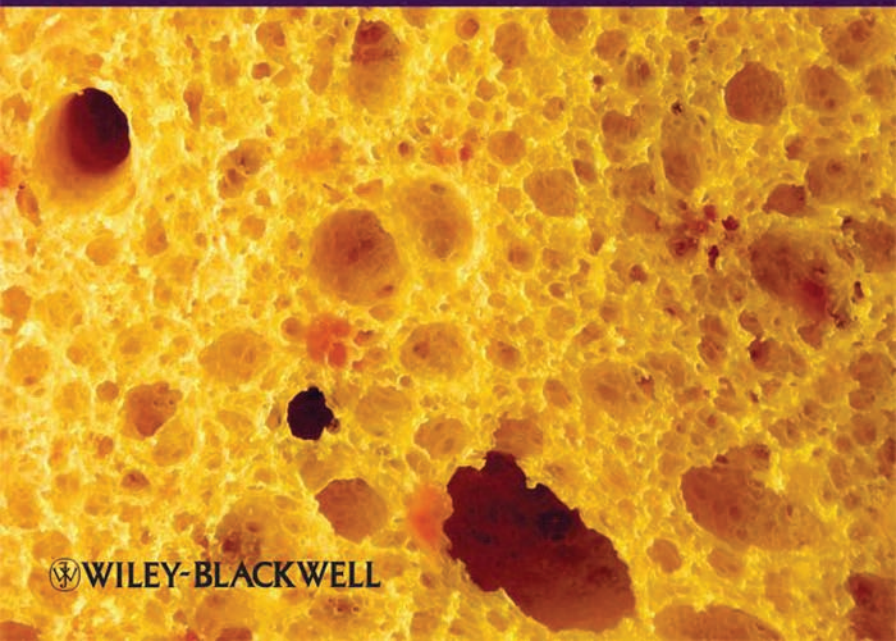
Gluten-Free Food Science and Technology

Eimear Gallagher



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WILEY-BLACKWELL
A John Wiley & Sons, Ltd., Publication

This edition first published 2009

Blackwell Publishing was acquired by John Wiley & Sons in February 2007.
Blackwell's publishing programme has been merged with Wiley's global Scientific, Technical, and Medical business to form Wiley-Blackwell.

Registered office
John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom

Editorial offices
9600 Garsington Road, Oxford, OX4 2DQ, United Kingdom
2121 State Avenue, Ames, Iowa 50014-8300, USA

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www.wiley.com/wiley-blackwell.

Library of Congress Cataloging-in-Publication Data

Gluten-free food science and technology / edited by Eimear Gallagher.
p. cm.
Includes bibliographical references and index.
ISBN 978-1-4051-5915-9 (hardback : alk. paper) 1. Gluten-free foods. I. Gallagher, Eimear.
TP451.D53G58 2009
664′.75–dc22

2008047433

A catalogue record for this book is available from the British Library.

Set in 10/12 pt Times by Aptara® Inc., New Delhi, India
Printed in Singapore

1 2009

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Preface

The incidence of coeliac disease or other allergic reactions/intolerances to gluten is rising, largely due to improved diagnostic procedures and changes in eating habits. The worldwide average of coeliac sufferers has been predicted to increase by a factor of 10 over the next number of years. This will result (the effects are already evident) in a growing market for high-quality gluten-free cereal products.

Gluten is the main structure-forming protein in flour. It is responsible for the elastic characteristics of dough, and contributes to the appearance and crumb structure of many baked products. The protein fractions in gluten are glutenin and gliadin. The former is a rough, rubbery mass when fully hydrated, while gliadin produces a viscous, fluid mass on hydration. Gluten, therefore, exhibits cohesive, elastic and viscous properties that combine the extremes of the two components. The gluten matrix is a major determinant of the important properties of wheat doughs (extensibility, resistance to stretch, mixing tolerance, gas holding ability) and finished products. Therefore, gluten removal results in major problems for bakers, and currently, many gluten-free products available on the market are of low quality and short shelf life, and exhibit poor mouthfeel and flavour. This presents a major challenge to the cereal technologist and baker alike, and has led to the search for alternatives to gluten in the manufacture of gluten-free bakery products.

The objective of this book is initially to introduce the concept of coeliac disease and show how its incidence is steadily increasing worldwide. Such a trend has led to increased research by cereal scientists in this area, and is paralleled by a growing demand for high-calibre gluten-free products by coeliac consumers.

The incidence of coeliac disease, its clinical presentation and the toxicity of grains are discussed in the opening three chapters. The nutritional value of gluten-free products is addressed in Chapter 4. Chapters 5 to 12 examine the diversity of ingredients that can be used to replace gluten and how the ingredient combinations and subsequent rheological and manufacturing properties of a range of gluten-free products, for example doughs, breads, biscuits and beer, may be manipulated.

This book will be a useful resource for ingredient manufacturers, bakers, cereal scientists and coeliac associations and societies. It should also be relevant to Food Science departments in Colleges and Universities to aid with teaching and research. In an era where coeliac disease and the availability of gluten-free foods have become so topical, few books dealing with this complex area are currently available.

As the editor, I would like to thank most sincerely all of the authors for their time, expertise and invaluable contribution to this book; I am most grateful. Also, thanks are due to the editorial team at Wiley-Blackwell for their advice and professionalism. Finally, I would like to thank my colleagues at Ashtown Food Research Centre, Dublin for their support, in

particular Dr Ronan Gormley, for his encouragement and helpful suggestions when the book was in its initial stages.

I hope that this book will be enjoyed, and that it will serve as a long-term source of knowledge and enlightenment for the reader.

Eimear Gallagher

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1 The Increasing Incidence of Celiac Disease and the Range of Gluten-Free Products in the Marketplace

Pamela Cureton and Alessio Fasano

1.1 INTRODUCTION

Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically susceptible individuals. Gluten is the protein component in wheat, rye and barley. Recent advances have increased our understanding of the molecular basis for this disorder. In the last 10 years, cutting edge scientific developments in this disease have led to the formulation of new concepts of epidemiology, pathophysiology and clinical manifestations. At present, the only available treatment for CD is a strict gluten-free diet (GFD).

The GFD is not an easy undertaking as gluten-containing grains, especially wheat, are the main ingredients in culturally popular foods such as bread, pasta and cakes. These grains are also widely used as additives, binders, preservatives and thickeners in a vast majority of processed foods such as broths, marinades, processed meats, canned goods, candy and medications. In 2004, the average American consumed 133 pounds (60.4 kg) of wheat and Canadians consumed 150 pounds (68 kg). In the United Kingdom, wheat consumption averages 167 pounds (76 kg) per year (Agriculture and Agri-Food Canada, 2004).

Fortunately, both medical knowledge and quality of the GFD continue to improve as awareness of CD increases throughout the world. At this time, people suffering from the effects of CD are being diagnosed more quickly than any other time in history. Manufacturers have also responded to the increased need for and potential profit in providing gluten-free foods.

1.2 HISTORY OF CD EPIDEMIOLOGY: INCREASE INCIDENCE OR INCREASING AWARENESS?

1.2.1 Epidemiology of CD in Europe

In the past four decades, a substantial number of epidemiological studies have been conducted in Europe to establish the frequency of CD, and interesting controversies have arisen. Earlier investigations measured the incidence of CD, namely the number of 'new' diagnoses in the study population during a certain period of time. One of the oldest epidemiological studies on CD conducted in 1950 established that the cumulative incidence of the disease in England and Wales was 1/8000, while an incidence of 1/4000 was detected in Scotland (Davidson and Fountain, 1950). The diagnosis at that time was entirely based on the detection of typical symptoms and confirmed by complicated and sometimes non-specific tests. The awareness

of the disease greatly increased in the 1960s when more specific tests for malabsorption and the pediatric peroral biopsy technique became available (Meeuwisse, 1970). Consequently, an elevated incidence of the disease (that in the mid-1970s reached peaks of 1/450–500) was reported in studies from Ireland (Mylotte et al., 1973), Scotland (Logan et al., 1986) and Switzerland (Van Stirum et al., 1982). This increased incidence of CD urged changes in the dietary habit, based on the hypothesis that delayed exposure to gluten could prevent the onset of the disease. For the first time in 25 years, a decrease in the incidence of CD was reported in the United Kingdom and Ireland (Littlewood et al., 1980; Dossetor et al., 1981; Stevens et al., 1987) following a late introduction of gluten in infant diet. Unfortunately, this decrease was deceptive, since subsequent screening studies demonstrated that the reduction of typical cases in infants was counterbalanced by the increase of atypical forms of CD with the onset of the symptoms occurring in older children or in adults (Greco et al., 1922). Due to the development of sensitive serological tests, it has recently become possible to evaluate the prevalence of CD (number of affected persons – including subclinical cases – in a defined population at a certain point in time). Screening studies have demonstrated a high prevalence of CD both among healthy children (Catassi et al., 1984, 1995; Mazzetti di Pietralata et al., 1992) and adults (Grodzinsky et al., 1992). The prevalence of CD throughout the old continent seems to be more homogeneous than previously thought (Table 1.1). Furthermore, these screenings revealed that CD is one of the most frequent genetically based diseases (Catassi et al., 1984; Ascher et al., 1991) occurring in 1 out of 130–300 in the European population (Catassi et al., 1996; Kolho et al., 1998) (Table 1.1). In a serological screening study involving more than 17 000 Italian schoolchildren, the prevalence of CD was 1 in 184 (Maki et al., 1988) and the ratio of known to undiagnosed CD cases resulted to be 1:7. The European experience taught that, despite common genetic and environmental factors, the clinical presentation of CD in neighboring countries may greatly diverge. A typical example of this phenomenon is the Danish epidemiological case. Until a few years ago, CD was regarded as rare in Denmark, with an estimated incidence based on clinical evidence (i.e. the presence of classical symptoms) of 1/10 000 (Weile and Krasilnifoff, 1993) (Table 1.1). At the same

