3.0	0.5	8.2
Finland	–	2.5	–	–	–
France	–	3.0	–	1.0	–
Germany	10.0	3.5	1.5–1.8	0.3	–
Greece	5–8	5.0	–	–	–
Israel	–	3.0	1.5	0.2–0.5	–
Italy	–	3.0	1.5–2.0	1.0	–
Kenya	–	2.25	–	1.25	8.5
Kuwait	–	3.0	0.5–3.0	0.5	–
Lebanon	–	3.0	–	–	8.2
Luxembourg	10.0	–	–	–	9.0
Netherlands	4.4	2.95–4.4	1.5–1.8	0.5	8.2–12.6
New Zealand	–	3.25	–	2.0	–
Portugal	–	3.0	0.5–3.0	0.5	8.2
Spain	–	2.0	–	–	0.5
Switzerland	–	3.0	1.5–2.5	0.5	–
Sweden	–	3.0	–	–	0.5
South Africa	–	3.3	1.5–2.5	0.1–0.5	8.3–8.6
U.K.	–	3.5	1.0–2.0	0.3	8.5
U.S.A.	–	3.0–3.8	2.0–2.8	1.0–0.5	8.25–8.3

Adapted from Robinson and Tamime (1976); Australian Standards (1978), Anon. (1977, 1983, 1988a, 1996) and Kuwait Standards Specifications (KSS, 1980).

most appropriate factor. Whatever the validity of this point, there is no doubt that it is a time-consuming procedure and most dairies rely on measurements of the infrared absorption spectra (Anon., 1987a; IDF, 1990c; AOAC, 1990; Andersen *et al.*, 1993). The advantages of this approach are that it measures true protein, unlike the reference method which includes the non-protein nitrogen fraction and the infrared absorption technique can be applied to the measurement of fat, lactose and water, so that one instrument can give an accurate and rapid analysis of all the relevant components (Briggs, 1979). Obviously the calibration has to be established for the type of material to be analysed, for example, the incoming milk or the yoghurt as it leaves the vat, but modern instruments can provide full analysis for up to 360 samples per hour.

The other significant component, namely fat, is of interest not only in relation to any legal standards, but also because: (a) many stirred yoghurts are designated to be low or very low fat and hence it is important that the description should not be misleading, (b) milk fat has a major impact on the mouthfeel of yoghurt, around 1 g 100g^{-1} being regarded as the minimum to produce the desired response from the consumer, and (c) it is anticipated that full fat natural yoghurt (3.0–3.5 g 100 g^{-1}), 'luxury' fruit yoghurts (>4.0 g 100 g^{-1}) and Greek-style yoghurts (>8.0–10 g 100 g^{-1}) will have high fat contents and again these expectations must be met.

The gravimetric methods of determining fat in yoghurt (e.g. the Röse Gottlieb method) are regarded as the most accurate (Davis, 1970) but for routine purposes, the normal Gerber method (BSI, 1989a; IDF, 1997a) using 11.3 g of yoghurt in a milk butyrometer is totally appropriate. All these examinations should be performed on the natural yoghurt prior to the addition of fruit and monitoring the acidity is also more straightforward in the absence of additives. However, while the Röse Gottlieb method remains the reference method (IDF, 1988c), fat is routinely monitored by light scattering photometry (Anon., 1987a) or within a multicomponent analysis using infrared absorption (Andersen *et al.*, 1993).

The production of lactic acid beyond the point of coagulation is monitored principally in relation to consumer preference and hence the selected end point will vary not only from country to country, but also with the type of yoghurt. Thus, in The Netherlands, for example, Bulgarian yoghurt may have an acidity of up to $1.48 \, \mathrm{g} \, 100 \, \mathrm{g}^{-1}$ lactic acid, while other types are usually sold with a maximum of $1.17 \, \mathrm{g} \, 100 \, \mathrm{g}^{-1}$ lactic acid (Netherlands Standards, 1967). The IDF (1992c) have suggested a minimum of 0.7 g lactic acid per 100 g of retail product and hence the measurement of acidity is an important feature of production. Although the configuration of the lactic acid can be important from a nutritional standpoint, it is usually assumed that culture selection will determine whether the D (−) or L (+) isomer will dominate (see also Anon., 1995b). However, in situations where the characteristics of the culture are not known, there are colorimetric methods available (Lunder, 1972) to determine the total level of lactic acid and, subsequently, of the L (+) isomer and HPLC can achieve the same separation (Olieman and de Vries, 1988); an enzyme-based biosensor could also be used to identify L (+) lactic acid specifically in yoghurt (Mulchandani *et al.*, 1995).

Although the relationship between titratable acidity and pH is not straightforward in a highly buffered system like yoghurt (Lück *et al.*, 1973), the direct electrometric determination of pH is extremely convenient (Harrison *et al.*, 1970). Thus, once a correlation has been established between pH and the desired characteristics of a particular type of yoghurt, then routine monitoring during manufacture can become a normal practice. However, to maintain a close check on the acidity of the retail product, it is usually desirable to test representative samples of the cooled yoghurt for titratable acidity.

The measurement is a composite one including the natural acidity of the milk and the developed acidity arising from bacterial activity but, as the natural acidity should not vary a great deal (assuming that the milk is standardised for total solids), titratable acidity is a reasonable indication of the performance of the starter culture. The problems of measuring acidity by direct titration have been discussed by Sherbon (1988) and, for the analysis of yoghurt, the approach is based on the technique employed for liquid milk. Thus, the normal method involves transferring a known volume or weight of natural yoghurt to an evaporating basin and then neutralising the acidity with caustic soda. A detailed summary of some of the suggested methods is shown in Table 10.9 and it is noticeable that the expression of results differs from country to country. In practice, these national preferences are not important but, for comparative purposes, a chart of the type shown in Appendix I can always be constructed.

The subjective nature of the end points is more relevant, because it implies that some variation between operators has to be accepted, and hence a comparison of results from different laboratories may not always be possible. It also means that in

Table 10.9 Selection of methods for determining the titratable acidity of milk or fermented milk

Component	BSI (1989b)	Danish[a]	Netherland[a]	Tamime (1977)	IDF (1991h)	AOAC (1990)
Sample size	10 ml	25 ml	10 ml	10 g	10 g	20 ml or g
Dilution	–	–	–	–	–	2:1
Phenolphthalein[b]	1 ml	13 drops	0.5 ml	1 drop	–	2 ml
Alkali[b] (M NaOH 1^{-1})	0.1	0.1	0.1	N/9	0.1	0.1
End point[b]	Pink to match cobalt (II) sulphate or reference colour solution	Constant pale red colour	Pink to match fuchsin standard	Light rose to persistent pink colour	Titrate to pH 8.3	First persistent pink colour
Expression of results	Alkali (ml) 10 = g lactic acid 100 ml^{-1}	Alkali (ml) × 4/100 ml	1/10 ml of alkali ml^{-1}	Alkali (ml) 10 = % lactic acid or g lactic acid 100 g^{-1}	Alkali (ml) × 0.9 10 g = g lactic acid 100 g^{-1}	Alkali (ml) 20 = % lactic acid or ml alkali 100 g^{-1}

[a] Adapted from Robinson and Tamime (1976). [b] For the preparation of reagents refer to standards.

Note: For conversion of degrees Dornic (°D), Thorner (°T) or Soxhlet-Henkel (°SH) to % lactic acid refer to Appendix I.

any given laboratory, the measurement of titratable acidity should be carried out under standardised conditions, that is, a specific location in the laboratory with a non-variable light source, and that the actual titration should be performed by the same person. If these restrictions can be met, then titratable acidity becomes a most useful measurement, because not only can the figures be linked fairly accurately to consumer preferences but, through the component for developed acidity, changes in performance of the starter bacteria can be manifest rapidly.

Monitoring of other milk components like lactose is probably not important as a routine, but Mistry *et al.* (1989) can be consulted for a list of available methods; sucrose levels in the milk base or the final product can be checked instrumentally (Anon., 1981). The introduction of legislation covering additives and colouring materials means that close inspection of ingredient specifications (see Table 10.10) and/or additional specialised analyses are required. Thus, the addition of a fruit puree containing starch to a yoghurt base already incorporating a compound sta-biliser could raise the total starch above the suggested $1\,g\,100\,g^{-1}$ level, and preser-vative levels would need to be similarly monitored, at least on an occasional basis. The timing and extent of such analyses will differ from company to company and standard texts, such as AOAC (1990) or Kirk and Sawyer (1991), should be con-sulted concerning appropriate methods and their application.

10.5.2 Assessment of physical characteristics

Yoghurt is normally retailed in one of three physical states, namely set yoghurt, stirred yoghurt and fluid or drinking yoghurt, and each type has quite distinctive characteristics. The typical gel structure of a set type, for example, could never really be mistaken for the semifluid form of the stirred variety, but the low viscosity of some stirred brands leaves the consumer with little option but to drink them. This degeneration of product image is obviously regrettable and, although the release of an occasional poor batch is inevitable, the question of desirable viscosity is always somewhat vexing. In practice, each manufacturer will probably adopt an agreed in-house standard for viscosity (or consistency in the case of set yoghurt) and then operate to this specification, so that the routine assessment of these physical fea-tures becomes a normal part of quality control.

10.5.2.1 Set yoghurt

The essential gel structure of set yoghurt means that assessment of the product must be approached in a manner that does not destroy the delicate coagulum. The falling sphere technique (Pette and Lolkema, 1951) can be used, but the most convenient test involves the use of the penetrometer (Hartman, 1976; see Fig. 10.3). The only special adaptation centres on the choice of spindle and cone, for these have to be selected so that, for the product in question, the depth of penetration of the cone does not exceed about 33% of the total depth of the retail sample. The risk of edge effects from the carton must be minimised by choosing a spindle with a diameter no greater than 50% of the diameter of the pot and, with these restrictions in mind, it becomes a simple matter to select a probe/spindle weight that is appropriate. The configuration of the probe (i.e. flat or cone-shaped) is also variable, so that products with $12–16\,g\,TS\,100\,g^{-1}$ can be handled with ease. In addition, the weight of the probe/spindle may be changed in relation to temperature of the product, for example, a light spindle might be selected for examination of a carton at $42°C$

Table 10.10 Some proposed or existing regulations concerning the introduction of non-dairy ingredients into stirred fruit yoghurts; no additives are usually permitted in natural set or stirred yoghurts

Country	Stabiliser	Fruit	Preservatives	Colours/flavours
Belgium	Gelatin Starch/modified starch[a] Pectin	–	Sorbic acid and salts	Anthocyan, caramel, carotenoids, chlorophyll, cochineal Xanthophyll Vanilla extract, ethyl vanillin
Denmark	nil	10–15%	nil	nil
France	–	Up to 30%	–	Anthocyan E 163, beet root red E 162, caramel E 150, carbo medicinalis vegetalis E 153, carotinoid E 160, chlorophyll E 140, cochenille E 120, curcumin E 100, indigotine E 132, lactoflavin E 101, xanthophyll E 161
Greece	nil	–	nil	nil
Italy	–	Up to 30%	Sorbic acid E 200 Sodium, potassium and calcium sorbate	Specified colours and listed preservatives may only be added with the fruit
Luxembourg	Permitted	<8%	Sorbic acid and salts	As with stabilisers, permitted from positive EU lists
Netherlands	Permitted	–	–	Permitted
Portugal	–	–	Sorbic acid and potassium sorbate	Carotene E 160a, bixin E 160b, cochenille – colours and preservatives may only be present in the fruit
United Kingdom	Starch[a] Pectin Gelatin Alginates[b] Agar Edible gums Celluloses	Up to 30%	Sulphur dioxide Benzoic acid Methyl-4-hydroxybenzoate Ethyl-4-hydroxybenzoate Propyl-4-hydroxybenzoate Sorbic acid	

[a] Any combination not to exceed 1.0%. [b] Any combination not to exceed 0.5%.

After: Robinson (1976) and Anon. (1989).

Fig. 10.3 Standard penetrometer of the type that can be employed to measure the consistency of set yoghurt

immediately after incubation and a heavier spindle for assessment of the firmer coagulum developed in yoghurt held subsequently at 7°C for 24 hours. These changes in spindle weight make it possible to discriminate, at a given temperature, between samples of different gel strengths and the fact that comparisons are possible at 42°C makes it feasible to predict the consistency of the retail product prior to final cooling.

The technique is, therefore, both reliable and versatile and hence standardising the physical properties of set yoghurt becomes a straightforward exercise. If the data are required for research and development purposes, the use of a computerised texture profile analyser (TPA) (see Fig. 10.4 A and B) may improve the repeatability of the measurements (Prentice, 1992; Benezech and Maingonnat, 1993, 1994; Vélez-Ruiz and Barbosa Cánovas, 1997). Other physical features or faults, for example lumpiness or the presence of nodules, usually become apparent during sensory analysis. These problems will be discussed later.

10.5.2.2 Stirred and fluid yoghurt

The range of methods that are available to measure the viscosity of fluid and semi-fluid products has been discussed by Sherman (1970) and Prentice (1992) and the choice of method is really a matter of operator preference. Thus, in the present context, interest centres on making an objective comparison between samples, or between a sample and an expected result representing product of acceptable quality. A number of simple techniques can be employed for this purpose.