Table 1.1 Comparison between prevalence on clinical diagnosis and on screening data in several countries worldwide.

Geographic area	Prevalence on clinical diagnosis[a]	Prevalence on screening data
Brazil	?	1:400
Denmark	1:10 000	1:500
Finland	1:1000	1:130
Germany	1:2300	1:500
Italy	1:1000	1:184
Netherlands	1:4500	1:198
Norway	1:675	1:250
Sahara	?	1:70
Slovenia	?	1:550
Sweden	1:330	1:190
United Kingdom	1:300	1:112
United States	1:10 000	1:133
Worldwide (average)	1:3345	1:266

Source: Reprinted from Fasano and Catassi (2001), p. 16, copyright 2001, with permission from Elsevier.

[a] Classical gastrointestinal symptoms.

time, the incidence of the disease in neighboring countries (including Sweden and Finland) that share similar genetic backgrounds increased following a decrease in breast-feeding practice and an increased consumption of gluten during infancy (Ascher et al., 1991; Maki et al., 1992). Subsequent serological screening studies suggested that CD is as frequent in Denmark as in Sweden, with a reported prevalence of 1/500 (Ascher and Kristiansson, 1994) (Table 1.1). These results suggest that in Denmark most cases of CD were previously undiagnosed, presumably because of lack of typical gastrointestinal symptoms. Factors such as type of cow's milk formulae, breast feeding, age at gluten introduction, quantity of gluten and quality of cereals, and quantity of wheat gluten may all influence the clinical presentation of the disease (Maki et al., 1992).

1.2.2 Epidemiology of CD in the United States

Within the American scientific community it was generally held that CD was a rare disorder in the United States, which was reflected by the limited number of scientific papers published from the new continent in the 30 years period 1965–1995 (Fasano, 1996). Remarkably, only two epidemiological studies on CD were published during this period, both between 1993 and 1994. The first study was conducted by Rossi et al. (1993) on a pediatric population from the western New York area with symptoms possibly related to CD, as chronic diarrhea, failure to thrive, short stature and diabetes. While the prevalence of CD among patients with symptoms possibly associated to the disease was lower than reported in Europe, the concurrence of CD and type 1 diabetes were comparable to that previously reported from the Old Continent. These data suggested that other atypical presentations of CD and eventually the late onset of the disease after an asymptomatic phase during childhood may account for the low occurrence of CD reported in this study. The second American epidemiological study published in 1994 was based on a retrospective evaluation (1960–1990) of the incidence of CD among the population of Olmsted County, Minnesota using the medical record of the Rochester Epidemiological Project (Talley et al., 1994). Case definition was limited to those individuals presenting typical gastrointestinal symptoms (i.e. chronic diarrhea and weight loss) or dermatitis herpetiformis whose intestinal biopsy showed a flat mucosa (Talley et al., 1994). Using these restrictive parameters, the authors identified only three cases among the pediatric population (calculated incidence rate: 0.4 per 100 000 person-year), while the overall age- and gender-adjusted incidence was 1.2 per 100 000 person-year. Based on these results, the authors concluded that CD is relatively rare in the United States (prevalence $\sim$1:10 000). Unfortunately, both studies failed to consider the protean clinical manifestations of CD. By focusing on specific symptoms, the authors may have missed what is currently defined as the submerged part of the so-called celiac iceberg. Recently, a series of epidemiological studies conducted by using more appropriate experimental designs and powerful screening tools have demonstrated that CD in the United States is as frequent as in Europe in both risks groups (Hill et al., 1995, 2000; Catassi et al., 2007) and the general population (Not et al., 1998; Berti et al., 2000; Fasano et al., 2003) (Table 1.1). Our center for celiac research has conducted a large, multicenter study on the prevalence of CD in both risk groups (i.e. subjects with either symptoms or complications associated to CD, first and second degree relatives of biopsy-proved CD patients, etc.) and the general population. Our results showed for the first time that the prevalence of CD in the United States is similar to that reported in Europe if not even higher, both among risk groups and the general population (Fasano et al., 2003) (Table 1.1).

1.2.3 Epidemiology of CD in the rest of the world

Since CD is the result of the interaction between genetic (both HLA (human leukocyte antigen) and non-HLA associated genes) and environmental factors (gluten-containing grains), it would be reasonable to evaluate the world distribution of these two components in order to identify area 'at risk' for CD. The coincidence of the CD HLA aplotypes and the level of wheat consumption clearly confirm Europe as a region at risk for CD (Fasano and Catassi, 2001). However, the co-existence of the two key components involved in CD pathogenesis is also notable in regions where CD has been historically considered rare. A more attentive analysis of these pathogenic factors can possibly explain this apparent paradox.

1.2.3.1 *CD causal factors show a worldwide distribution*

The principal genetic and environmental factors responsible for the development of CD show a worldwide distribution (Fasano and Catassi, 2001). The consumption of gluten-containing cereals is widespread in Europe, North Africa, North and South America and part of the Asian continent. In India, wheat consumption is higher in the so-called 'celiac belt', that is, the North-Western group of states where this cereal is the staple diet (Punjab, Haryana, Delhi, Rajasthan and Uttar Pradesh) (Yachha, 2006). This finding explains why in India CD has mostly been described in the Northern part of the country, a situation that might change in the future due to Westernization of the diet. In India, as well in other Asian countries, there is a clear slowing of per capita consumption of rice and an increased consumption of wheat-based products. Rising income and urbanization are driving forces in the rise of wheat consumption. Whereas wheat is considered an inferior good in Western societies, in the traditional rice-eating countries in Asia, wheat is becoming a preferred staple. Because of these alimentary trends, an increasing incidence of CD in India should be expected in the near future.

The major HLA-related predisposing genotypes (DQ2 and DQ8) have consistently been found in most populations of the world, with a few exceptions (e.g. Highlanders in Papua New Guinea). However, the geographical distribution of the different HLA-DQ predisposing genotypes is still incomplete. A variable frequency of either high- (e.g. DQ2 in homozygosity) or low/moderate risk (e.g. DQ8) genotypes could contribute to explaining the variable prevalence of CD that has been reported in different parts of the world.

1.2.3.2 *CD is increasingly reported from the developing world*

Until recently, the geographical distribution of CD was mostly restricted to Europe and other developed countries, such as the United States, Canada and Australia. New epidemiological studies have brought evidence that this disorder is common in many developing countries as well, showing that the 'global village of celiac disease' has indeed a worldwide distribution (Catassi et al., 2005).

The presence of CD is long established in many South American countries that are mostly populated by individuals of European origin. Among Brazilian blood donors, the prevalence of CD ranged between 1:681 (Gandolfi et al., 2000) and 1:214 (Oliveira et al., 2007). It is worth noting that studies on blood donors tend to underestimate the prevalence of CD, as these individuals represent the 'healthiest' segment of the population and are mostly males (while CD is more common among women). In Argentina, Gomez et al. (2001) found an

overall prevalence of 1 on 167 on 2000 adults involved in a prenuptial examination (Gomez et al., 2001).