At one end of the scale, some small producers rely on extremely simple techniques, such as:

- scooping a sample of yoghurt onto the back of a spoon, and then gently inclining the spoon downwards – the rate at which the yoghurt drips from the spoon is a reflection of its viscosity; the same technique will also reveal any irregularities in the coagulum;

(A)   (B)

Fig. 10.4   Texture profile analysers (A: Stevens compression response analyser; B: TA-XTA texture analyser) suitable for measuring the firmness of yoghurt

- inserting a plastic teaspoon into a typical retail carton of yoghurt – if the spoon remains upright, the product has an acceptable, spoonable viscosity;

and, although these approaches are subjective in the extreme, they do offer the experienced operative a guide to the quality of the end product.

It is more usual, however, to rely on more reproducible techniques and a number of these are available. Thus, Davis (1970) has described the use of a rotating cylinder which could be tilted until the yoghurt began to pour; the angle necessary to initiate flow can be taken as a measure of product viscosity. The time taken for a standard metal sphere to descend a certain distance through a prescribed volume of yoghurt has also provided a convenient method of comparison (Ashton, 1963; Bottazzi, 1976), as has the flow rate of yoghurt through funnels of prescribed orifice sizes; in the Posthumus funnel, for example, the time taken for the yoghurt "surface" to pass between the starting point and the centrally located pointer gives a measure of the viscosity of the product (Posthumus, 1954). A similar approach has been employed in The Netherlands (Galesloot, 1958), in South Africa (Ginslov, 1970) and in Sweden (Storgards, 1964). The time taken for a yoghurt sample of known volume to flow down an inclined plane, with or without weirs, has also been advocated, as has the "plummet" (Hilker, 1947), but perhaps the most universally accepted approach is that employing a rotational viscometer (see Fig. 10.5), or the torsion wire apparatus. Another empirical method used to measure the rheological property of stirred yoghurt is known as the Bostwick consistometer. The unit resembles a rectangular channel made from stainless steel and fitted with removable slot or door. The consistometer can be used on-site in the production area where a sample of yoghurt is placed in the slotted compartment; the door is removed and the rate

(A)   (B)

Fig. 10.5   Rotational viscometers (A: Brookefield Synchro-lectric; B: Haake) that would
be suitable for comparing the viscosity of different samples of stirred yoghurt

Note: With the Haake, the geometry of the system is constant and circulating water allows for accurate
temperature control.

of flow per time is measured on a scale. The thinner or low viscous yoghurt tends
to flow faster (Fig. 10.6).

The ease of operation makes the rotational viscometer, such as the Brookfield
Synchro-Lectric, a popular choice, and once the type of spindle and its speed of rota-
tion have been established for a given product, comparison between successive
batches presents few problems; the Helipath system in which the spindle rises ver-
tically through the sample while rotating is preferable since there is less risk of the
spindle causing local syneresis and an artificially low reading (Abrahamsen and
Holmen, 1980; see also Hellinga *et al.*, 1986). It is reasonable to stipulate, therefore,
that a stirred yoghurt should have a viscosity that falls within certain preset limits
and the physical nature of a fluid yoghurt could be similarly described; for thick,
stirred yoghurts (e.g. labneh or Greek-style yoghurts) Tamime *et al.* (1989, 1991)
have suggested that the use of a Stevens-LFRA texture or compression response
analyser should be considered (see Fig. 10.4). Handling batches that fall outside
these categories is a matter for company policy, but clearly, monitoring this aspect
of product quality can be undertaken on a routine basis.

However, although methods of this type have the speed and simplicity essential
for routine quality control, some authorities argue that the actual figures do not
reflect the true nature of the product, since the shearing effect of the spindle
destroys the integrity of the coagulum. Obviously processing alone has disturbed
the original gel of stirred yoghurts but even so, the coagulum does tend to reform
to some extent during cooling. Consequently, it has been suggested that as stirred
yoghurt is a viscoelastic material, that is, it has some of the properties of a viscous

Fig. 10.6 A Bostwick consistometer

liquid and some of an elastic solid (Ozer *et al.*, 1997), dynamic oscillatory testing would be more appropriate (Steventon *et al.*, 1990; Vlahopoulou and Bell, 1990; Xiong and Kinsella, 1991; Rönnegård and Dejmek, 1993). Thus, yoghurt gels are particulate structures composed mainly of caseins (Dickinson, 1990) and, in general, continuously connected strands of protein produce a heterogeneous three-dimensional gel network which holds free water. Any factors which affect the properties of the gel network by changing the nature and number of protein interactions will also affect the water holding capacity of the gel. The gel structure is known to involve both covalent (thiol/disulphide interchange) and non-covalent bonds (Roefs, 1986; Brendehaug, 1987; Mottar *et al.*, 1989; Roefs and van Vliet, 1990; Langton, 1991; Amice-Quemeneur *et al.*, 1995). Dickinson (1994) claimed that the physical characteristics of particulate gels are determined by the strong, permanent (covalent) bonds formed during the aggregation of protein particles. Furthermore, the structure of the final gel is also dependent upon the number of weak, reversible interactions that occur between the particles prior to formation of the permanent bonds. In effect, the numerical balance between the strong and weak bonds controls the rheology of the gel (Dickinson, 1994).

Another factor that affects the physical characterisitics of yoghurt is the distribution of protein–protein bonds over the gel network (Walstra, 1997). A number of studies have investigated the relationship between protein concentration, the distribution of protein–protein bonds and the rheology of the resultant gels (Bremmer *et al.* 1990; Walstra *et al.* 1990). Thus, although in homogeneous gels all the components contribute to the network (Roefs and van Vliet, 1990), in non-homogeneous materials like yoghurt, thick protein "nodes" including more than one protein junction point are evident; the contribution of the protein–protein bonds to the elasticity of the gel decreases as the number of stress-carrying strands is reduced.

When the texture of a set yoghurt is measured with a penetrometer or texture analyser or the viscosity of a stirred yoghurt is determined by one of the conventional techniques (Ozer *et al.*, 1997), the disturbance breaks the chains of casein micelles that form the three-dimensional network which immobilizes the liquid phase (Heertje *et al.*, 1985). However, by examining such a structure with a

controlled-stress rheometer, two parameters which indicate the elastic and viscous characteristics of the gel can be determined with minimal disturbance to the inherent structure.

The Rheotech international controlled-stress rheometer (see Fig. 10.7) is typical of the instruments that can be used to make dynamic measurements; the oscillating surface is a parallel plate of 20 mm diameter. The gap between this plate and the stationary surface is variable but, for a soft gel like yoghurt, a gap of around 10 mm is normal. In practice, the frequency and amplitude ranges of the sinusoidal waves generated by the moving plate are established for the product in question, so that all readings fall within the so-called linear viscoelastic region (LVE) (Ferry, 1980). In this region, both shear moduli are independent of strain and stress and the plateau ends at the applied strain required to cause the material to break down. Some typical conditions for yoghurt are: (a) set yoghurt: frequency 0.25 Hz; amplitude 0.015 mN m (minimum), 0.15 mN m (maximum) and (b) stirred yoghurt: frequency 0.25 Hz; amplitude 0.008 mN m (minimum), 0.08 mN m (maximum) with all measurements being made at 25°C.

Once the conditions have been established, the storage modules G' can be measured. This parameter expresses the energy stored in the material from rearrangements in the structure that take place during the oscillation period; solids tend to return to the original state after a stress is released, and hence G' confirms the elastic characteristics of the product. Then, the loss modulus G'' can be determined and this value records the energy lost during the cycle of deformation, so indicating the viscous component of the material. These measurements should, therefore, give an accurate picture of the gel structure (van Marle and Zoon, 1995).

An example of the application of this technique is shown below and the materials are concentrated yoghurts $(23 \, \text{g} \, \text{TS} \, 100 \, \text{g}^{-1})$ produced from normal yoghurt $(16 \, \text{g} \, \text{TS} \, 100 \, \text{g}^{-1})$ by the traditional (cloth bag) system, UF (Tamime *et al.*, 1989, 1991) and RO. The protein content $(\text{g} \, 100 \, \text{g}^{-1})$ of the product derived by the traditional process was 8.00, by UF 8.13 and reverse osmosis (RO) 6.38. As a high protein

Fig. 10.7 Overall view of a rheometer linked to a computer to enable dynamic measurements of yoghurt structure to be made

content leads to an increased number of protein interactions and protein–protein bonds, so the elastic character of the gel (G') should increase and, as shown in Fig. 10.8, the traditional and UF samples had higher storage moduli; the enhanced protein levels made the gels less prone to breakdown with increasing amplitude. Exactly why the traditional product showed increased elastic properties was not determined, but it seems likely that slow drainage under gravity altered some aspect of the protein–protein interactions.

The same increase in concentration of protein causes the space occupied by the protein network to increase, so restricting the mobility of free water; higher values for G'' (viscous component) may be expected (see Fig. 10.9). It is noticeable also that the values for the storage moduli (G') are higher than those of the loss moduli

Fig. 10.8   Storage moduli of samples of yoghurt concentrated by the traditional (cloth bag) method (◆), ultrafiltration (●) and reverse osmosis (▲) to $23\,g\,TS\,100\,g^{-1}$; the points indicate the amplitude strain at the stress in question, and the arrows show the position at which structural breakdown was noted

Data compiled from Ozer (1997)

Fig. 10.9   Loss moduli of samples of yoghurt concentrated by the traditional (cloth bag) method (◆), ultrafiltration (●) and reverse osmosis (▲) to $23\,g\,TS\,100\,g^{-1}$

Compiled from Ozer (1997)

over the range measured, so confirming that yoghurt has, as widely reported, a weak viscoelastic structure. In addition, it is fair to assume that the stronger protein bonds contribute to the elastic character of viscoelastic gels, whereas the loss modulus (G'') reflects the number and/or distribution of weak bonds. Both the number and distribution of protein bonds throughout the gel network seem to be dependent on the protein content (Ozer *et al.*, 1997). As the changes in storage moduli (G') as a function of amplitude were paralleled by changes in the values for loss moduli, considerable differences in the loss tangent (tan δ) figures were recorded, especially at the higher amplitudes (see Fig. 10.10). This suggests that the nature of the interactive forces were essentially dependent on the variables examined; the loss tangent (tan δ = G''/G') is indicative of the nature of the interaction forces in a gel (Ferry, 1980) or the methods used to fortify milk solids with skimmed milk powder (SMP) or UF retentate (Biliaderis *et al.*, 1992) or the level of fat in the milk base (de Lorenzi *et al.*, 1995). However, as shown in Chapter 2 the processing conditions can influence the rheological properties of yoghurt. Some selected studies include: (a) a study shawing that the shear rate and time dependency were influenced by both the presence of pectin and strawberry concentrate (Ramaswamy and Basak, 1991a, b; Basak and Ramaswamy, 1994; Butler and McNulty, 1995), (b) the influence of heat treatment of the milk base on the firmness of the gel, reported by Schmidt *et al.* (1985), Parnell-Clunies *et al.* (1986a, b) and Gebhardt *et al.* (1996), and (c) the rheological properties of yoghurt made with encapsulated non-EPS (exopolysaccharide) cultures or the effect of humectants (e.g. NaCl, sucrose or sorbitol), have been detailed by Hassan *et al.* (1996a, b) and Lacroix and Lachance (1988), respectively. All these rheological studies on yoghurt have been carried out on packaged products, but Picque and Corrieu (1988) and Doi *et al.* (1992) described techniques to make in-line measurements of gel characteristics and to monitor milk curd formation continuously. The inverse photoelectric method had been used to characterise yoghurt through the measurement of its thermal effusivity or measurement of penetration coefficient $(k\rho c)^{1/2}$. The results suggest that the sensitivity of the technique is influenced by the fat : water ratio (Bicanic *et al.*, 1994).

Certainly, dynamic rheometry does expose differences in the rheological charac-

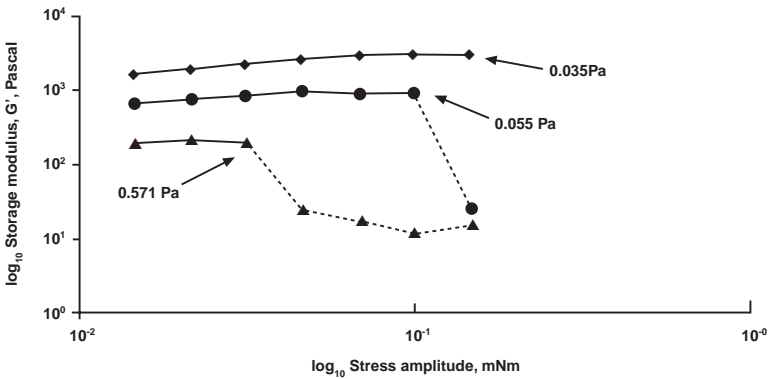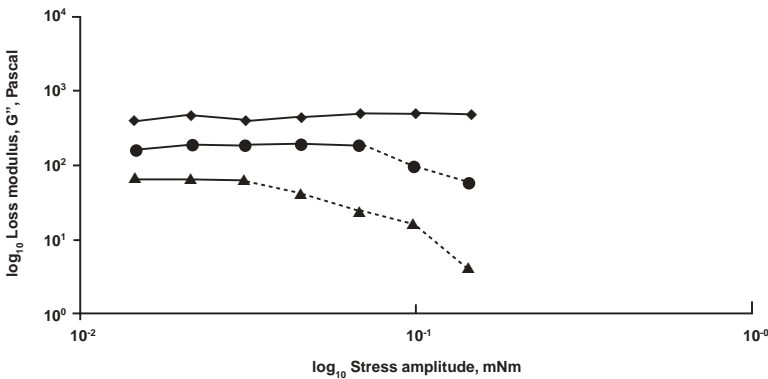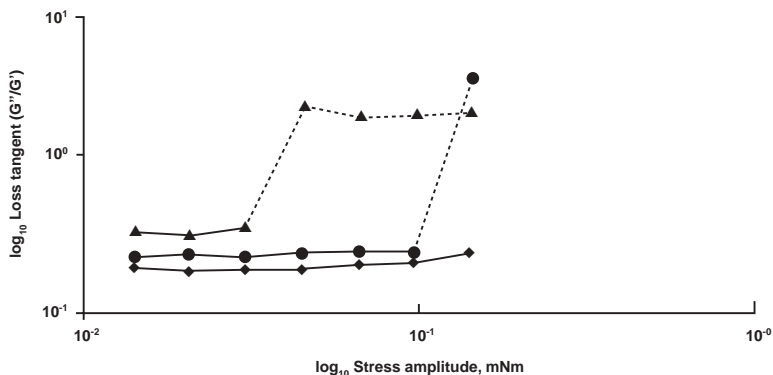**Fig. 10.10**   Loss tangent (G''/G' = tan δ) of samples of yoghurt concentrated by the traditional (cloth bag) method (◆), ultrafiltration (●) and reverse osmosis (▲) to 23 g TS 100 g^{-1}

Compiled from Ozer (1997)

teristics of stirred yoghurts that would not be apparent on the basis of destructive measurements with a viscometer (Teggatz and Morris, 1990; Rohm, 1992, 1993; Skriver *et al.*, 1993, 1995; Rohm and Schmid, 1993; Rohm and Kovac, 1994; Skriver, 1995; Rawson and Marshall, 1997; Hess *et al.*, 1997; Ozer *et al.*, 1998). However, the disadvantages of this approach are that the equipment (see Fig. 10.7) is expensive in contrast to the cost of a viscometer, and that taking the measurements can be technically demanding, and hence for routine operation in quality control, it is a system that is unlikely to find much application.

10.5.3 Microbiological analysis

While the techniques for measuring physical properties and chemical composition can be applied to any type of yoghurt, a microbiological examination of the finished product may include checks on the survival of the starter organisms, as well as for the presence of undesirable spoilage or pathogenic organisms. The tests for pathogens will, of course, be used for many dairy products but as the specific microfloras of yoghurt and bio-yoghurt may well be different, the two types of product will be discussed separately.

10.5.3.1 Standard yoghurt

Interest in an examination for the bacteria of starter origin stems from the fact that low population levels of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* may be associated with excessively long incubation times and poor development of the typical yoghurt flavour. However, excessively high levels can result in:

- too rapid or excessive acidification,
- syneresis in set yoghurts,
- an imbalance of flavour components,
- spoilage from continued acid production during storage, even at low temperature.

In addition, it has been suggested that "yoghurt should contain abundant and viable organisms of starter origin" (FAO/WHO, 1990) and, whatever the precise definition of these words, there is a general agreement that yoghurt should contain live bacteria unless specifically designated as pasteurised or heat-treated (see also IDF, 1992d). Many countries stipulate also that the term yoghurt should only be applied to a milk fermented with *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* alone (Bourlioux, 1986; Anon., 1989), and there is a continuing debate as to whether the word "yoghurt", alone or qualified, should be used for any product that does not contain *L. delbrueckii* subsp. *bulgaricus*. From an historical perspective, this proposal makes sense, but the framing and enforcement of such a regulation could prove more difficult. For example, Table 10.11 shows some typical figures for starter bacteria in retail yoghurts, and most reports suggest that counts of this order are common (Hamann and Marth, 1984a; Sinha *et al.*, 1989; Roberts and Maust, 1995). The question does arise, however, regarding whether a yoghurt must have counts of this order, or whether a few cells of *L. delbrueckii* subsp. *bulgaricus* ml^{-1} of product will suffice.

The methods available for examining the starter flora of normal yoghurt have been discussed elsewhere (see Starter cultures for standard yoghurt, Section 10.4.3) and an indication of the results that might be anticipated is shown in Table 10.11.

Table 10.11 Indication of the numbers of starter bacteria that have been isolated from retail cartons of yoghurt and some suggested standards relating to both contaminants and desirable organisms

Organism	Yoghurt		
	Natural	Strawberry	Blackcurrant
S. thermophilus $\times 10^6$ cfu ml^{-1}	10–820	35–1100 54–250	80–1850
L. delbrueckii subsp. *bulgaricus* $\times 10^6$ cfu ml^{-1}	11–680	5–360 <1–150	5–400
Suggested advisory standards:			
	Satisfactory	Doubtful	Unsatisfactory
S. thermophilus $\times 10^6$ cfu ml^{-1}	>100	100–10	<10
L. delbrueckii subsp. *bulgaricus* $\times 10^6$ cfu ml^{-1}	>100	100–10	<10
Coliforms	<1	1–10	>10
Yeasts } cfu ml^{-1}	<10	10–100	>100
Moulds	<1	1–10	>10

After: Davis and McLachlan (1974) and Robinson (1976).

The wide variations are a reflection of both between batch and between brand differences, but the standard suggested by Davis and McLachlan (1974) for a satisfactory yoghurt should be readily attainable. Obviously, it is not suggested that unsatisfactory counts are a cause for concern but, at the same time, it is probably true that if flavour and acid development are satisfactory, then figures of 10×10^6 cfu ml^{-1} species will be an inevitable consequence; results of this order are, therefore, a fair indication that the organoleptic properties of the yoghurt will be satisfactory as well. At the other extreme, a tendency towards extremely high counts may raise problems later, particularly if the refrigeration chain is substandard; consumer complaints relating to excessively sour yoghurts can imply that acid production by *L. delbrueckii* subsp. *bulgaricus* has been poorly controlled.

An examination of yoghurt for contaminant organisms is, as indicated earlier, concerned with protection of the consumer from any potentially pathogenic species and assurance that the material will not undergo microbial spoilage during its anticipated shelf life (Stannard, 1997). These issues are of vital importance to any company. Thus, apart from the moral obligation that a company has to its customers, the financial losses that can accrue from the release of suspect products are motivation enough to give microbial quality control a high priority. A rapid estimation of the total numbers of lactic acid bacteria in yoghurt can be determined using the electric conductivity method (Yoshida *et al.*, 1987).

As far as pathogens are concerned, yoghurt with an acidity of around $1 \text{ g} 100 \text{ g}^{-1}$ lactic acid is a fairly inhospitable medium and really troublesome pathogens like *Salmonella* spp. and *Listeria monocytogenes* will be incapable of growth (Hobbs, 1972). A degree of survival of *L. monocytogenes* at pH 4.5 in labneh has been reported (Gohil *et al.*, 1996) but, even under severe test conditions, the counts

declined rapidly within 24 hours, that is, long before the product would have reached the consumer. Schaack and Marth (1988a–c) observed that *L. monocytogenes* was inhibited during a yoghurt fermentation, but Choi *et al.* (1988), Massa *et al.* (1991), Khattab *et al.* (1993), Zuniga Estrada *et al.* (1995) and Ribeiro and Carminati (1996) suggested that the final pH of the product was important, as was the precise strain of *L. monocytogenes* (see also Siragusa and Johnson, 1988; Ahmed, 1989; Greenwood *et al.*, 1991). However, bacteriological methods for the detection of contaminating micro-organisms and pathogens in milk and milk products have been reported by IDF (1991g, 1994b, 1995d).

Coliforms should also be inactivated by the low pH (Feresu and Nyati, 1990) and, in addition, some species may be susceptible to antibiotics released by the starter organisms (see Chapter 7). The acid sensitivity of *Campylobacter* spp. suggests that contaminants will not survive a normal fermentation (Cuk *et al.*, 1987; Uradzinski, 1990; Ionkova, 1990), but whether *Staphylococcus* spp., and in particular coagulase-positive strains (Masud *et al.*, 1993), can survive in yoghurt is a matter of some dispute (Arnott *et al.*, 1974; Alkanahl and Gasim, 1993). To date there have been no records of staphylococcal food poisoning being associated with the consumption of yoghurt in the United Kingdom (Gilbert and Wieneke, 1973; Keceli and Robinson, 1997) and Attaie *et al.* (1987) showed that a virulent strain of *Staphylococcus aureus* was inhibited during fermentations involving either yoghurt cultures or *L. acidophilus*. For this reason, an examination for staphylococci is not normally required for yoghurt (see Table 10.11) and even the test of fresh yoghurt for coliforms is probably of more value as an indicator of plant hygiene than as a warning that the product may constitute a health risk.

However, this general confidence does have to be tempered with caution, because a recent report linked an outbreak of *Escherichia coli* 0157 with the consumption of yoghurt (Morgan *et al.*, 1993; see also Kornacki and Marth, 1982; Reinheimer *et al.*, 1990; Martin and Marshall, 1995), so that it should be remembered that low starter activity and/or post-heat treatment contamination can lead to problems even with this traditionally safe product (Al-Mashhadi *et al.*, 1987; Ibrahim *et al.*, 1989). This latter point has been emphasised by studies with some of the so-called emerging pathogens like *Yersinia enterocolitica* and *Aeromonas hydrophila*, in which survival in yoghurt or in bio-yoghurt has been shown to be closely correlated with pH (Mantis *et al.*, 1982; Ahmed *et al.*, 1986; El-Kholy, 1992; El-Gmiey *et al.*, 1994; Aytac and Ozbac, 1994a, b; Ozbac and Aytac, 1995, 1996); the behaviour of *Bacillus cereus* in yoghurt will follow a similar pattern (Wong and Chen, 1988; Sultan *et al.*, 1988; Stadhouders and Driessen, 1992). However, many researchers have studied the behaviour of yoghurt starter cultures with antibacterial properties against pathogens, and the following reports are recommended for further reading (Mohran and Said, 1990; Bodana and Rao, 1990; Mohammed and Younis, 1990; Prasad and Ghodeker, 1991; Amin *et al.*, 1991; Bielecka *et al.*, 1994a, b; Balasubramanyam and Varadaraj, 1995; Ebringer *et al.*, 1995; Massa *et al.*, 1997; Yang *et al.*, 1997).

The freak occurrence of *Clostridium botulinum* in hazelnut yoghurt also highlights the need for vigilance (O'Mahony *et al.*, 1990; Collins-Thompson and Wood, 1993), but perhaps of more significance is the finding by Leyer and Johnson (1992) that *Salmonella typhimurium* can display a degree of adaptation to acidity (see also Hosoda *et al.*, 1992; Nadathur *et al.*, 1994). Clearly there is major difference between adaptation to a pH above 5.0 and adaptation to the pH of 4.0–4.2 found in yoghurt,

but with some mild yoghurts being produced at around pH 4.6, the situation needs to kept under observation.

More significant from the producer's standpoint is the examination for yeasts and moulds, for these organisms are capable of spoiling yoghurt well within an anticipated sell-by date. Thus, many fungi are little affected by low pH and with ample sucrose and/or lactose available as energy sources, unacceptable deterioration can be rapid. Yeasts, whether lactose utilisers like *K. marxianus* var *marxianus* and *K. marxianus* var *lactis* or more cosmopolitan species, such as *Saccharomyces cerevisiae* or *Torulopsis candida* (Jordano *et al.*, 1991b) are a major concern (Fleet, 1990a, b; see also Giudici *et al.*, 1996). In order to avoid in-carton fermentation – often manifest by a "doming" of the lid of a carton or collapse of the carton (Anon., 1987b; Foschino *et al.*, 1993), Davis *et al.* (1971) have suggested that yoghurt, at the point of sale, should contain ≤ 100 viable yeast cells ml^{-1}. Above $1.0 \times 10^3 cfu\,ml^{-1}$ would imply a serious risk of deterioration for, although serious gas production and off-flavour development may not be manifest until the yeast population reaches $1.0 \times 10^5 cfu\,ml^{-1}$, such counts can be achieved quite easily within a 2–3 week shelf life (Jordano and Salmeron, 1990; Jordano *et al.*, 1991a). A rapid test method for the detection of one yeast cell per pot in cultured milk products within 24 hours was reported by de Groote *et al.* (1995).

Moulds tend, on the whole, to develop more slowly than the yeasts and although some genera like *Aspergillus* can form button-like colonies within a coagulum, most fungi require oxygen for growth and sporulation. Hence, moulds are usually visible only in retail cartons of set yoghurt, since the surface of stirred yoghurt rarely remains undisturbed for any length of time. Nevertheless, occasional problems can arise from such genera as *Mucor*, *Rhizopus*, *Aspergillus*, *Penicillium* or *Alternaria* and the unsightly superficial growths of mycelium will lead to consumer complaints (Garcia and Fernandez, 1984). For this reason, a mould count of up to $10\,cfu\,ml^{-1}$ of retail product has been rated as doubtful quality by Davis *et al.* (1971).