Although the frequency of CD in many parts of Africa is still unknown, it is nowadays clear that this condition is present in the African continent. The highest CD prevalence in the world has indeed been described in an African population originally living in Western Sahara, the Saharawi, of Arab-Berber origin. In a sample of 990 Saharawi children screened by EMA (anti-endomysial antibody) testing and intestinal biopsy, the prevalence of CD was found to be 5.6%, which is almost tenfold higher than in most European countries (Catassi et al., 1999). The reasons for this spiking CD frequency are unclear but could be primarily related to genetic factors, given the high level of consanguinity of this population. The main susceptibility genotypes, HLA-DQ2 and -DQ8, exhibit one of the highest frequencies in the world in the general background Saharawi population (Catassi et al., 2001). Gluten consumption is very high as well, since wheat flour is the staple food of the Saharawi refugees. A recently completed screening project on school children in Cairo City, Egypt, on both a general population sample and at-risk subjects showed that CD is a common and usually undiagnosed disorder among Egyptian children (Abu-Zekry et al., 2008). The prevalence in the general population was 0.53%, that is 1:187, a figure that overlaps with European, North American and North African data. The Egyptian study confirmed that most screening-detected CD cases in North Africa are subclinical, with a mild degree of growth failure and anemia as frequent findings. A significantly higher prevalence of CD was found in the same study in children hospitalized for diarrhea and/or malnutrition (2.36%) and children affected with type 1 diabetes (6.4%), confirming that subjects presenting with these at-risk conditions should always be serologically screened for CD. In a recent mass screening for CD performed in Ariana, Tunisia, on 6284 children tested by tTGA and EMA plus small bowel biopsy in suspected cases, a prevalence of 1:157 was found (Ben Hariz et al., 2007). Most of the screened Tunisian children were asymptomatic or had atypical CD. Indirect evidence suggests that CD is not a rare disorder in other Northern African countries. Large series of clinically diagnosed patients have been reported from Algeria (Mediene et al., 1995) and Libya (al-Tawaty and Elbargarthy, 1998). Furthermore, CD is one of the most common disorders diagnosed in children born from North African immigrants in European countries.

CD is a frequent disorder in Middle East and along the ‘silk road’ countries. One of the higher prevalence of CD in blood donors has indeed been reported from Iran (1 in 167). In the same country, 12% of cases with a diagnosis of irritable bowel syndrome for many years were actually affected by CD (Shahbazkhani et al., 2003). In studies from Iran, Iraq, Saudi Arabia and Kuwait, CD accounted for 20% and 18.5% of cases with chronic diarrhea in adults and children, respectively. In a study from Jordan, the high incidence of CD was related to the large wheat consumption of the population (135 kg/head/year) (Rawashdeh et al., 1996).

With the availability of improved and more accessible diagnostic tools, CD is being more and more frequently recognized in India, both in children and in adults. By using a case-finding approach (serological testing on symptomatic subjects), Sood et al. (2006) reported a prevalence of newly diagnosed CD of 1 in 310 children on a sample of 4347 school-age children from Punjab, India. CD in Indian children is predominantly associated with the DQ2 allele, often in linkage disequilibrium with the A26-B8-DR3 alleles (the so-called Indian haplotype, a variant of the ancestral Caucasian haplotype A1-B8-DR3-DQ2) (Kaur et al., 2002).

Finally, there are only anecdotal reports of CD in Far East Countries. Given the low prevalence of HLA predisposing genes DQ2/DQ8 and the low/absent gluten consumption, reduced disease prevalence should be expected in those populations.

1.2.3.3 *CD frequency is largely underestimated in the developing world*

The burden of CD in developing countries is largely underestimated. This situation depends on several reasons, particularly the common belief that CD does not exist in developing countries, poor awareness of the clinical variability of CD, scarcity of diagnostic facilities and more emphasis on other causes of small intestinal damage, such as intestinal tuberculosis and environmental enteropathy. It is also possible that the prevalence of CD is increasing in some developing countries because of the widespread diffusion of Western dietary habits, with increasing consumption of gluten-containing cereals. The abrupt modification of dietary habits may be one of the causes of the huge prevalence of CD among the Saharawis (Catassi et al. 1999, 2001). Historically, the Bedouin diet was based on prolonged breast feeding, camel milk and meat, dates, sugar and small amounts of cereals and legumes. Over the last century, however, the Saharawi dietary habits have changed dramatically because of the European colonization, and products made with wheat flour, especially bread, have become the staple food.

1.3 EVOLUTION OF THE GLUTEN-FREE DIET

The treatment for CD has long been suspected to be linked to the diet. The GFD has evolved over the past 100 years to its current form; however, the first attempts to control the disease by diet were based on less than palatable products. With advances in medicine technology leading to a better understanding of the cause of the disease also came improvements in the GFD.

In 1888, Samuel Jones Gee, an English physician and pediatrician, published the first complete modern description of the clinical picture of CD in a lecture at the Hospital for Sick Children, Great Ormond Street in 1888. Gee theorized on the importance of diet in the treatment for CD as follows:

> There is a kind of chronic indigestion which is met with in persons of all ages, yet is especially apt to affect children between one and five years old. Signs of the disease are yielded by the faeces; being loose, not formed, but not watery; more bulky than the food taken would seem to account for; pale in colour, as if devoid of bile; yeasty, frothy, an appearance probably due to fermentation; stinking, stench often very great, the food having undergone putrefaction rather than concoction.

Gee identified food as the main part of treatment for CD, and states, '[I]f the patient can be cured at all, it must be by means of diet.' Gee prescribed a diet free of milk, highly starched foods, rice, sago, fruits and vegetables. Raw meat and thin slices of toasted bread were recommended, with the caveat that children 'cannot bear this diet for more than one season' (Gee, 1988).

Improving upon Gee's dietary recommendations, Haas (1924), an American pediatrician, reported a banana diet resulted in positive effects for children with CD. Parents were instructed to feed their children a diet of chicken, rice and bananas, over the course of several years. When their symptoms resolved, these children were started back on a diet containing gluten,

as at that time, physicians believed that the condition was curable. However, symptoms of CD redevelop, sometimes as late as age 50 or 60, with notable complications. A patient recently seen at the University of Maryland School of Medicine writes of her celiac experience:

> Then on September 21, 1937, my mother dropped me off at the hospital a second time and was told to not come to see me for 6 weeks. I am sure they questioned my survival, although my mother did not. I ended up staying here in this hospital until June 28, 1938 – 9 months and 1 week. Doctor Loring Joslin was my pediatrician and diagnosed my problem as Celiac Disease. My diet became what we knew as baked bananas and Bulgarian butter milk. There was another little boy in the hospital with me with the same problems. We were documented in a 1938 university medical journal as being first survivors. We were known as the Banana Babies.
>
> I lived on the baked bananas and Bulgarian buttermilk for 2–3 years, and in all that time, my mother never tasted it, as it looked so terrible. Bananas were not easy to buy back then either. A family member or a friend, had to go to Baltimore each week to purchase the weekly supply – 21 bananas for 3 a day.
>
> My mother was told I would out grow the condition, so other foods were gradually added to my diet and seemed to be tolerated. Dairy products were the last to be added, so by age 6, I could finally eat ice cream.

This diet remained in vogue until the actual cause of CD was determined in the 1950s. While carbohydrates had been suspected from early investigations, the link with wheat was made by the Dutch pediatrician Dr. Willem Dicke in 1950. He noted clinical improvement of his patients during the Dutch famine of 1944 (van Berge-Henegouwen and Mulder, 1993). During this time, wheat, rye, oats and barley were seized for use by the German armies and the Dutch population was living on bread made with flour ground from tulip bulbs. After the war ended and wheat consumption resumed, children's symptoms of CD recurred.

Until the groundbreaking multicenter study on the prevalence of CD in the United States was published in 2003, CD was considered rare in that country, and few patients were correctly diagnosed. With no demand for special foods, no gluten-free products were manufactured until 1978, when small companies began manufacturing wheat-free, gluten-free breads. After this study's finding that 1 in 133 people (1% of the US population) suffered from CD, government agencies became involved on both the medical and manufacturing aspects of the treatment of CD (Fasano et al., 2003).

1.4 US GOVERNMENT AGENCIES BECOME INVOLVED IN CD

1.4.1 NIH consensus conference held on CD

The National Institutes of Health (NIH) convened a Consensus Development and State-of-the-Science Conference on CD in June 2004. Consensus conferences are convened to review the scientific evidence on a biomedical or public health topic, for the purpose of resolving a particular controversial issue in clinical or public health practices. The objective of the conference was to provide health care providers, patients and the general public with a responsible assessment of currently available data regarding CD (NIH Consensus Statement on Celiac Disease, 2004). The panel concluded that CD was not rare in the United States and actually affected up to 3 million Americans, with the vast majority being undiagnosed. The

panel recommended the following:

- Education of physicians, dietitians, nurses and the public about CD by a trans-NIH initiative, to be led by the National Institute of Diabetes and Digestive and Kidney Diseases, in association with the Centers for Disease Control and Prevention.
- Standardization of serologic tests and pathologic criteria for the diagnosis of CD.
- Adoption of a standard definition of a GFD based on objective evidence such as that being developed by the American Dietetic Association.
- Development of an adequate testing procedure for gluten in foods and definition of standards for gluten-free foods in the United States to lay the foundation for rational food labeling.
- Formation of a federation of CD societies, CD interest groups, individuals with CD and their families, physicians, dietitians and other health care providers for the advancement of education, research and advocacy for individuals with CD (NIH Consensus Statement on Celiac Disease, 2004).