It has been reported by Jordano *et al.* (1989) that some strains of *Aspergillus flavus* isolated from commercial yoghurts were aflatoxigenic but, although the sucrose content of fruit yoghurt would be sufficient to support aflatoxin production (Ahmed *et al.*, 1997), it has not been suggested that aflatoxin synthesis does occur in yoghurt. Aflatoxin M_1 has been identified on occasion in the milk for yoghurt production, but even this contamination may, depending on the pH of the product, decline during fermentation (Wiseman and Marth, 1983; Sharaf *et al.*, 1988; El-Deeb, 1989; Batish *et al.*, 1989; Karunaratne *et al.*, 1990; Rasic *et al.*, 1991; Hassanin, 1994; Gourama and Bullerman, 1995a, b; Garcia *et al.*, 1995; El-Nezami and Ahokas, 1998). However, in a recent survey in Italy, the occurrence of aflatoxin M_1 in yoghurt ($n = 91$) was 80% of the samples, and the amounts ranged between <1 and $497\,ng\,l^{-1}$; only two samples had levels that exceeded the Swiss legal limit ($>50\,ng\,l^{-1}$) (Galvano *et al.*, 1998).

At one time, fruit was the major source of fungal contamination (Fleischer *et al.*, 1984), but now that most sources will be heat treated prior to use, infection from this source should have been eliminated. Airborne spores or yeast cells can prove more difficult to control, particularly during certain months of the year and, unless a serious lapse in plant hygiene is suspected, high yeast or mould counts usually indicate contaminants in the atmosphere. The unexpected variety of yeast species isolated from yoghurt by Tilbury *et al.* (1974) and Suriyarachchi and Fleet (1981) can probably be explained by this type of chance contamination and protection

of the filling area is a top priority. Regular monitoring of the air in the processing area may help to identify the route taken by airborne propagules (Lück and Gavron, 1990) and the examination of representative samples of the end product employing acidified malt agar or Rose Bengal agar (Bridson, 1990), yoghurt whey agar (Yamani, 1993) or chloramphenicol agar (yeasts) (IDF, 1990d) can provide a warning of impending problems. Alternatively, impedance measurements can be employed to determine low levels of yeast in yoghurt (Shapton and Cooper, 1984; Pettipher, 1993; Bolton and Gibson, 1994; Kleiss *et al*., 1995) and the direct epifluo-rescent filter technique (DEFT) has been used succesfully by Rowe and McCann (1990) and McCann *et al.* (1991). More recently, the Petrifilm™ method has been recommended by Vlaemynck (1994) for enumerating yeasts and moulds in yoghurt, as has the ISO-GRID membrane filtration system in conjunction with YM-11 agar (Entis and Lerner, 1996; see also Salih *et al*., 1990).

In commercial practice, any yeast infection is regarded with dismay, and eradi-cation of the source becomes a priority. On occasion, this search may require the identification of the dominant spoilage yeast and the classic texts of Kreger-van Rij (1984) or Barnett *et al.* (1990) can prove valuable sources of information. However, traditional taxonomic methods can be extremely time consuming, and more rapid systems relying on oligonucleotide probes or polymerase chain reaction (PCR) fingerprinting have now been developed (IDF, 1998).

Overall, therefore, it is clear that well-made yoghurt should not present a manufacturer with many complaints as far as microbiological quality is concerned, although some small producers have yet to match the standards of the major sup-pliers (Tamime *et al.*, 1987).

10.5.3.2 Bio-yoghurts
All the yoghurts in this group, whether labelled as "bio" or "B/A" products, should contain high counts of a "health promoting" culture, such as *L. acidophilus*, *Bifido-bacterium* spp. or similar organisms, where high means above the therapeutic minimum discussed earlier. However, few commercial products contain just *L. acidophilus* and/or *Bifidobacterium*, so that any system for quality control must be able to cope with the presence of *S. thermophilus* and perhaps *L. delbrueckii* subsp. *bulgaricus* as well. Consequently, a range of media has evolved for examining bio-yoghurts for the presence of *L. acidophilus* alone in a fermented milk, or in the presence of other genera, and a selection of these media are shown in Table 10.12 (see also Lim *et al*., 1995; Lankaputhra *et al*., 1996; Dave and Shah, 1997; Micanel *et al*., 1997).

The choice of medium does, as mentioned elsewhere, depend on the personal preference of the operator, whether the medium needs to be selective for automatic counting, or whether differentiation between a number of species on one plate is an advantage and the strains of the species concerned, because the reactions in any medium are usually strain dependent. Some media, such as TPPYPB, are good for differentiating the normal yoghurt microflora, but offer the additional advantage of enabling an inoculum of a yoghurt culture, bifidobacteria and *L. acidophilus* to be enumerated on one medium (Ghoddusi and Robinson, 1996). Thus, colonies of *S. thermophilus* appear as small pale blue colonies with a thin blue zone in RCPB agar, while *Bifidobacterium* spp. give rise to white colonies. *L. delbrueckii* subsp. *bul-garicus* produce small, shiny white colonies surrounded by a wide, royal blue zone and *L. acidophilus* are readily distinguished as large, pale blue colonies surrounded

Table 10.12 Some of the media which can be employed to enumerate *L. acidophilus* and other "health-promoting" lactobacilli when growing either alone in milk, or in the presence of other lactic acid bacteria

Culture medium	Species	Comments
L. acidophilus alone		
MRS agar	*L. acidophilus*	Non-selective
Tomato juice agar	*L. acidophilus*	Non-selective
L. acidophilus in the presence of *Leuconostoc* or *Lactococcus* spp.		
Aesculin-cellobiose agar	*L. acidophilus*	The ability of *L. acidophilus* to hydrolyse aesculin and ferment cellobiose at 40°C makes this medium selective.
X-Glu-agar	*L. acidophilus*	Mesophiles lack the β-D-glucosidase enzyme which is the basis for the colour reaction produced by *L. acidophilus*.
L. acidophilus in the presence of thermophilic lactic acid bacteria		
MRS-bile agar	*L. acidophilus*	Bile salts inhibit yoghurt cultures and aerobic incubation restricts the growth of bifidobacteria.
TGV agar	Non-selective	Colonies distinguished on basis of colour and morphology.
LA agar	Non-selective	Colonies distinguished on basis of colour and morphology.
TPPYPB agar	Non-selective	Colonies distinguished on basis of colour and morphology.

Note: The media may have to be modified for counting of *L. reuteri* or *L. rhamnosus*; some species of bifidobacteria can utilise cellobiose.

After: IDF (1995a) and Ghoddusi and Robinson (1996).

by a wide, royal blue zone; Rybka and Kailasapathy (1996) proposed a similar scheme employing RCPB agar. It must be emphasised again that differences between strains may prove to be important and it may be that a number of the media mentioned in Tables 10.5 to 10.7 are capable of further modification and improvement.

If *Bifidobacterium* spp. alone are the organisms of interest, then species of human origin grow well on a number of media (see Table 10.13). However, in the presence of other lactic acid bacteria, selective agents have to be employed and the choice and level of the agents can have a dramatic impact on recovery. Thus as shown in Table 10.14, on blood liver agar supplemented with neomycin sulphate, paramomycin sulphate, nalidixic acid and lithium chloride (NPNL), only *Bifidobacterium bifidum* showed acceptable growth. The same contrast in growth responses was observed on modified rogosa agar with NPNL added at $50\,ml\,l^{-1}$ of medium, and also with TPY agar. Successful growth of both species of *Bifidobacterium* was recorded in the presence of lithium chloride/sodium propionate, but unfortunately both mesophilic and other thermophilic lactic acid bacteria grow on the same medium. The most promising option, therefore, appears to be the use of TPY agar with $20\,ml\,l^{-1}$ NPNL which gives good recovery for both *B. bifidum* and *Bifidobacterium adolescentis* while, at the same time, eliminating the growth of other cultures.

Table 10.13 Some of the media which can be employed to enumerate *Bifidobacterium* spp. growing either alone in milk or in the presence of other lactic acid bacteria under anaerobic conditions at 37°C

Culture medium (agar)	Selective supplement	Response of *Bifidobacterium* sp.
Blood liver	Nil	Excellent growth
Modified rogosa	Nil	Excellent growth
de Man rogosa sharpe	Nil	Limited growth
Tomato juice	Nil	Excellent growth
Trypticase phytone yeast	Nil	Excellent growth
Lithium chloride/Na-propionate	Nil	Excellent growth[a]
AMC[f]	–	Specified for *Bifdobacterium longum*
Blood liver	NPNL[c]	Good growth/selectivity[b]
Modified rogosa	NPNL	Good growth/selectivity
Modified rogosa	PPNL[d]	Excellent growth/selectivity
Trypticase phytone yeast	NNL[e]	Excellent growth/selectivity

[a] This medium may NOT be selective against certain strains of cheese or yoghurt starter cultures. [b] Recovery of *Bifidobacterium* spp. depends on the species, strain of the species and the concentration of NPNL. [c] NPNL (mg 100 ml^{-1} of stock solution): neomycin sulphate (10), paramomycin sulphate (20), nalidixic acid (1.5), lithium chloride (300) with an addition rate of 2–5 ml of stock solution 100 ml^{-1} of medium. [d] PPNL (mg 100 ml^{-1} of stock solution): sodium propionate (6000), paramomycin sulphate (200), neomycin sulphate (800), lithium chloride (12 000) with an addition rate of 5 ml 100 ml^{-1} of medium. [e] NNL (mg 100 ml^{-1} of stock solution): neomycin sulphate (200), nalidixic acid (30), lithium chloride (6000) with an addition rate of 5 ml 100 ml^{-1} of medium. [f] AMC from work by Arroyo *et al.* (1995).

After de Man *et al.* (1960), Shimada *et al.* (1977), Teraguchi *et al.* (1978), Scardovi (1986), Rasic (1990), Samona and Robinson (1991), Lapierre *et al.* (1992), Arroyo *et al.* (1995) and Tamime *et al.* (1995).

Table 10.14 Total colony counts ($\times 10^6$ cfu ml^{-1}) of the organisms indicated on a range of general and selective media that have been recommended for the enumeration of bifidobacteria

Medium (agar)	Supplement	*B. bifidum*	*B. adolescentis*
Blood liver	–	510	850
	NPNL[a]	280	0
	PPNL[a]	0	0
Modified rogosa	–	202	720
	NPNL	180	0
	PPNL	0	0
TPY	–	250	790
	NPNL	340	0
	PPNL	0	0
	NPNL[b]	190	680
	LP[c]	630	1140

[a] The compositions of these supplements are shown in Table 10.13. [b] The NPNL concentration is 2 ml 100 ml^{-1}. [c] LP (g 100 ml^{-1}): lithium chloride (0.2) and sodium propionate (0.3); each composite solution was added to the basic medium at a rate of 5 ml 100 ml^{-1}; this supplement is NOT selective against all strains of yoghurt or cheese cultures.

Note: The plates were incubated anaerobically at 37°C.

After Ghoddusi (1996).

However, the need to check any proposed medium against the strains being employed in the factory is emphasised by the fact that, while *Lactococcus* species (NCDO 276) grew on TPY agar with 20 ml l^{-1} NPNL, *Lactococcus* species (NCDO 763) was inhibited; similar patterns could probably observed with strains of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* as well. Unless colony morphology/colour eliminates confusion, it is important to note that checks on typical colonies under the microscope may not prove helpful. Thus, while the cells of bifidobacteria are usually Y-shaped or bone-shaped depending upon the species (Tamime *et al.*, 1995), they may become coccoid if the growing conditions so dictate (Samona and Robinson, 1991). Consequently, the observation of Gram-positive cocci in a breed smear of a colony from a plate of a medium presumed to be selective could result from the presence of either normal *S. thermophilus* or abnormal *Bifidobacterium* sp.; cell shape and size would provide little help. It may be worth mentioning, however, that the change in morphology tends to occur over several generations, so that the examination of a bio-yoghurt manufactured with a direct-to-vat culture should reveal typical Y-shaped or bone-shaped cells.

In addition, it should be emphasised that the concentration of any antibiotic mixture can have an impact on the total level of recovery of bifidobacteria (Samona and Robinson, 1991) and, bearing in mind that health claims should be supported by high cell counts in the product, media selection becomes a vital issue.

10.5.4 Assessment of organoleptic characteristics

The ultimate judge of any product in a free society is the consumer and although brand awareness only accounts for some 20% of decisions of purchase (Kroger and Fram, 1975), deliberate avoidance of a brand as the result of dissatisfaction represents a completely separate situation. To some extent, the chemical and physical analyses suggested earlier (e.g. titratable acidity and viscosity) will provide a reasonable indication that the normal in-house standards have been achieved, but the use of some form of taste panel to perform a final check is usual practice. The composition of such panels can range from "one man and a plastic teaspoon" through to a full panel of trained tasters selected and organised along the lines proposed by Amerine *et al.* (1965), Stone and Sidel (1985) and Lyon *et al.* (1992). Obviously no one would dispute the skill of the individual (Harper, 1962, 1972, 1977), but the more objective and quantifiable the acquired data can be made, the easier the task of maintaining standards over a long period of time.