1.4.2 Food and Drug Administration institutes a new food labeling law

Historically, finding safe, gluten-free foods in the market place has been an enormous challenge for people with CD. Grocery shopping became extremely time-consuming, confusing and unproductive. The food labels provided little help in determining whether products were gluten free. Shopping for gluten-free foods takes an average family between 10 and 20 h per month longer than average consumers, which includes contacting food manufacturers, reading product labels and searching the Internet to identify foods that are free from gluten ingredients and cross-contamination (Gluten Intolerance Group, 2005).

In 2003, the celiac community, including support group leaders, researchers, health care providers and industry representatives, organized a task force with the specific goal of working toward the passage of a new food labeling law. The American Celiac Task Force (now named The American Celiac Disease Alliance) works in conjunction with the Food Allergy and Anaphylaxis Network to change the requirements of food labeling. In 2004, the Food Allergen Labeling and Consumer Protection Act (FALCPA) of 2004 was signed into law. The FALCPA requires that the labels of all FDA-regulated food products, labeled on or after 1 January 2006, clearly state whether a food contains any 'major food allergen'. This law mandates that food manufacturers label, in plain English, the use of any of the top eight allergens in their products, including milk, eggs, fish, shellfish, tree nuts, peanuts, soybeans and **wheat** (Gomez et al., 2001). The FALCPA greatly simplifies label reading and reduces the possibility of hidden wheat gluten in most packaged foods. FALCPA does not, however, include barley and rye, making it possible for these sources of gluten to be hidden in flavorings and other additives.

1.4.3 Part two of the FALCPA

Currently, there is no US Federal regulation that defines the term 'gluten free' in labeling of foods. Based on comments and other information the FDA received during its public meeting on gluten-free food labeling held in August 2005, there is no universal understanding among United States food manufacturers or consumers about the meaning of foods labeled as 'gluten free'. The FDA believes that establishing a definition for the term 'gluten free' and uniform conditions for its use in the labeling of foods will ensure that persons with CD are not misled and are provided with truthful and accurate information.

Fortunately, FALCPA addresses this concern as well, directing that no later than 4 years after the date of enactment of FALCPA, the Secretary of Health and Human Services shall issue a final rule to define and permit use of the term 'gluten free' on the labeling of foods. The FDA is proposing to define 'gluten free' to mean that a food bearing this claim **does not** contain any of the following:

- An ingredient that is a 'prohibited grain', which refers to any species of wheat (e.g. durum wheat, spelt wheat or kamut), rye, barley or their crossbred hybrids.
- An ingredient (e.g. wheat flour) that is derived from a 'prohibited grain' and that has not been processed to remove gluten.
- An ingredient (e.g. wheat starch) that is derived from a 'prohibited grain' that has been processed to remove gluten, if the use of that ingredient results in the presence of 20 μg or more gluten per gram of food.
- Or 20 μg or more gluten per gram of food.

A food that bears the claim 'gluten free' or a similar claim (e.g. 'free of gluten', 'without gluten' and 'no gluten') in its labeling and fails to meet the conditions specified in the proposed definition of 'gluten free' would be deemed misbranded (Department of Health and Human Services Food and Drug Administration, 2007).

Internationally, there is no agreement on the definition for the term 'gluten free' or universal symbol to designate a product that is gluten free. In several countries (e.g. Canada), foods are labeled gluten free only if they are made without any gluten-containing grains and do not contain any detectable levels of gluten. The Canadian Food Inspection Agency, however, uses a testing method that has an analytical limitation of 20 parts per million (ppm) gluten, leading to a 'gluten-free' label in this country as indicating tested to less than 20 ppm (Case, 2006).

The International Codex standard, used in much of Europe, is in the process of revising the standards for gluten-free foods, but has been unsuccessful thus far, as there was no consensus on acceptable gluten-free levels and the method of testing for gluten. The original 1983 Codex standard defines 'gluten-free' foods as follows:

(a) consisting of or made only from ingredients which do not contain any prolamins from wheat, rye, barley or their crossbred varieties with a gluten level not exceeding 20 ppm; or
(b) consisting of ingredients from wheat, rye, barley, oats, spelt or their crossbred varieties which have been rendered 'gluten free', with a gluten level not exceeding 200 ppm; or
(c) any mixture of the two ingredients in (a) and (b) with a gluten level not exceeding 200 ppm (Codex Alimentarius Commission, 2008).

When clear standards are in place from the government, food manufacturers can then provide information and safe manufacturing practice needed to give confidence to the celiac consumer needs to find safe gluten-free foods in the marketplace.

1.5 GROWTH IN THE GLUTEN-FREE MARKETPLACE

Increased awareness along with proposed regulations across the world for gluten-free labeling has impacted the market, resulting in rapid growth and availability of gluten-free products. A new report from market-research publisher Packaged Facts, New York, estimated that sales of gluten-free products in the United States would top $696 million in 2006. With projected

growth of 25% annually over the next 4 years, the gluten-free market is predicted to expand to $1.7 billion by the end of 2010, growing from a modest $210 million in 2001 (Kuntz, 2006).

1.5.1 From humble beginnings: the US experience

In 1978, Ener-G Foods, a small, two-employee flour packaging company, started production of wheat free, gluten-free bread. The company had previously developed a low-protein bread, with a base of wheat starch, for patients with renal failure awaiting dialysis. Dr Cyrus Rubin, inventor of the Rubin Biopsy Tube and professor of Gastroenterology at the University of Washington and his dietitian, Elaine Hartsook, PhD, requested that the company make gluten-free bread with the restriction that no wheat starch would be used. In the next 30 years, Ener-G Foods would increase their product line to over 150 gluten-free products, pioneering gluten-free foods for people with CD in the United States. Company sales records indicate that during the past 4 years, business has more than doubled (J. Colburn (Ener-G Foods Sales and Marketing Manager), personnel communication, 3 January 2008).

1.5.2 Gluten-free foods go mainstream

Consumers have historically looked to natural foods retailers or the Internet for gluten-free food options however, with the demand for gluten-free foods growing, conventional markets currently account for 63% of gluten-free products purchased. These trends may be due, in part, to increasing availability of gluten-free products. For example, in 2006, the number of gluten-free items increased to over 2400 in natural supermarkets, and to over 1400 in conventional supermarkets (Rourke and Tirone, 2007).

1.5.3 Growth in the gluten-free market

Mintel Global New Products Database (GNPD) tracks new product development trends around the world. Mintel Executive Summary on Food Allergies and Intolerance – US – April 2007 states that the most common conditions currently impacting the market are CD and lactose intolerance and therefore the products currently seeing the greatest growth on the market are directly relational to the diagnoses of CD and lactose intolerance. Mintel's market analyst of the 'free-from' (i.e. gluten-free, lactose-free) market has already enjoyed sales growth of over 300% since 2000. Sales of products such as wheat-free breads and cakes have grown by almost 120% over the last 3 years alone, reaching €48 million ($65 million; see Table 1.2) with the most interest in snack food and bakery items (see Table 1.3). An even

Table 1.2 New products claiming 'gluten free' in the United States by year.

Year	Number of new food and beverage products
2004	202
2005	332
2006	610
2007*	636

Source: Reprinted with permission of Mintel Global New Products Database (GNPD).
*2007 includes only products tracked from January 2007 through December 28, 2007.

Table 1.3 Top categories for gluten free in 2007[a].

Category	Number of new food and beverage products
Snacks	174
Bakery	94
Dairy	62
Confectionery	56
Sauces and seasonings	51
Processed fish, meat and egg products	45
Beverages	43
Meals and meal centres	28
Side dishes	27
Desserts and ice cream	24

Source: Reprinted with permission of Mintel Global New Products Database (GNPD).
[a] The year 2007 includes only products tracked from January 2007 to 28 December 2007.

greater increase in new gluten-free product development was seen worldwide (see Tables 1.4 and 1.5) during this time.

1.5.4 Future needs of gluten-free market development

Research is underway to find alternative treatments to the GFD. Such advances, although promising, are still in the future. The past decade has brought advances to the medical arena in the ability to diagnose CD, resulting in rapid growth of both the quantity and quality of gluten-free foods. Despite these advances, there is still much to be done to improve the quality of life for people with CD. One major area of concern that must be addressed is the high cost of the GFD. In a survey conducted by The Gluten Intolerance Group, a large celiac support group in the United States, patients indicated that taste and cost were the most important factors when making purchasing decisions for gluten-free products (C. Kupper (RD, CD, Executive Director Gluten Intolerance Group) personnel communication, 10 October 2007). The average cost of a gluten-free food item compared with its gluten-containing counterpart is five times as great (see Table 1.6). Dr Robert Anderson, the chief gastroenterologist for one of Australia's two CD clinics, estimates a GFD costs about $1000 per year more than a traditional diet for people with CD living in Australia (Coeliac UK Prescriptions, 2007).

Low-income families experience even greater difficulty in finding comparable gluten-free foods due to their high cost. Currently in the United States and Australia, there is no government subsidy for special dietary foods as in some European countries (e.g. United Kingdom, Italy, Finland) and in New Zealand. People medically diagnosed with CD in the

Table 1.4 New food and beverage products claiming 'gluten free' by year.

Year	Argentina	Australia	Brazil	Canada	Egypt	Ireland	Israel	Italy	UK
2004	3	60	56	26	3	21	9	48	118
2005	49	130	49	74	6	19	15	19	334
2006	93	179	307	150	3	30	12	144	180
2007	158	269	418	266	8	41	22	111	421

Source: Reprinted with permission of Mintel Global New Products Database (GNPD).

Table 1.5 Top categories for gluten-free food and beverage products launched in 2007.

Category	Argentina	Australia	Brazil	Canada	Egypt	Ireland	Israel	Italy	UK
Snacks	5	44	42	81	2	4	1	7	50
Bakery	17	39	24	29	0	21	5	26	41
Sauces and seasonings	19	37	54	15	0	4	2	4	60
Dairy	61	22	76	9	0	1	3	11	11
Baby food	2	27	0	1	1	4	3	12	129
Beverages	15	4	99	22	1	0	0	3	9
Processed fish, meat and egg products	9	21	22	14	0	0	0	23	6
Side dishes	1	12	19	11	1	2	1	21	15
Desserts and ice cream	9	6	14	21	0	0	0	3	18
Confectionery	3	11	15	24	0	0	0	0	18
Spreads	8	13	23	7	0	0	1	0	12
Meals and meal centers	0	12	0	21	0	1	1	0	18
Breakfast cereals	1	12	1	4	0	3	0	1	17
Fruit and vegetables	8	3	18	0	0	1	0	0	2
Soup	0	4	1	2	0	0	5	0	12
Sweeteners and sugar	0	2	10	0	2	0	0	0	0
Pet food	0	0	0	5	1	0	0	0	3

Source: Reprinted with permission of Mintel Global New Products Database (GNPD).

Table 1.6 Cost comparison between wheat product and gluten-free products.[a]

Wheat flour	$0.34/lb	Brown rice flour	$1.89/lb
Wheat bread	$1.09/loaf	Gluten-free bread	$6.00/loaf
Wheat pasta	$0.87/lb	Gluten-free pasta	$3.69/lb
Chocolate chip cookie	$2.69/lb	Gluten-free chocolate chip	$12.83/lb
Wheat crackers	$1.63/lb	Rice crackers	$9.12/lb

[a] Based on US Department of Labor, Bureau of Labor: consumer price index.

United Kingdom are entitled to receive gluten-free foods on prescription. A survey in 2006 found that over 90% of people with CD in the United Kingdom obtained their gluten-free food on prescription (Herald Sun, 2008).

The cost of treatment for CD in developing countries presents a significant challenge to World Health Organization and other relief agencies. It is possible that the prevalence of CD is increasing in some developing countries as a result of increasing consumption of gluten-containing cereals as provided by such agencies as seen in the Saharawi population (Catassi et al., 1999). These organizations are presented with the challenge that humanitarian interventions should focus on the goal of supplying enough food while being aware of the nutritional outcome to the recipients. Wheat and other gluten-containing grains should be replaced with naturally gluten-free cereals that are present in the developing country. The relief of symptoms people suffer as a result of untreated CD must be available to all who suffer, not only those who can afford the cost of its treatment.

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2 The Clinical Presentation and Diagnosis of Celiac Disease

Peter H. R. Green

2.1 INTRODUCTION

Celiac disease has been traditionally considered a malabsorption syndrome, and as such appears in medical textbooks in the chapters devoted to that topic. However, the disease now more resembles a multisystem disorder with almost any organ involved, rather than a primary diarrheal illness. In addition, celiac disease was considered primarily a pediatric condition, however, it is now mainly an adult disorder diagnosed most frequently in the 4th to 6th decade (Green et al., 2001).

2.2 MODES OF PRESENTATION OF CELIAC DISEASE

The classification of the main modes of presentation of adults with celiac disease into 'classical' – diarrhea predominant and 'silent' is widely accepted (Green and Jabri, 2003). The silent group includes atypical presentations and those presenting with complications of celiac disease as well as truly asymptomatic individuals picked up through screening high-risk groups.

2.2.1 Presentation in children

The original modern descriptions of celiac disease were in infants and children (Haas, 1924; Andersen, 1938, 1947; Dickie, 1950; Anderson, 1961). More recently it has become clear that children may present with a classical, diarrhea predominant presentation or a more subtle, atypical way. The presentation varies with age of the child as well as whether there was a history of breast feeding (D'Amico et al., 2005). Infants and young children present with diarrhea, abdominal distension and failure to thrive, the so-called classical presentation. However, vomiting, irritability, anorexia and even constipation are common. Older children often present with extra-intestinal manifestations, such as short stature, neurologic symptoms or anemia (D'Amico et al., 2005). Breast feeding appears to delay the manifestations, and results in more atypical, less classical presentations (Maki et al., 1988; D'Amico et al., 2005).

2.2.2 Presentation in adults

In order to assess the clinical spectrum of celiac disease in the United States, we obtained data on 1138 people with biopsy proven celiac disease (Green et al., 2001). Our results demonstrated that the majority of individuals were diagnosed in their 4th to 6th decades.

Females predominated (2.9:1); however, the female predominance was less marked in the elderly. Diarrhea was the main mode of presentation, occurring in 85%. Most strikingly, symptoms were present a mean of 11 years prior to diagnosis.

In order to assess whether the presentation had changed over time we analyzed the mode of presentation for a series of patients seen in the Celiac Center at Columbia University in New York (Lo et al., 2003). There were 227 patients with biopsy proven celiac disease. We noted that females again predominated, in a ratio of 1.7:1. Mean age at diagnosis was similar in men and women 46.4 $\pm$ 1.0 years (range 16–82 years). Females were slightly younger and had a longer duration of symptoms compared to the males. Diarrhea was the main mode of presentation (in 62%) with the remainder classified as silent (38%). This later group included anemia or reduced bone density as presentations (15%), screening first-degree relatives (13%), and incidental diagnosis at endoscopy performed for such indications as reflux or dyspepsia (8%). We compared those diagnosed before and after 1993 (when serologic testing was first seen in patients), and noted a reduction in those presenting with diarrhea, 73% versus 43% ($p = 0.0001$) and a reduction in the duration of symptoms, from 9.0 $\pm$ 1.1 years to 4.4 $\pm$ 0.6 years ($p < 0.001$). These results suggested that the use of serologic testing was responsible for more patients being detected with celiac disease having presented in non-classical ways, after a shorter duration of symptoms. Further analysis of the patients seen at the Celiac Disease Center has revealed that progressively fewer patients are presenting with diarrhea, less than 50% of those diagnosed in the last 10 years (Rampertab et al., 2006).

In a population-based study from Minnesota, Murray et al. (2003) noted a tenfold increase in the incidence of celiac disease from the 1950 to 2001. This was accompanied by a decrease in the clinical severity of the disease with fewer people with diarrhea and weight loss at presentation. Only 54% had diarrhea at diagnosis while 34% complained of abdominal pain and 30% bloating. Obesity was present in 27%.

Many patients with celiac disease (36% in our series), and 29% of Canadians with celiac disease, have had a previous diagnosis of irritable bowel syndrome (Green et al., 2001; Cranney et al., 2007). In fact, screening of patients seen in irritable bowel referral clinics in England and Iran reveals celiac disease in a significant number of patients (Sanders et al., 2001; Shahbazkhani et al., 2003). The majority of patients with celiac disease, detected in a primary care screening study had symptoms attributable to an irritable bowel syndrome (Catassi et al., 2007).