This latter approach is, of course, time-consuming and may involve:

- convening a panel of at least five judges (with alternatives) on the basis of their knowledge of the product and their willingness to participate on a regular basis;
- obtaining agreement among the judges on the characteristics of a good quality yoghurt, with the definition of good quality being solely related either to products from the factory in question or to a specific brand image;
- obtaining agreement among the judges about what is meant by the terminology that might be applied to certain faults or defects; and
- the derivation of a scheme of assessment that can be employed as part of a routine quality control procedure.

The ultimate selection of a scheme will rest with the panel concerned, but three typical schemes that have been proposed and/or employed in various countries are shown in Tables 10.15, 10.16 and 10.17 (Fütschik, 1963; Bergel, 1971a; Pearce and

Heap, 1974; Bodyfelt *et al.*, 1988; see also Grab, 1983). The over-riding factors must be operational simplicity and the ability of the procedure to discriminate between samples.

A few practice runs will quickly establish the preferences of a particular panel and it should then be possible to accept, perhaps with modification, one of the available schemes. It is worth noting, however, that the description of defects can be a valuable part of the exercise, because the quality controller may then be in a position to indicate why the particular batch of yoghurt has scored poorly in certain respects (see also Bodyfelt *et al.*, 1988; Ogden, 1993; IDF, 1997d). Thus, as shown in Table 10.18, some degree of association between a recognised faults and likely causes does exist, and hence an accurate description from a taste panel can speed up the implementation of remedial action.

In some instances the causes of defects are not readily identifiable, the apparently seasonal occurrence of granulation in yoghurt is a case in point (Robinson, 1981). Thus, although there is evidence linking poor process control with the formation of small, protein-rich lumps in yoghurt, a fault especially noticeable in fruit yoghurts, there have been reports that the defect is most prominent during the spring and autumn months (Cooper *et al.*, 1974). Whether this periodicity is linked with seasonal changes in milk composition has not been established, nor is it clear

Table 10.15   Yoghurt Evaluation – Scheme I – The Karl Ruher Nine Point Scheme

Score	Judgement	Quality	Range	Class	Overall classification
9	Excellent	–		Upper	
8	Very good	Very good	I	Medium	Free of objections
7	Good	Good		Lower	
6	Satisfactory	Satisfactory		Upper	
5	Mediocre	Average	II	Medium	Still acceptable
4	Sufficient	Sufficient		Lower	in commerce
3	Imperfect	Bad		Upper	
2	Bad	Bad	III	Medium	Unsaleable
1	Very bad	Bad		Lower	

Table 10.16   Yoghurt Evaluation – Scheme II

Date:
Taster: Code No:
    a. Appearance and colour   ....
        Defects   ....
    b. Body and Texture   ....
        Defects   ....
    c. Flavour   ....
        Defects   ....
                Overall score   ....

Judge the three characteristics on a 1–5 scale of: 5 excellent; 4 very good; 3 good; 2 fair; 1 poor.

The overall score is obtained by multiplying the flavour score by 2 and then adding that score to the rest. An excellent yoghurt gives an overall score of 20.

Possible defects: (a) appearance and colour (extraneous matter, lack of uniformity, unnatural colour, surface discoloration, wheying-off, fat separation, gassiness), (b) body and texture (too thin, gelatinous, chalky, lumpy or granular, slimy), and (c) flavour (excess acid, excess sugar, excess stabiliser, excess milk powder, yeasty, unclean).

Table 10.17 Yoghurt Evaluation – Scheme III – Approved by the American Dairy Science Association

Yoghurt Score Card	
Attribute	Perfect score
Flavour	10
Body and Texture	5
Appearance	5
Total	20

Defects: (a) flavour (acetaldehyde – coarse, bitter, cooked, foreign, high and/or acid, lacks flavour; flavouring – freshness and/or sweetness, old ingredient, oxidised, rancid, too high flavouring and/or sweetness, unnatural flavour, unclean), (b) body and texture (gel-like, grainy, ropy, too firm, weak), and (c) appearance (atypical colour, colour leaching, excess fruit, free whey, lacks fruit, lumpy, shrunken, surface growth).

why some manufacturers observe the problem more than others, why changes in starter culture can often solve the problem, and why reversion to the original culture after 2–3 weeks does not lead to reoccurrence of the problem; applying a high shear to yoghurt after manufacture reduced nodulation, but also reduced viscosity (Guirguis *et al*., 1987). Furthermore, it may be likely that during the fermentation stage, the starter organisms clump and generate a region of low pH causing isoelectric precipitation of casein in and around the clumps (Weeks *et al*., 1997).

Schemes of the type cited earlier have the further attraction of being easy to operate once the panel has become familiar with the product and the use of the form but, at the end of the day, they do remain essentially subjective. For this reason, there has been much attention paid to the possibility of imposing a more rigid framework within which the taste panel might operate. To this end, Robinson (1988) applied qualititative descriptive analysis (QDA) as described by Powers (1988) to natural yoghurts produced with different starter cultures. Ten terms were employed to describe the flavour or mouthfeel of the yoghurts, and the attribute profiles were easily distinguished (see Fig. 10.11). The scheme is equally applicable to stirred fruit/flavoured yoghurts and, once the terms covering a typical retail sample are agreed by a panel and the profile drawn, the profiles for subsequent samples can be compared by superimposition. By performing this operation on a weekly basis, changes in perceived quality can be readily detected; experience will soon indicate whether an observed difference between the sample and the standard profiles is significant.

Multidimensional scaling procedures (i.e. KYST and SINDSCAL) have been used by Poste and Patterson (1988) to identify yoghurt characteristics by trained panelists. Nine selected attributes were selected by the panellists, and the presence of fruit, sweetness, acidity and lumpiness of yoghurt appeared to be predominant in the perceived interrelationships. However, Tuorila *et al.* (1993) reported the sensory results of a trained panel ($n = 14$) and consumer panel ($n = 41$), and while perceived sweetness and creaminess were interrelated, acid taste was not; yoghurt samples with sucrose $10\,g\,100\,g^{-1}$ and fat $3.5\,g\,100\,g^{-1}$ received the highest scores, whilst more men than women preferred sweet and creamy yoghurts.

In an attempt to determine which organoleptic attributes were important with respect to consumer perceptions of quality, Muir and Hunter (1992) asked a panel

Table 10.18 Some common defects of yoghurt and an indication of some possible causes and remedies

Defect	Possible causes	Remedy
Syneresis	Low SNF or fat content	Adjust formulation
	High mineral content in milk	Blend with milk of low mineral content
	Insufficient heat treatment/ homogenisation of milk	Adjust process conditions
	Too high incubation temperature	Reduce temperature to 42°C
	Low acidity, e.g. pH 4.8	Ensure pH 4.4
	Adventitious enzymes capable of coagulating protein	Eliminate source
	Disturbance of coagulum prior to cooling	Adequate cooling
	Unspecified	Addition of stabiliser
		Change to culture of viscous type
Low viscosity	Low total solids	Adjust formulation
	Insufficient heat treatment/ homogenisation of milk	Adjust process conditions
	Too low incubation temperature	Raise temperature to 42°C
	Too low inoculation rate	Raise inoculum to $2\,ml\,100\,ml^{-1}$ or equivalent
	Prolonged agitation	Improve mechanical handling system
	Unspecified	Addition of stabiliser
		Change culture to viscous type
Bubbles in coagulum (in gel)	Poor storage conditions	Check temperature of cool stores
	Contamination with yeasts	Eliminate source of infection
	Contamination with coliforms	Poor plant or starter hygiene
	Excessive aeration of mix	Control agitation
Granular coagulum	Poor mixing of milk powder	Adjust process conditions
	Agitation prior to cooling	Adequate cooling
	Too high incubation temperature	Reduce temperature to 42°C
	Too low inoculation rate	Raise inoculum to $2\,ml\,100\,ml^{-1}$ or equivalent
	Unspecified	Change culture to viscous type
Flavour problems	Insipid	Lower inoculum to $2\,ml\,100\,ml^{-1}$ or equivalent
		Extend incubation time
	Unclean	Raise inoculum to $2\,ml\,100\,ml^{-1}$ or equivalent
		Reduce incubation time
		Check for contamination with coliforms
	Bitter	Lower inoculum to $2\,ml\,100\,ml^{-1}$ or equivalent
		Change starter culture
	Sour	Lower inoculation rate
		Check temperature to storage
	Malty/yeasty	Suspect contamination by yeasts
	Rancid	Check quality of raw milk

See also Connolly (1990).
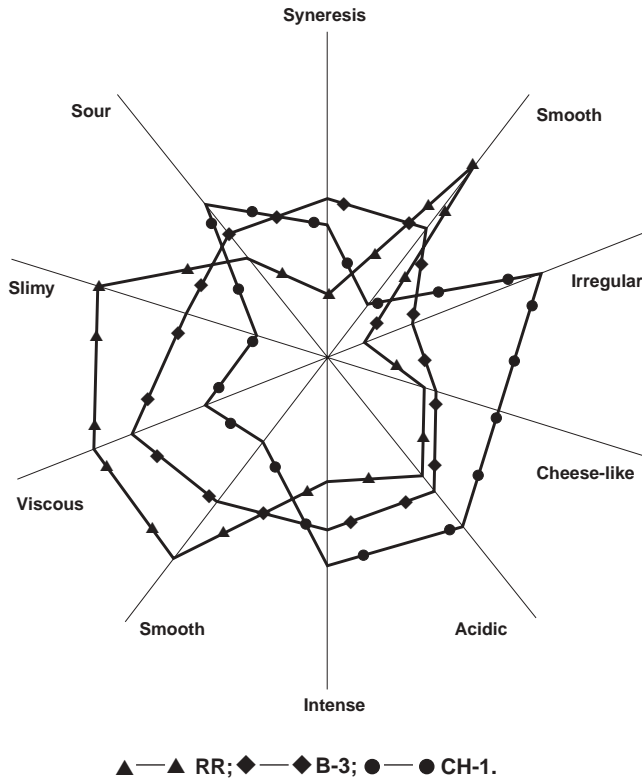
Fig. 10.11   Attribute profiles of natural yoghurts made with three different cultures

Culture RR was obtained from NIZO in the Netherlands and cultures B-3 and CH-1 from Chr. Hansen's Laboratory in the UK.

Reproduced by courtesy of *Dairy Industries International*.

of 20 judges familiar with fermented milks to examine nine different types of commercial natural yoghurt/yoghurt-style product, and suggest terms that described the sensory properties of one or more of the samples. The samples ranged from low fat fromage frais through to Greek-style yoghurt with $10\,g\,fat\,100\,g^{-1}$ and the consensus was that the following terms were important:

Odour	Flavour	Aftertaste	Texture
Intensity	Intensity	Intensity	Firmness
Sour	Sour/acid	Bitter	Creaminess
Fruity	Fruity	Sour/acid	Viscosity
Buttery	Buttery	Other	Sliminess
Yeasty	Rancid		Curdy
Creamy	Creamy		Mouth-coating
Sweet	Salty		Chalky
Other	Bitter		Serum separation
	Lemon		
	Sweet		
	Chemical		
	Other		

The application of QDA under rigorously controlled conditions (MacFie *et al.*, 1989) provided data for analysis by principal component analysis (Piggott, 1988) and the results of Muir and Hunter (1992) revealed a number of important points with respect to the sensory analysis of yoghurt, namely:

- giving the panel the option of the term "other" generates a plethora of descriptors that are not helpful;
- the data could be simplified into five principal components responsible for over 90% of the total variance, i.e.

Odour:	Flavour:	After-taste:	Texture:
Sweet	Intensity	Acid	Firmness
	Acid/sour		Creamy
	Fruity		Viscosity
	Creamy		Curdy
	Lemon		Chalky
	Sweet		Serum separation

In other words, it would appear that five attributes are important in discriminating between and/or describing fermented milks: acidity, curd character, sweetness, creamy character and chalkiness. However, the further application of this conclusion would merit caution, because consumer reaction to acidity, for example, can be positive or negative depending on the market (Greig and van Kan, 1984). The reaction to sweetness can again vary with the market and the growing demand for mild flavoured, sweet yoghurts in the U.K. suggests that a large sector of the public link sucrose content with acceptability (McGregor and White, 1986). Evidence from the retail sector would support the view that a creamy mouthfeel is strongly correlated with a perception of quality and many stirred fruit yoghurts now contain over $1.0 \, g \, 100 \, g^{-1}$ milk fat as routine. The isolation of curd character and chalkiness is probably a reflection of the restriction of the procedure to natural yoghurts, often of the set variety, and the wider usefulness of these terms might merit further study.

It is of note also that none of the panellists appears to have suggested the term "yoghurt-like", because the flavour of natural yoghurt, based upon acetaldehyde and similar components, is quite unique among the fermented milks (Hruskar *et al*., 1995). Thus, the use of a generic name to describe the flavour of a product has much to recommend it (Harper, 1962) and it would be interesting to know what panellists were actually describing as intensity. Nevertheless, this more objective approach to sensory analysis will help to define those characters of a product that are important with respect to acceptability, so enabling manufacturers to refine their own routine assessments.