2.2.3 Iron deficiency anemia

Iron deficiency anemia was the mode of presentation in 8% of the individuals seen by us (Lo et al., 2003), and was reported as a prior diagnosis in 40% of Canadians with celiac disease (Cranney et al., 2007). In a study from the Mayo Clinic, celiac disease was identified as the cause of iron deficiency in 15% of those undergoing endoscopic assessment for iron deficiency (Oxentenko et al., 2002). While in a prospective study of adults, mean age in their 50s, Karnam et al. (2004) found 2.8% to have celiac disease. Anemia in celiac disease is typically due to iron deficiency, though vitamin B12 and folic acid deficiency may also be present and contribute to anemia (Halfdanarson et al., 2006). Anemia in celiac disease, however, may be multifactorial in origin; patients may even have anemia of chronic disease secondary to the inflammatory process in the small intestine (Harper et al., 2007). We noted that treatment of patients with anemia with a gluten-free diet resulted in normalization of the ferritin values (Harper et al., 2007). Those with anemia of chronic disease and an elevated

ferritin were noted to have an improvement in anemia and a fall in ferritin values, presumably due to a reduction in the intestinal inflammation. Whereas those with iron deficiency anemia experienced an increase in serum ferritin due to improved absorption of iron.

2.2.4 Reduced bone density

Osteoporosis in celiac disease is considered multifactorial in origin. Calcium malabsorption and secondary hyperparathyroidism are common (Selby et al., 1999). Failure to obtain maximum bone density due to celiac disease in childhood and adolescence, premature menopause and circulating cytokines and anti-bone antibodies may all be factors (Sugai et al., 2002; Moreno et al., 2005).

A diagnosis of celiac disease may be made during the evaluation of reduced bone density (osteopenia or osteoporosis) (Meyer et al., 2001). In our study reduced bone density was more severe in men than women (Meyer et al., 2001). Certainly men and premenopausal women with osteoporosis should be evaluated for celiac disease even if they lack evidence of calcium malabsorption, though the yield in menopausal women is low (Gonzalez et al., 2002). Studies have shown that reduced bone density in celiac disease is associated with an increased fracture risk (Fickling et al., 2001; West et al., 2003; Moreno et al., 2004). It is generally recommended that estimation of the bone density be performed after the diagnosis of celiac disease in adults (Green and Cellier, 2007). Though this is probably not necessary in childhood celiac disease because metabolic bone disease rapidly improves with a gluten-free diet (Zanchi et al., 2008).

2.2.5 Incidental recognition of villous atrophy at endoscopy

An increasingly important mode of presentation is the recognition of endoscopic signs of villous atrophy in individuals who undergo endoscopy for symptoms not typically associated with celiac disease. These endoscopic signs include reduction in duodenal folds, scalloping of folds and the presence of mucosal fissures. The indications for upper gastrointestinal symptoms include dyspepsia, upper abdominal pain or gastroesophageal reflux. This presentation accounted for 10% of those who were diagnosed with celiac disease in our series (Lo et al., 2003). Interestingly, symptoms of gastroesophageal reflux may resolve after starting a gluten-free diet (Green et al., 2000). This is thought to be due to resolution of an accompanying motility disorder (Usai et al., 1995). These endoscopic abnormalities of the duodenal mucosa are neither specific nor sensitive markers of celiac disease (Shah et al., 2000; Oxentenko et al., 2002).

There is an argument for the routine biopsy of the duodenum in anyone undergoing upper gastrointestinal endoscopy to detect celiac disease, irrespective of the appearance of the duodenal mucosa (Green and Murray, 2003).

2.2.6 Screening detected celiac disease

Screening of high-risk groups, especially relatives of patients with celiac disease, is a major mode of presentation. Studies reveal that 5–10% of first-degree relatives of patients with celiac disease have serologic and biopsy evidence of the disease (Marsh, 1992; Fasano et al., 2003). A single testing of relatives does not suffice to detect all those with celiac disease. In one particular study, among those who were initially negative, 3.5% were positive a mean of 2 years after their initial testing (Goldberg et al., 2007). Other groups that are frequently

screened for celiac disease include those with type 1 diabetes (Mahumd et al., 2005), Down syndrome (Henderson et al., 2007) and primary biliary cirrhosis (Dickey and McMillan, 1998).

2.2.7 Atypical presentations

Among the atypical presentations that we have encountered are neurologic problems. We have found that 8% of those attending a peripheral neuropathy center, for evaluation of peripheral neuropathy, had celiac disease (Alaedini et al., 2002; Chin et al., 2003). The neuropathy is typically sensorial in type, involving the limbs and sometimes the face. Nerve conduction studies are frequently normal; however, skin biopsies reveal nerve damage in small fibers. We have also identified patients with severe ataxia or balance problems (Sander et al., 2003). We have not identified patients with epilepsy, a neurologic manifestation that may be more common in childhood celiac disease (Arroyo et al., 2002).

Other, less common presentations, are abnormalities of blood chemistry determinations such as elevated serum amylase, secondary to macroamylasemia (two amylase molecules bound together by Iga) (Rabsztyn et al., 2001), hypoalbuminemia, hypocalcemia, vitamin deficiency states (Mauro et al., 1991) and evidence of hyposplenism (malfunctioning of the spleen usually associated with a reduction in the splenic size). We have seen patients referred because of dental enamel defects (Aine, 1994). Many females diagnosed with celiac disease have a history of infertility and there is a yield of screening infertile individuals for celiac disease (Sher and Mayberry, 1994; Collin et al., 1996; Kolho et al., 1999).

2.3 ETHNIC ORIGINS OF PATIENTS WITH CELIAC DISEASE

Celiac disease is common in populations of European origin. However, the greatest reported prevalence is in a North African refugee population (Catassi et al., 1999) and the disease is frequently recognized in the Middle East and India as well as South America (Araya et al., 2000; Gandolfi et al., 2000; Gomez et al., 2001; Sood et al., 2001; Shahbazkhani et al., 2003). While not commonly recognized in African Americans, Hispanics or Asians in North America, there are reported cases of celiac disease identified from these ethnic groups indicating that the disease should be considered in any ethnic group (Sagaro and Jimenez, 1981; Freeman, 2003; Brar et al., 2006), not only in residents but also immigrants from many diverse countries around the world (Nelson et al., 1973; Cataldo et al., 2004).

2.4 DIAGNOSIS OF CELIAC DISEASE

The diagnosis of celiac disease depends on the finding of characteristic small intestinal pathological abnormalities together with clinical or histologic improvement on a gluten-free diet (Walker-Smith et al., 1990). People come to biopsy because of either clinical suspicion of the disease, positive serologic tests or because the patient is undergoing an endoscopy.

2.4.1 Serology testing

The widespread availability of serologic tests has permitted the diagnosis of celiac disease to be considered and tested for by any physician. The most sensitive tests are based on the use of

IgA isotypes. The available tests include antigliadin antibodies as well as connective tissue antibodies: endomysial and tissue transglutaminase antibodies. The lower sensitivity and specificity of the antigliadin antibodies (70–80%) have resulted in their use being called into question for the diagnosis of celiac disease (NIH, 2004). There has however been the recent availability of new generation antigliadin antibodies that are directed against deamidated (by tissue transglutaminase) synthetic gliadin peptides (Prince, 2006). These antibodies have high sensitivity and specificity for celiac disease detection (Sugai et al., 2006; Volta et al., 2007).

The current gold standard in celiac serologies remains the IgA endomysial antibody (EMA) (Chorzelski et al., 1984; Rossi et al., 1988). This is based on its very high specificity for celiac disease that approaches 100%. This high specificity has prompted some investigators to suggest that in the presence of a positive EMA, a duodenal biopsy is not necessary for diagnosis (Valdimarsson et al., 1996). Overall the sensitivity of the EMA is excellent with the majority of reports indicating approximately 90% sensitivity (Rostom et al., 2004). The titer of EMA correlates with the degree of mucosal damage (Sategna-Guidetti et al., 1993), and accordingly, the sensitivity declines when a greater number of patients with lesser degrees of villous flattening are included in the studies (Dickey et al., 2000; Tursi, et al., 2001; Abrams et al., 2004). The EMA is an observer-dependent immunofluorescence test that requires expertise in reading it and the use of either primate esophagus or human umbilical cord as tissue substrate.

The recognition of the enzyme tissue transglutaminase (tTG) as the autoantigen for the EMA (Dieterich et al., 1997) allowed development of an enzyme-linked immunoassay (ELISA; Dieterich et al., 1998; Sulkanen et al., 1998). Initially, the antigen in the assay was tTG derived from guinea pig liver (GP-tTG): subsequently human tTG (H-tTG), either recombinant or derived from human red cells, has replaced the assays using GP-tTG. Different kits for assaying tTG have different characteristics and resultant sensitivities and specificities (Wong et al., 2002; Van Meensel et al., 2004). While there are differences among the available H-tTG kits, overall they perform better than the GP-tTG derived kits (Wong et al., 2002).