To this end, some additional sensory studies on yoghurt have included first, preference mapping that allows the investigator to relate the preference responses of consumers to a map where the results can be related to product formulations (Anon., 1998b). Gains and Gutteridge (1991) evaluated different British yoghurts using this technique and they reported that the rank order of the yoghurts preferred by consumers were population 1 – thick and creamy, population 2 – natural, and population 3 – low fat. Second, using different types of starter cultures to make yoghurt, Rohm *et al*. (1994) observed that the trained assessors could easily identify differences in each sensory categories of these products except gel firmness using a hedonic scale. Multiple regression analysis revealed that the results were mainly determined by flavour and EPS cultures, showing positive and negative

weightings, respectively. Third, Stoer and Lawless (1993) concluded from their organoleptic study (*n* = 920) of dairy products including yoghurt that both single product scaling and relative-to-product scaling methods of assessment by trained and untrained panellists were equal in their efficiency for sensory evaluation. Fourth, detailed studies of the sensory ratings of commercial yoghurts (plain and fruit flavoured) by a consumer panel (*n* = up to 180) and a descriptive panel (*n* = 11) have been reported by Barnes *et al.* (1991a, b) and Harper *et al.* (1991) (see also Muir *et al.*, 1997; Kähkönen *et al.*, 1997). Fifth, taste and health claims for yoghurt had the largest influence on buying intent of American consumers, whilst brand had little influence (Vickers, 1993).

10.6　Conclusions

If the essential requirements for manufacturing a high quality yoghurt were to be tabulated (see also Lewis and Dale, 1994), then it is likely that the list might look rather like this:

- milk of good quality and adequate SNF;
- correct heat treatment;
- an active, well-balanced and contaminant-free starter culture;
- a clean and well-maintained plant;
- correct inoculation rate;
- correct incubation times and temperatures;
- avoidance of rough handling of set yoghurts;
- the use of high quality fruit or other ingredients;
- correct storage of retail product below 5°C,

and what is important about this list is that all these areas should form part of the commitment to good manufacturing practice. The actual degree of surveillance will vary in the light of experience in a particular plant, but the principle remains the same, namely that someone in authority must have an accurate picture of the entire operation, for without this, the smooth running of the plant and the quality of the end product will be at risk.

10.7　References

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Appendix I

Different ways in which titratable acidity is expressed and their relative values to % lactic acid

% Lactic acid	Soxhlet-Henkel (°SH)	Thorner (°T)	Dornic (°D)	% Lactic acid	Soxhlet-Henkel (°SH)	Thorner (°T)	Dornic (°D)
0.0000	0	0.0	0.00	0.6975	31	77.5	69.75
0.0225	1	2.5	2.25	0.7200	32	80.0	72.00
0.0450	2	5.0	4.50	0.7425	33	82.5	74.25
0.0675	3	7.5	6.75	0.7650	34	85.0	76.50
0.0900	4	10.0	9.00	0.7875	35	87.5	78.79
0.1125	5	12.5	11.25	0.8100	36	90.0	81.00
0.1350	6	15.0	13.50	0.8325	37	92.5	83.25
0.1575	7	17.5	15.75	0.8550	38	95.0	85.50
0.1800	8	20.0	18.00	0.8775	39	97.5	87.75
0.2025	9	22.5	20.25	0.9000	40	100.0	90.00
0.2250	10	25.0	22.50	0.9225	41	102.5	92.25
0.2475	11	27.5	24.75	0.9450	42	105.0	94.50
0.2700	12	30.0	27.00	0.9675	43	107.5	96.75
0.2925	13	32.5	29.25	0.9900	44	110.0	99.00
0.3150	14	35.0	31.50	1.0125	45	112.5	101.25
0.3375	15	37.5	33.75	1.0350	46	115.0	103.50
0.3600	16	40.0	36.00	1.0575	47	117.5	105.75
0.3825	17	42.5	38.25	1.0800	48	120.0	108.00
0.4050	18	45.0	40.50	1.1025	49	122.5	110.25
0.4275	19	47.5	42.75	1.1250	50	125.0	112.50
0.4500	20	50.0	45.00	1.1475	51	127.5	114.75
0.4725	21	52.5	47.25	1.1700	52	130.0	117.00
0.4950	22	55.0	49.50	1.1925	53	132.5	119.25
0.5175	23	57.5	51.75	1.2150	54	135.0	121.50
0.5400	24	60.0	54.00	1.2375	55	137.5	123.75
0.5625	25	62.5	56.25	1.2600	56	140.0	126.00
0.5850	26	65.0	58.50	1.2825	57	142.5	128.25
0.6075	27	67.5	60.75	1.3050	58	145.0	130.50
0.6300	28	70.0	63.00	1.3275	59	147.5	132.75
0.6525	29	72.5	65.25	1.3500	60	150.0	135.00
0.6750	30	75.0	67.50	1.3725	61	152.5	137.25

% Lactic acid	Soxhlet-Henkel (°SH)	Thorner (°T)	Dornic (°D)	% Lactic acid	Soxhlet-Henkel (°SH)	Thorner (°T)	Dornic (°D)
1.3950	62	155.0	139.50	1.8000	80	200.0	180.00
1.4175	63	157.5	141.75	1.8225	81	202.5	182.25
1.4400	64	160.0	144.00	1.8450	82	205.0	184.50
1.4625	65	162.5	146.25	1.8675	83	207.5	186.75
1.4850	66	165.0	148.50	1.8900	84	210.0	189.00
1.5070	67	167.5	150.75	1.9125	85	212.5	191.25
1.5300	68	170.0	153.00	1.9350	86	215.0	193.50
1.5525	69	172.5	155.25	1.9575	87	217.5	195.75
1.5750	70	175.0	157.50	1.9800	88	220.0	198.00
1.5975	71	177.5	159.75	2.0025	89	222.5	200.25
1.6200	72	180.0	162.00	2.0250	90	225.0	202.50
1.6425	73	182.5	164.25	2.0475	91	227.5	204.75
1.6650	74	185.0	166.50	2.0700	92	230.0	207.00
1.6875	75	187.5	168.75	2.0925	93	232.5	209.25
1.7100	76	190.0	171.00	2.1150	94	235.0	211.50
1.7325	77	192.5	173.25	2.1375	95	237.5	213.75
1.7550	78	195.0	175.50	2.1600	96	240.0	216.00
1.7775	79	197.5	177.75	2.1825	97	242.5	218.25

Appendix II

Temperature conversion*

	Centigrade/Celsius °C = 5/9 (F − 32) = 0.555 (F − 32)			Fahrenheit °F = (9/5 × C) + 32 = (1.8 × C) + 32			
°C		°F	°C		°F	°C		°F
−31.7	−25	−13.0	−15.6	4	39.2	0.6	33	91.4
−31.6	−24	−11.2	−15.0	5	41.0	1.1	34	93.2
−30.6	−23	−9.4	−14.4	6	42.8	1.7	35	95.0
−30.0	−22	−7.6	−13.9	7	44.6	2.2	36	96.8
−29.4	−21	−5.8	−13.3	8	46.4	2.8	37	98.6
−28.9	−20	−4.0	−12.8	9	48.2	3.3	38	100.4
−28.3	−19	−2.2	−12.2	10	50.0	3.9	39	102.2
−27.8	−18	−0.4	−11.7	11	51.8	4.4	40	104.0
−27.2	−17	1.4	−11.1	12	53.6	5.0	41	105.8
−26.7	−16	3.2	−10.6	13	55.4	5.6	42	107.6
−26.1	−15	5.0	−10.0	14	57.2	6.1	43	109.4
−25.6	−14	6.8	−9.4	15	59.0	6.7	44	111.2
−25.0	−13	8.6	−8.9	16	60.8	7.2	45	113.0
−24.4	−12	10.4	−8.3	17	62.6	7.8	46	114.8
−23.9	−11	12.2	−7.8	18	64.4	8.3	47	116.6
−23.4	−10	14.0	−7.2	19	66.2	8.9	48	118.4
−22.8	−9	15.8	−6.7	20	68.0	9.4	49	120.2
−22.2	−8	17.6	−6.1	21	69.8	10.0	50	122.0
−21.7	−7	19.4	−5.6	22	71.6	10.6	51	123.8
−21.1	−6	21.2	−5.0	23	73.4	11.1	52	125.6
−20.6	−5	23.0	−4.4	24	75.2	11.7	53	127.4
−20.0	−4	24.8	−3.9	25	77.0	12.2	54	129.2
−19.4	−3	26.6	−3.3	26	78.8	12.8	55	131.0
−18.9	−2	28.4	−2.8	27	80.6	13.3	56	132.8
−18.3	−1	30.2	−2.2	28	82.4	13.9	57	134.6
−17.8	0	32.0	−1.7	29	84.2	14.4	58	136.4
−17.2	1	33.8	−1.1	30	86.0	15.0	59	138.2
−16.7	2	35.6	−0.6	31	87.8	15.6	60	140.0
−16.1	3	37.4	0	32	89.6	16.1	61	141.8

°C		°F	°C		°F	°C		°F
16.7	62	143.6	47.2	117	242.6	77.8	172	341.6
17.2	63	145.5	47.8	118	244.4	78.3	173	343.4
17.8	64	147.2	48.3	119	246.2	78.9	174	345.2
18.3	65	149.0	48.9	120	248.0	79.4	175	347.0
18.9	66	150.8	49.4	121	249.8	80.0	176	348.8
19.4	67	152.6	50.0	122	251.6	80.6	177	350.6
20.0	68	154.4	50.6	123	253.4	81.1	178	352.4
20.6	69	156.2	51.1	124	255.2	81.7	179	354.2
21.1	70	158.0	51.7	125	257.0	82.2	180	356.0
21.7	71	159.8	52.2	126	258.8	82.8	181	357.8
22.2	72	161.6	52.8	127	260.6	83.3	182	359.6
22.8	73	163.4	53.3	128	262.4	83.9	183	361.4
23.3	74	165.2	53.9	129	264.2	84.4	184	363.2
23.9	75	167.0	54.4	130	266.0	85.0	185	365.0
24.4	76	168.8	55.0	131	267.8	85.6	186	366.8
25.0	77	170.6	55.6	132	269.6	86.1	187	368.6
25.6	78	172.4	56.1	133	271.4	86.7	188	370.4
26.1	79	174.2	56.7	134	273.2	87.2	189	372.2
26.7	80	176.0	57.2	135	275.0	87.8	190	374.0
27.2	81	177.8	57.8	136	276.8	88.3	191	375.8
27.8	82	179.6	58.3	137	278.6	88.9	192	377.6
28.4	83	181.4	58.9	138	280.4	89.4	193	379.4
28.9	84	183.2	59.4	139	282.2	90.0	194	381.8
29.4	85	185.0	60.0	140	284.0	90.6	195	383.6
30.0	86	186.8	60.6	141	285.8	91.1	196	384.6
30.6	87	188.6	61.1	142	287.6	91.7	197	386.6
31.1	88	190.4	61.7	143	289.4	92.2	198	388.4
31.7	89	192.2	62.2	144	291.4	92.8	199	390.2
32.2	90	194.4	62.8	145	293.0	93.3	200	392.0
32.8	91	195.8	63.3	146	294.8	93.9	201	393.8
33.3	92	197.6	63.9	147	296.6	94.4	202	395.6
33.9	93	199.4	64.4	148	298.4	95.0	203	397.4
34.4	94	210.2	65.0	149	300.2	95.6	204	399.2
35.0	95	203.0	65.6	150	302.0	96.1	205	401.0
35.6	96	204.8	66.1	151	303.8	96.7	206	402.8
36.1	97	206.6	66.7	152	305.6	97.2	207	404.6
36.7	98	208.4	67.2	153	307.4	97.8	208	406.4
37.2	99	210.2	67.8	154	309.2	98.3	209	408.2
37.8	100	212.0	68.3	155	311.0	98.9	210	410.0
38.3	101	213.8	68.9	156	311.8	99.4	211	411.8
38.9	102	215.6	69.4	157	314.6	100.0	212	413.6
39.4	103	217.4	70.0	158	316.4	100.6	213	415.4
40.0	104	219.2	70.6	159	318.2	101.1	214	417.2
40.6	105	221.0	71.1	160	320.0	101.7	215	419.0
41.1	106	222.8	71.7	161	321.8	102.2	216	420.8
41.7	107	224.6	72.2	162	323.6	102.8	217	422.6
42.2	108	226.4	72.8	163	325.4	103.3	218	424.4
42.8	109	228.2	73.3	164	327.2	103.9	219	426.2
43.3	110	230.0	73.9	165	329.0	104.4	220	428.0
43.9	111	231.9	74.4	166	330.8	105.0	221	429.8
44.4	112	233.6	75.0	167	332.6	105.6	222	431.6
45.0	113	235.4	75.6	168	334.4	106.1	223	433.4
45.6	114	237.2	76.1	169	336.2	106.7	224	435.2
46.1	115	239.0	76.7	170	338.2	107.2	225	437.0
46.7	116	240.8	77.2	171	339.8	107.8	226	438.8

Continued

°C		°F	°C		°F	°C		°F
108.3	227	440.6	122.2	252	485.6	136.1	277	530.6
108.9	228	442.4	122.8	253	487.4	136.7	278	532.4
109.4	229	444.2	123.3	254	489.2	137.2	279	534.2
110.0	230	446.0	123.9	255	491.0	137.8	280	536.0
110.6	231	447.8	124.4	256	492.8	138.3	281	537.8
111.1	232	449.6	125.0	257	494.6	138.9	282	539.6
111.7	233	451.4	125.6	258	496.4	139.4	283	541.4
112.2	234	453.2	126.1	259	498.2	140.0	284	543.2
112.8	235	455.0	126.7	260	500.0	140.6	285	545.0
113.3	236	456.8	127.2	261	501.8	141.1	286	546.8
113.9	237	458.6	127.8	262	503.6	141.7	287	548.6
114.4	238	460.4	128.3	263	505.4	142.2	288	550.4
115.0	239	464.4	128.9	264	507.2	142.8	289	552.2
115.6	240	464.0	129.4	265	509.0	143.3	290	554.0
116.1	241	465.8	130.0	266	510.8	143.9	291	555.8
116.7	242	467.6	130.6	267	512.6	144.4	292	557.6
117.2	243	469.4	131.1	268	514.4	145.0	293	559.4
117.8	244	471.2	131.7	269	516.2	145.6	294	561.2
118.3	245	473.0	132.2	270	518.0	146.1	295	563.0
118.9	246	474.8	132.8	271	519.8	146.7	296	564.8
119.4	247	476.6	133.3	272	521.6	147.2	297	566.6
120.0	248	478.4	133.9	273	523.4	147.8	298	568.4
120.6	249	480.2	134.4	274	525.2	148.3	299	570.2
121.1	250	482.0	135.0	275	527.0	148.9	300	572.0
121.7	251	483.8	135.6	276	528.8			

* Find the known temperature to be converted in the "boxed" column, then read the conversion to the left for °C and/or right for °F.
Example: Convert the following known temperature, i.e. 50

10.0 | 50 | 122.0

∴ 50°C = 122.0°F
or 50°F = 10.0°C

Appendix III

Volume units

Metric (SI) (Prefixes)		*Imperial (Imp) and US*
Kilo-	= 1000	Gallon
Hecto-	= 100	Yard
Deca-	= 10	Foot
Deci-	= 0.1	Inch
Centi-	= 0.01	Pint
Milli-	= 0.001	Fluid ounce
Micro-	= 0.000 001	Drams

	To convert volume	*Multiply by*
Millilitres to	gallons (Imp)	0.00022
	gallons (US)	0.00026
	fluid ounces (Imp)	0.03520
	fluid ounces (US)	0.03380
Cubic centimetres to	cubic inches	0.06100
Cubic metres to	cubic inches	61.0×10^{-3}
	cubic feet	35.3000
	gallons (Imp)	220.000
	gallons (US)	264.1700
Litres to	cubic feet	0.03500
	cubic inches	61.03000
	fluid ounces (Imp)	35.19600
	fluid ounces (US)	33.81400
	gallons (Imp)	0.21990
	gallons (US)	0.26420
	pints (Imp)	1.75980
	pints (US)	2.11340
	quarts (Imp)	0.87880
	quarts (US)	1.05670
Fluid ounces (US) to	cubic inches	1.80500
	gallons (US)	0.00780
	litres	0.02950
	millilitres	29.57000
	fluid ounces (Imp)	1.04100

Fluid ounces (Imp) to	gallons (Imp)	0.00630
	millilitres	28.4100
	fluid ounces (US)	0.96000
Pints (Imp) to	litres	0.56800
	pints (US)	0.83270
Pints (US) to	litres	0.47320
	pints (Imp)	1.20090
Quarts (Imp) to	litres	1.13650
Quarts (US) to	litres	0.94600
Cubic feet to	cubic inches	1728.0000
	gallons (Imp)	6.4810
	gallons (US)	7.4810
	litres	28.3200
Cubic inches to	cubic centilitres	16.3870
	cubic feet	0.00058
	gallons (Imp)	0.0036
	gallons (US)	0.0043
	litres	0.0164
	fluid ounces (US)	0.5540
Fluid drams to	fluid ounces (US)	0.1250
Fluid ounces to	drams (US)	8.0000
Fluid ounces to	litres (Imp)	0.0208
Fluid ounces (US) to	litres	0.0296
Gallons (Imp) to	cubic feet	0.1600
	cubic inches	277.3000
	litres	4.5460
	gallons (US)	1.2009
	millilitres	4546.0000
	cubic metres	0.0046
	ounces (Imp)	160.0000
Gallons (US) to	cubic feet	0.1337
	cubic inches	231.3000
	litres	3.7853
	gallons (Imp)	0.8327
	millilitres	3785.0000
	cubic metres	0.0038
	ounces (US)	128.0000

Appendix IV

Weight/mass units

Metric (SI)	Imperial
Tonnes	Ton
Kilograms	Pound
Grams	Ounce
	Grains

	To convert weight	Multiply by
Gram to	grain	15.4300
	ounce	0.0353
	pound	0.0022
Kilogram to	ounce	35.2700
	pound	2.2040
Ounce to	gram	28.3500
	grain	437.0000
	pound	0.0625
Pound to	gram	453.6000
	grain	7000.0000
	kilogram	0.4500
	ounce	16.0000
Grain to	ounce	0.0023
	pound	0.00014
	gram	0.0648

	To convert weight per volume	Multiply by
Gram/litre to	grain/gallon (Imp)	70.1140
	grain/gallon (US)	58.4000
	grain/ounce (US)	0.4600
	pound/gallon (US)	0.0083
Gram/millilitre to	pounds/gallon (US)	8.3450
Pound/cubic foot to	gram/cubic centimetre	0.0160
	pound/gallon (US)	0.1337
Pound/gallon (US) to	gram/millilitre	0.1198
	pound/cubic foot	7.8410
	gram/litre	119.9470

Appendix V

Miscellaneous units

To convert to SI units	*Multiply by*
1. Velocity	
$cm\,s^{-1}$	$1.0000 \times 10^{-2}\,m\,s^{-1}$
$m\,hour^{-1}$	$2.7778 \times 10^{-4}\,m\,s^{-1}$
$ft\,s^{-1}$	$3.0480 \times 10^{-1}\,m\,s^{-1}$
$ft\,hour^{-1}$	$3.4667 \times 10^{-5}\,m\,s^{-1}$
$mile\,hour^{-1}$	$4.4704 \times 10^{-1}\,m\,s^{-1}$
2. Volumetric flow	
$cm^3\,s^{-1}$	$1.0000 \times 10^{-6}\,m^3\,s^{-1}$
$m^3\,hour^{-1}$	$2.7778 \times 10^{-4}\,m^3\,s^{-1}$
$ft^3\,s^{-1}$	$2.8317 \times 10^{-2}\,m^3\,s^{-1}$
$cm^3\,min^{-1}$	$1.6667 \times 10^{-8}\,m^3\,s^{-1}$
$1\,min^{-1}$	$1.6667 \times 10^{-5}\,m^3\,s^{-1}$
$ft^3\,min^{-1}$	$4.7195 \times 10^{-4}\,m^3\,s^{-1}$
$ft^3\,hour^{-1}$	$7.8658 \times 10^{-6}\,m^3\,s^{-1}$
$gal\,(Imp)\,min^{-1}$	$7.5766 \times 10^{-5}\,m^3\,s^{-1}$
$gal\,(Imp)\,hour^{-1}$	$1.2628 \times 10^{-6}\,m^3\,s^{-1}$
$gal\,(US)\,min^{-1}$	$6.3089 \times 10^{-5}\,m^3\,s^{-1}$
$gal\,(US)\,hour^{-1}$	$1.0515 \times 10^{-6}\,m^3\,s^{-1}$
3. Viscosity	
A. Dynamic	
$g\,cm^{-1}\,s^{-1}$	$1.000 \times 10^{-1}\,kg\,m^{-1}\,s^{-1}$
$kg\,m^{-1}\,hour^{-1}$	$2.7778 \times 10^{-4}\,kg\,m^{-1}\,s^{-1}$
$lb\,ft^{-1}\,s^{-1}$	$1.4882\,kg\,m^{-1}\,s^{-1}$
$lb\,ft^{-1}\,hour^{-1}$	$4.1338 \times 10^{-4}\,kg\,m^{-1}\,s^{-1}$
B. Kinematic	
$cm^2\,s^{-1}$	$1.0000 \times 10^{-4}\,m^2\,s^{-1}$
$m^2\,hour^{-1}$	$2.7778 \times 10^{-4}\,m^2\,s^{-1}$
$ft^2\,s^{-1}$	$9.2903 \times 10^{-2}\,m^2\,s^{-1}$
$ft^2\,hour^{-1}$	$2.5806 \times 10^{-5}\,m^2\,s^{-1}$
4. Density	
$g\,cm^{-3}\,{}^{\circ}C^{-1}$	$1.0000 \times 10^{3}\,kg\,m^{-3}$
$lb\,ft^{-3}\,{}^{\circ}F^{-1}$	$1.6018 \times 10\,kg\,m^{-3}$
$lb\,gal^{-1}\,(Imp)$	$9.9779 \times 10\,kg\,m^{-3}$
$lb\,gal^{-1}\,(US)$	$1.1983 \times 10^{2}\,kg\,m^{-3}$

Appendix VI

Work/energy and other related units

Quantity	SI factor
Energy	
cal	$4.1868\,J$
kcal	$4.1868 \times 10^3\,J$
Btu	$1.0551 \times 10^3\,J$
Horse power (hp) hour^{-1}	
metric	$2.6477 \times 10^6\,J$
hp hour^{-1} (Imp)	$2.6845 \times 10^6\,J$
kW hour^{-1}	$3.6000 \times 10^6\,J$
ft lb^{-1}	$1.3558\,J$
Therm	$1.0551 \times 1^8\,J$
Thermic	$4.1855 \times 10^6\,J$
Calorific value (volumetric)	
cal cm^{-3}	$4.1868 \times 10^6\,J\,m^{-3}$
kcal m^{-3}	$4.1868 \times 10^3\,J\,m^{-3}$
Btu ft^{-3}	$3.7260 \times 10^4\,J\,m^{-3}$
Therm ft^{-3}	$3.7260 \times 10^9\,J\,m^{-3}$
Coefficient of expansion (volumetric)	
g cm^{-3} $^\circ$C^{-1}	$1.0000 \times 10^3 \times kg\,m^{-3}\,^\circ C^{-1}$
lb ft^{-3} $^\circ$C^{-1}	$28.8330 \times kg\,m^{-3}\,^\circ C^{-1}$
Heat flux	
cal s^{-1} cm^{-2}	$4.1868 \times 10^4\,W\,m^{-2}$
kcal hour^{-1} m^{-2}	$1.1630\,W\,m^{-2}$
Btu hour^{-1} ft^{-2}	$3.1546\,W\,m^{-2}$
Heat release rate	
A. Mass	
cal s^{-1} g^{-1}	$4.1868 \times 10^3\,W\,kg^{-1}$
kcal hour^{-1} kg^{-1}	$1.1630\,W\,kg^{-1}$
Btu hour^{-1} lb^{-1}	$6.4612 \times 10^{-1}\,W\,kg^{-1}$
B. Volumetric	
cal s^{-1} cm^{-3}	$4.1868 \times 10^6\,W\,m^{-3}$
kcal hour^{-1} m^{-3}	$1.1630\,W\,m^{-3}$
Btu hour^{-1} ft^{-3}	$1.0350 \times 10\,W\,m^{-3}$
Heat transfer coefficient	
cal s^{-1} cm^{-2} $^\circ$C^{-1}	$4.1868 \times 10^4\,W\,m^{-2}\,^\circ C^{-1}$

kcal hour^{-1} m^{-2} °C^{-1} 1.1630 W m^{-2} °C^{-1}
Btu hour^{-1} ft^{-2} °F^{-1} 5.6704 W m^{-2} °C^{-1}

Power

cal s^{-1} 4.1868 W
kcal hour^{-1} 1.1630 W
Btu s^{-1} 1.0551×10^3 W
Btu hour^{-1} 2.9308×10^{-1} W
hp (metric) 7.3548×10^2 W
hp (Imp) 7.4570×10^2 W
ft lb s^{-1} 1.3558 W

Specific enthalpy

cal g^{-1} 4.1868×10^3 J kg^{-1}
Btu l^{-1} 2.3260×10^3 J kg^{-1}

Specific heat

cal g^{-1} °C^{-1} 4.1868×10^3 J kg^{-1} °K^{-1}
Btu lb^{-1} °F^{-1} 2.3260×10^3 J kg^{-1} °K^{-1}

Thermal conductivity

cal s^{-1} cm^{-2} (°C cm^{-1}) 4.1868×10^2 W m^{-2}
kcal hour^{-1} m^{-2} (°C cm^{-1}) 1.1630 W m^{-2}
Btu hour^{-1} ft^{-2} (°F ft^{-1}) 1.7308 W m^{-2}
Btu hour^{-1} ft^{-2} (°F in^{-1}) 1.4423×10^{-1} W m^{-2}