Overall the sensitivity of both the EMA and tTG is greater than 90% (Rostom et al., 2004). While the specificity of the EMA is considered to be virtually 100%, the tTG test does not achieve that degree of specificity. There are numerous reports of positive tTG results in the absence of celiac disease (Dahele et al., 2001; Clemente et al., 2002; Freeman, 2004; Lock et al., 2004; Weiss et al., 2004). They may be seen in type 1 diabetes (Lampasona et al., 1999), chronic liver disease (Vecchi et al., 2003), psoriatic or rheumatoid arthritis (Spadaro et al., 2002) and heart failure (Peracchi et al., 2002), though biopsy has not been performed in most of these studies.

New generation tests that detect antibodies to deamidated gliadin peptides show promise (Prince, 2006), and in one study were positive in patients with dermatitis herpetiformis who lacked villous atrophy (Sugai et al., 2006).

2.4.2 Selective IgA deficiency

Selective IgA deficiency (SigAD) is a genetically determined near absence of IgA. This occurs more commonly in patients with celiac disease than the general population (Collin et al., 1992). As a result, patients with celiac disease lack IgA-EMA, IgA-tTG and IgA-antigliadin antibodies (Rittmeyer and Rhoads, 1996; Cataldo et al., 1997). To detect celiac disease in those with SigAD, a total IgA level should be incorporated into the testing for celiac

disease (Cataldo et al., 1997), as well as an IgG antibody-based test, either IgG-antigliadin or IgG-tTG (Korponay-Szabo et al., 2003).

2.4.3 Seronegative celiac disease

Several studies have demonstrated that serologic studies may lack sensitivity when used in the practice setting (Rostami et al., 1999; Dickey et al., 2000; Ashabani, 2001; Dahele et al., 2001). Reliance on EMA as a single test has, in fact, underestimated the prevalence of celiac disease by at least 20–25% (Dickey et al., 2000; Dahele et al., 2001; Sanders et al., 2001; Shamir et al., 2002). This is mainly due to the inclusion of patients with mild mucosal changes, a situation when patients may not express an EMA (Sategna-Guidetti et al., 1993; Rostami et al., 1998, 1999a,b; Abrams et al., 2004). A similar situation occurs with tTG, with titers decreasing as the mucosal lesion becomes less marked (Rostami et al., 2003; Tursi et al., 2003).

Anti-smooth muscle and anti-actin antibodies are detected in the sera of patients with celiac disease (Lasagni et al., 1999; Clemente et al., 2000, 2004; Granito et al., 2004) and the titer of anti-actin antibodies correlates with the degree of villous damage (Clemente et al., 2004). The role of these antibodies in the assessment of patients considered to have celiac disease is to be determined.

2.4.4 Role of HLA DQ2/DQ8 assessment

HLA DQ2 is found in up to 90–95% of patients with celiac disease, while most of the remaining patients have HLA DQ8 (Ploski et al., 1993; Johnson et al., 2004). The HLA (human leukocyte antigen) system is the name of the major histocompatibility complex in humans. This group of genes resides on chromosome 6, and encodes cell-surface antigen-presenting proteins and many other genes. However, these HLA alleles that encode for DQ2 or DQ8 are found in up to 40% of the general population. They appear to be a necessary, but not sufficient, factor in the pathogenesis of celiac disease. The role of determining whether an individual carries HLA DQ2 or DQ8 in the assessment of celiac disease lies in their high negative predictive value (Kaukinen et al., 2002). The main uses of the determination of HLA DQ2 or DQ8 status are in determining whether family members require screening for celiac disease. Those that are negative are very unlikely to ever get celiac disease and do not need serial serologic assessment. Another role is in excluding celiac disease when patients are already on a gluten-free diet and in the situation where the diagnosis of celiac disease is unclear.

2.4.5 Biopsy and histology

Biopsy of the small intestine remains the gold standard in the diagnosis of celiac disease. Due to the patchy nature of villous changes in celiac disease (Scott and Losowsky, 1976; Siegel et al., 1997; Bonamico et al., 2004) multiple biopsies are necessary, though standard sized forceps are sufficient (Dandalides et al., 1989).

The recognition of the spectrum of histological changes in celiac disease, as classified by Marsh (Marsh, 1992; Marsh, 1992a), or modifications of this classification (Rostami et al., 1999b; United European Gastroenterology, 2001) have provided a major advance in the diagnosis of celiac disease. The earliest lesion, Marsh I, is characterized by normal

villous architecture with an intraepithelial lymphocytosis (>30 lymphocytes per 100 enterocytes). An intraepithelial lymphocytosis is not however specific for celiac disease and may be seen in tropical sprue, parasitic infection with *Giardia lamblia*, acute infective enteropathy after viral gastroenteritis, *Helicobacter pylori* gastritis, Crohn's disease, during non-steroidal anti-inflammatory drug usage, and in various autoimmune disorders (Marsh, 1992; Shah et al., 2000; Kakar et al., 2003; Lebwohl et al., 2003; Memeo et al., 2005). A Marsh II lesion is identified when the intraepithelial lymphocytosis is accompanied by crypt hypertrophy. The majority of patients diagnosed with celiac disease fall into the category of Marsh III that includes moderate to very severe reduction in villous height; commonly classified as partial, subtotal or total villous atrophy.

A major pitfall in the diagnosis of celiac disease is in the over-interpretation of villous morphology leading to a mistaken report of mucosal flattening in poorly oriented biopsies (Shidrawi et al., 1994). It is unclear whether attempts at orientation (attempts to ensure that villi are oriented upwards) of the small biopsies taken at endoscopy, prior to fixation, could alleviate this problem (Pais et al., 2008). Another problem arises when biopsies in patients with a high likelihood of celiac disease, and positive serologies, are reportedly normal. The first step is to have the biopsy reviewed by an experienced gastrointestinal pathologist. Other reasons for histologic findings that are milder than expected, include a reduced amount of gluten in the diet as often happens in a family in which there are members with celiac disease.

2.4.6 Use of stool and salivary antibody testing to diagnose celiac disease

This is a controversial area. While antigliadin antibodies, EMA and tTG antibodies are recovered in duodenal secretions and stool of patients with celiac disease (Arranz and Ferguson, 1993; Dahele et al., 2002; Picarelli et al., 2002) as well as normal individuals their role in the diagnosis of celiac disease is controversial (Arranz and Ferguson, 1993; Dahele et al., 2002). IgA-tTG antibodies are also recovered in saliva of patients with celiac disease (Bonamico et al., 2004) and have been advocated as a non-invasive screening test for celiac disease by some investigators (Bonamico et al., 2004), but not all (Baldas et al., 2004). Recently both salivary and fecal serologic testing for celiac disease have been studied in a group of patients with celiac disease and controls: the value of the procedures for the diagnosis of celiac disease was hampered by overlapping results between both groups (Halblaub et al., 2004).

2.5 CONCLUSION

In summary, adults with celiac disease usually present after a long duration of symptoms, though this duration is decreasing. Non-diarrhea predominant presentations, or those with silent celiac disease, are the most frequent presentation these days.

The diagnosis of celiac disease depends on the finding of characteristic small intestinal pathological abnormalities together with clinical or histologic improvement on a gluten-free diet. Lesser degrees of mucosal change must be recognized as reactions to gluten in sensitized individuals and must therefore not be ignored or dismissed as 'non-specific'. EMA and tTG antibodies have high sensitivity and specificity toward celiac disease, though there are some pitfalls in their use.

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3 Classification of Proteins in Cereal Grains: What Is Toxic and How Is It Measured in Foods?

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3.1 WHAT IS TOXIC?

3.1.1 What is meant by toxicity?

Coeliac disease (CD) is an inflammatory response in the small intestinal mucosa exacerbated by dietary gluten. It is accepted by most authors that there is an inappropriate response of the immune system, rather than a direct toxic effect of gluten. However, the term toxicity has been so widely used that it has now become the accepted terminology. Any discussion on toxicity normally involves aspects of small intestinal inflammatory responses, although a number of extraintestinal manifestations may result from gluten ingestion by susceptible individuals, for example, the rash of dermatitis herpetiformis. We must assume that the same gluten epitopes are responsible for such extraintestinal manifestations, although little data exists.