Appendix VII

Force and pressure units

Quantity	Multiplication factor
Force	
dyn	$1.0000 \times 10^{-5}\,\text{N}$
kg force	$0.9867\,\text{N}$
lb force	$4.4482\,\text{N}$
ton force	$9.9640 \times 10^{3}\,\text{N}$
Pressure	
dyn cm^{-2}	$1.0000 \times 10^{-5}\,\text{N}\,\text{m}^{-2}$
kgf m^{-2}	$9.8067\,\text{N}\,\text{m}^{-2}$
standard atmosphere	$1.0133 \times 10^{5}\,\text{N}\,\text{m}^{-2}$
atmosphere or kgf cm^{-1}	$9.8067 \times 10^{4}\,\text{N}\,\text{m}^{-2}$
bar	$1.0000 \times 10^{5}\,\text{N}\,\text{m}^{-2}$
lbf ft^{-2}	$4.7880 \times 10\,\text{N}\,\text{m}^{-2}$
lbf in^{-1}	$6.8948 \times 10^{3}\,\text{N}\,\text{m}^{-2}$
mm mercury (Hg)	$1.3333 \times 10^{2}\,\text{N}\,\text{m}^{-2}$
inch Hg	$3.3866 \times 10^{3}\,\text{N}\,\text{m}^{-2}$

Appendix VIII

Length and area units

Metric (SI)	*Imperial*
Kilometre (km)	Mile (mi)
Metre (m)	Yard (yd)
Centimetre (cm)	Foot (ft)
Millimetre (mm)	Inch (in)

$$1 \text{ metre} = 100 \text{ cm} = 1000 \text{ mm}$$
$$1 \text{ kilometre} = 1000 \text{ m}$$

$$1 \text{ metre} = 39.4 \text{ in} = 3.28 \text{ ft} = 1.09 \text{ yd} = 0.621 \times 10^{-3} \text{ mile}$$
$$1 \text{ yard} = 3 \text{ ft} = 36 \text{ in}$$
$$1 \text{ mile} = 1760 \text{ yd}$$

To convert length	*Multiply by*
Inches to centimetres	2.5400
Feet to metres	0.3048
Yards to metres	0.9144
Miles to metres	1609.0000
Centimetres to inches	0.3940
Metres to feet	3.2810
Metres to yards	1.0936
Kilometres to miles	0.6213

To convert area	*Multiply by*
Square inches to square centimetres	6.4520
Square feet to square metres	0.0929
Square yards to square metres	0.8360
Square centimetres to square inches	0.1550
Square metres to square feet	10.7640
Square metres to square yards	1.1970

Appendix IX

Pearson square and algebraic methods

Pearsons square method

If the raw materials $(\mathrm{g}\,100\,\mathrm{g}^{-1})$ used for the manufacture of yoghurt are: skimmed milk (solids-not-fat 9 and water 91), skimmed milk powder (solids-not-fat 97 and water 3) and cream (fat 50), calculate the quantitites of the above raw materials required to produce a 500 l batch of yoghurt with total solids 16 and fat 1.5 in the final product. Calculate first the quantities of skimmed milk and skimmed milk powder required to give the desired level of solids-not-fat $14.5\,\mathrm{g}\,100\,\mathrm{g}^{-1}$.

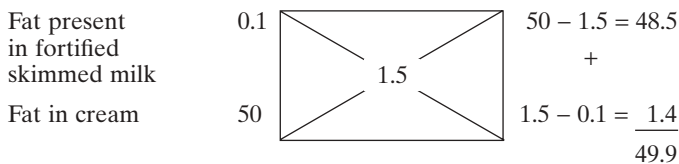|			
SNF in Skimmed milk	9		$97 - 14.5 = 82.5$
		14.5	+
SNF in skimmed milk powder	97		$14.5 - 9 = \underline{\ 5.5}$
			$\underline{88.0}$
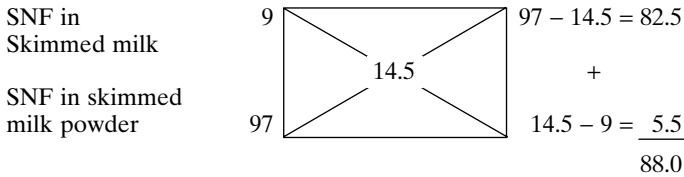
The amount of skimmed milk required $= \dfrac{82.5 \times 500}{88} = 468.75\,\text{litres}$

The amount of skimmed milk powder required $= \dfrac{5.5 \times 500}{88} = \underline{31.25\,\text{kg}}$

$\qquad\qquad\qquad$ Total $\qquad$ $\underline{500.00\,\text{litres}}$

Since the above mix contains only small quantities of fat, e.g. $0.1\,\mathrm{g}\,100\,\mathrm{g}^{-1}$, the balance of the required fat comes from the cream as follows:

Fat present in fortified skimmed milk	0.1		$50 - 1.5 = 48.5$
		1.5	+
Fat in cream	50		$1.5 - 0.1 = \underline{\ 1.4}$
			$\underline{49.9}$

The amount of fortified skimmed milk required $= \dfrac{48.5 \times 500}{49.9} = 485.97 \, \text{litres}$

The amount of cream required $= \dfrac{1.4 \times 500}{49.9} = \underline{14.03 \, \text{litres}}$

Total $\quad \underline{500.00 \, \text{litres}}$

Although the above calculation does not take into consideration the amount of solids-not-fat present in the cream ($4.5 \, \text{g} \, 100 \, \text{g}^{-1}$) and the starter culture inoculum ($12 \, \text{g} \, 100 \, \text{g}^{-1}$), the accuracy is sufficient for most practical purposes. However, as an additional check, the final composition of the yoghurt can be calculated as follows:

Product	Weight (l or kg)	Weight of fat supplied (kg)	Weight of solids-not-fat supplied
Skimmed milk	485.97	$\dfrac{0.1 \times 485.97}{100} = 0.49$	$\dfrac{9 \times 485.97}{100} = 43.74$
Skimmed milk powder	31.25	***	$\dfrac{97 \times 31.25}{100} = 30.31$
Cream	14.03	$\dfrac{50 \times 14.03}{100} = 7.02$	$\dfrac{4.5 \times 14.03}{100} = 0.63$
Starter culture at 3% rate of inoculation	15.00	***	$\dfrac{12 \times 15}{100} = 1.8$
Total	546.25	7.51	76.48

$\therefore$ the % of fat in yoghurt $= \dfrac{100 \times 7.51}{546.25} = 1.37$

The % of solids-not-fat in yoghurt $= \dfrac{76.48 \times 100}{546.25} = 14.00$

Hence, the difference in the composition ($\text{g} \, 100 \, \text{g}^{-1}$) is fat 0.13 and solids-not-fat 0.5, and such small margin of error is due to the fact that the cream (SNF) and the starter inoculum (SNF) are not considered. If such small % of fat and SNF is compensated for, then the prepared milk base will have the desired level of fat and SNF.

The algebraic method

This method of calculation takes into consideration all the raw materials used for the manufacture of yoghurt in order to obtain exactly the quantities required for a balanced mix, an approach which is similar to that used in the ice-cream industry (Hyde and Rothwell, 1973). For example, if the aim is to prepare a mix for yoghurt production which has the following chemical composition ($\text{g} \, 100 \, \text{g}^{-1}$) (fat 1.5 and SNF 14.5) and the dairy materials used are whole milk, skimmed milk, skimmed milk powder and a liquid starter culture, the composition of the raw materials can be taken as:

X = kg of whole milk (fat 3.5, SNF 8.5 and water 88.0);

Y = kg of skimmed milk (fat 0.1, SNF 9.0 and water 90.0);

Z = kg of skimmed milk powder (SNF 97 and water 3).

If the inoculation rate ($\text{g} \, 100 \, \text{g}^{-1}$) of the starter culture (SNF 12 and water 88) is 3, then in a batch of 100 units, 3 kg of culture would be used containing (0.36 kg SNF and 2.64 kg of water).

For convenience, the liquid ingredients could be measured in litres and the procedure of calculation is as follows:

The source of fat is whole milk ($3.5\,\mathrm{g}\,100\,\mathrm{g}^{-1}$) and skimmed milk ($0.1\,\mathrm{g}\,100\,\mathrm{g}^{-1}$) and the level in the mix is $1.5\,\mathrm{g}\,100\,\mathrm{g}^{-1}$

$$\frac{3.5X}{100} + \frac{0.1Y}{100} = 1.5 \tag{1}$$

The source of SNF ($\mathrm{g}\,100\,\mathrm{g}^{-1}$) is whole milk (8.5), skimmed milk (9) and skimmed milk powder (97), and the level in the mix is 14.5; however, the amount of SNF (0.36 kg) which originates from the starter culture must be deducted, i.e.

$$\frac{8.5X}{100} + \frac{9Y}{100} + \frac{97Z}{100} = 1.45 - 0.36 = 14.14 \tag{2}$$

The source of water ($\mathrm{g}\,100\,\mathrm{g}^{-1}$) is whole milk (88.0), skimmed milk (90.9), skimmed milk powder (3.0) and the weight of the water from the starter culture; therefore, the formula becomes:

Amount of water present in the mix is equal to:

$$100 - (\text{wt. of fat} + \text{wt. of SNF} + \text{wt. of water from the starter culture})$$

$$100 - (1.5 + 14.5 + 2.64) = 81.36$$

$$\frac{88X}{100} + \frac{90.9Y}{100} + \frac{3Z}{100} = 81.36 \tag{3}$$

Multiply equations (1), (2) and (3) by their denominator, i.e. 100

$$3.5X + 0.1Y = 150 \tag{4}$$

$$8.5X + 9Y + 97Z = 1414 \tag{5}$$

$$88X + 90.9Y + 3Z = 8136 \tag{6}$$

Calculate the value of X from equation (4)

$$X = \frac{150 - 0.1Y}{3.5} \tag{7}$$

Substitute the value of X (7) in equation (5) and (6)

$$8.5\frac{(150 - 0.1Y)}{3.5} + 9Y + 97Y = 1414 \tag{8}$$

$$88\frac{(150 - 0.1Y)}{3.5} + 90.9Y + 3Z = 8136 \tag{9}$$

Multiply equation (8) by its denominator, i.e. 3.5

$$8.5\,(150 - 0.1Y) + 31.5Y + 339.5Z = 4949$$

$$1275 - 0.85Y + 31.5Y + 339.5Z = 4949$$

$$31.5Y - 0.85Y + 339.5Z = 4949 - 1275$$

$$30.65Y + 339.5Z = 3674 \tag{10}$$

Multiply equation (9) by its denominator, i.e. 3.5

$$88(150 - 0.1Y) + 318.15Y + 10.5Z = 28476$$

$$13200 - 8.8Y + 318.15Y + 10.5Z = 28476$$

$$318.15Y - 8.8Y + 10.5Z = 28476 - 13200$$

$$309.35Y + 10.5Z = 15276 \tag{11}$$

Divide the value (Z) in equation (10) by the value (Z) in equation (11) in order to calculate the multiplication factor by which the value (Y) can be calculated

$$\frac{339.5Z}{10.5Z} = 32.33$$

Multiply equation (11) by the factor 32.33

$$10001.29Y + 339.47Z = 493\,873.08 \tag{12}$$

Subtract equation (10) from equation (12)

$$10001.29Y + 339.47Z = 493\,873.08$$

$$30.65Y + 339.5Z = 3674$$

$$9970.64Y + zero = 490\,199.08$$

(The value of Z in equations (10) and (12) is approximately equal)

$$\therefore Y = \frac{490\,199.08}{9970.64}$$
$$= 49.16\,\text{kg or l of skimmed milk required}$$

Substitute the value of Y in equation (10) to calculate the value of Z

$$49.16(30.65) + 339.5Z = 3674$$

$$1506.75 + 339.5Z = 3674$$

$$339.5Z = 3674 - 1506.75 = 2167.25$$

$$\therefore Z = \frac{2167.25}{339.5}$$
$$= 6.38\,\text{kg of skimmed milk powder required}$$

Substitute the value of Y in equation (7) to calculate the value of X

$$X = \frac{150 - 0.1(49.16)}{3.5}$$
$$= \frac{150 - 4.916}{3.5}$$
$$= \frac{145.08}{3.5}$$
$$= 41.45\,\text{kg or l of whole milk required}$$

Therefore add the weights of the raw materials required:

whole milk	41.45
skimmed milk	49.16
skimmed milk powder	6.38
starter culture	3.00
Total	99.99

The above total should amount to exactly 100, but the slight discrepancy is due to various approximations made in the above calculations; however, a second check from the above weights of raw materials can be made to confirm the chemical composition of the final yoghurt:

Product	Weight in kg	Weight of fat supplied	Weight of SNF supplied
Whole milk	41.45	$\dfrac{3.5 \times 41.45}{100} = 1.45$	$\dfrac{8.5 \times 41.45}{100} = 3.52$
Skimmed milk	49.16	$\dfrac{0.1 \times 49.16}{100} = 0.05$	$\dfrac{9 \times 49.16}{100} = 4.42$
Skimmed milk powder	6.38	***	$\dfrac{97 \times 6.38}{100} = 6.19$
Starter culture	3.00	***	$\dfrac{3 \times 12}{100} = 0.36$
	99.99	1.5	14.48

The above example could be applied to calculate exactly the weight of any dairy raw material which could be used for the manufacture of yoghurt (see Chapter 2) and since the quantity or unit of a 100 is used, it can be easily converted to a much larger volume of production.

Reference

HYDE, K.A. and ROTHWELL, J. (1973) In *Ice Cream*, Churchill Livingstone, London.