The only existing treatment for CD is a gluten-free diet (GFD) with total avoidance of wheat, rye, barley and possibly oats. Various novel approaches to treatment have been explored. One of the key 'toxic' gluten peptides is resistant to breakdown by human intestinal proteases; however, this 33-mer is degraded by bacterial prolyl endopeptidases (Shan et al., 2002). High concentrations of these enzymes can reduce the amount of immunostimulatory peptide reaching the mucosa when used ex vivo in an Ussing Chamber system (Matysial-Budnik et al., 2005). To date, no in vivo studies have confirmed the use of such enzymes as supplements. In some instances, it might be possible to add enzymes to foods at the processing level to degrade immunostimulatory sequences. This would be possible in situations where a partial hydrolysis of gluten would not affect the processing or palatability of the eventual product; in other words where formation of dough was not essential. The production of beers or other malted products is a good example. Selected lactobacilli with specialised peptidases were used to make sourdough bread (Di Cagno et al., 2004). Preliminary clinical toxicity studies suggested that this might be better tolerated by CD patients, although the gold standard method for testing for coeliac toxicity (see below) was not used. The possibility of producing cereals with no, or lower, toxicity has been raised. Some ancient wheat varieties have been shown to have fewer toxic sequences, while some modern varieties may lack certain epitopes (Molberg et al., 2005; Spaenij-Dekking et al., 2005). This information might be exploited in selective breeding programmes; alternatively, genetic manipulation could be used to detoxify certain epitopes (Vader et al., 2003), and/or allow transfer of selected gluten genes into non-toxic species such as maize (Bretteschneider et al., 2003; Gahrtz et al., 2003).

3.1.2 How is toxicity assessed?

To be able to understand the arguments in this chapter, it is necessary to be acquainted with the methods used to assess whether a food, protein or peptide is coeliac toxic. These can be divided into in vitro and in vivo methods.

3.1.2.1 *In vitro testing*

It is relatively easy to collect multiple biopsies directly from the small intestine of patients who are being investigated for CD. It is then possible to investigate the effects of gluten on the small intestinal mucosa ex vivo, thus causing no systemic effect for the patient. The histology of the small intestinal epithelium from untreated patients with CD improves when the biopsies are placed in an organ culture chamber in the absence of gluten (Browning and Trier, 1969). This improvement is inhibited by the addition of gluten (Townley et al., 1973). Similarly, biopsies from patients with treated CD show significant deterioration in the presence of gluten. The method has been used to show toxicity of certain gluten peptides (De Ritis et al., 1988). The method has been validated, in that peptides shown to be toxic in organ culture have also been verified as toxic in vivo, as discussed below (Sturgess et al., 1994; Shidrawi et al., 1995; Fraser et al., 2003; Martucci et al., 2003).

Healthy individuals do not express a small intestinal T-cell response to gluten. It is, however, possible to isolate and grow up gluten sensitive T lymphocytes from small intestinal biopsies of coeliac patients. These will multiply rapidly in the presence of gluten, but stay quiescent in its absence. The rate of multiplication is known as the stimulation index (SI). A positive SI reflects in vivo toxicity (Anderson et al., 2000; Arentz-Hansen et al., 2000; Fraser et al., 2003). However, certain peptides which are toxic in vivo (Sturgess et al., 1994), do not elicit a small intestinal T-cell response (Arentz-Hansen et al., 2000).

3.1.2.2 *In vivo testing*

While in vitro methods are of great value in assessing the toxicity of large numbers of proteins or peptides, the in vivo challenge is the gold standard for assessment of toxicity and has been used since the early 1950s to study the role of cereal fractions in CD. While early studies relied on crude indicators of coeliac toxicity, such as faecal fat analysis (Dicke et al., 1953), the advent of small intestinal biopsy and sophisticated immunohistochemical techniques allowed accurate studies to be performed. In essence, a biopsy of the duodenum is taken at baseline and assessed for the standard morphometrical parameters, villus height to crypt depth (VH:CD), enterocyte cell height (ECH) and intra-epithelial cell counts (IEL). In vivo gluten challenge with bread, over, usually, 4 weeks may be performed where there is doubt about the diagnosis of CD in an individual. Indeed, the challenge of treated patients with CD and induction of relapse of the disease was once mandatory for the diagnosis of CD. Longer-term challenges are used to determine the suitability of foodstuffs for CD patients. Short-term challenge involving instillation of the test substance via one of three possible routes is normally performed as a research tool in order to ascertain the coeliac toxicity of a particular peptide or protein fraction. In all these situations, a repeat biopsy is performed at the end of the study period for comparison with the baseline data.

Short-term challenge

Short-term challenge via direct instillation requires relatively small quantities of material, applied directly to the gastrointestinal (GI) mucosa. This remains the gold standard for

the assessment of coeliac toxicity. The test substance can be dripped onto the duodenal mucosa via a tube attached to a Quinton multiple biopsy capsule (Ciclitira et al., 1984; Sturgess et al., 1994; Fraser et al., 2003). The capsule may remain in place for 6 h while the duodenum is biopsied at intervals. Under x-ray control, it is possible to ensure that the area of duodenum biopsied is the same as the area challenged with test substance. Challenge of the small intestinal mucosa is a time-consuming and relatively invasive procedure. Thus, other methods have been sought. Direct infusion of the rectum with gluten has been shown to result in an increase in mucosal intra-epithelial lymphocytes, occurring only in coeliac patients (Loft et al., 1990). Later, it was shown that the oral mucosa could be similarly challenged and biopsied, offering a less invasive and potentially more acceptable technique (Lähteenoja et al., 2000). Both these techniques have so far only been applied for research, rather than diagnostic purposes.

Long-term in vivo challenge

This technique is useful in assessing the appropriateness of certain foodstuffs, which may be of low toxicity for coeliac patients and therefore inappropriate for use in acute challenge studies. Wheat starch is a good case in point. A number of gluten-free products are based on this wheat flour derivative, and legislation within the European Union allows the use of this product in CD patients, even though it is recognised that it contains trace amounts of gluten (Skerritt and Hill, 1992). Both wheat starch and oats have been the subject of a large number of this type of study, as discussed below.

3.2 CLASSIFICATION OF CEREAL PROTEINS

Wheat grains have three major constituents that are separated by milling: the outer husk or bran, the germ and the endosperm or white flour, which constitutes 70–72% of the whole grain by weight and which contains the toxic components. The storage proteins of wheat fall into two major groups: the ethanol-soluble fraction termed prolamins because of their unusually high proportions of glutamine and proline, and the polymeric glutenins (Wieser, 1995). The glutenin polymers are insoluble in aqueous and ethanolic solutions. Solubilisation of these polymers can be achieved with dilute acetic acid and 2% SDS (sodium dodecyl sulfate), neither of which gives a product which is highly compatible with biological test systems. Once the inter-chain bonds are broken, using reducing agents such as dithioerythritol, or beta-mercaptoethanol, the glutenins, like gliadins become soluble in 40–70% ethanol. Again, however, the presence of such reducing agents may interfere in subsequent biochemical processes; care must be taken to test the compatibility of reagents. The most common route around the solubility problem is to employ partial hydrolysis. The experiments of Frazer et al. (1959) showed that partial digestion of wheat gluten with pepsin and trypsin from porcine intestine yielded a product which was water-soluble and which retained coeliac toxicity. This data is now clarified by the finding of Shan et al. (2002) and Arentz-Hansen et al. (2002), who showed that important gliadin epitopes are resistant to digestion by human proteases, probably due to the presence of proline residues. This is very important because it allows the production of physiological solutions of gluten proteins which retain toxicity, for use in the studies detailed above.

The gliadins may be further subdivided into α, ß, γ and ω subfractions, either according to their relative electrophoretic mobility, or α, γ and ω according to their *N*-terminal amino

Table 3.1 Nomenclature of prolamins from cereals.

Cereal	Prolamin	Wheat equivalent	Prolamin type
Rye	Gamma secalins	Gamma gliadins	Sulfur rich
	Omega secalins	Omega gliadins	Sulfur poor
	HMW secalin	HMW-GS	HMW prolamins
Barley	Gamma hordeins	Gamma gliadins	Sulfur rich
	B hordeins	LMW-GS	Sulfur rich
	C hordeins	Omega gliadins	Sulfur poor
	D hordeins	HMW-GS	HMW prolamins

acid sequences. Molecular masses from the gliadins range from 32–58 kilodaltons. Much information is available on the amino acid structure of the gliadins. Complete sequences are available (Kasarda et al., 1984), which have facilitated studies using sequenced or synthetic peptides. Wheat glutenins comprise low molecular weight glutenin subunits (LMW-GS) and high molecular weight glutenin subunits (HMW-GS). The major dough forming HMW-GS are 1Dx5, 1Dy10, 1Dx4 and 1Dy9, the former two being the most important.

Wheat gluten proteins can also be classified as sulfur-poor prolamins, that is, the omega gliadins, sulfur rich prolamins, that is, the alpha, gamma gliadins and the LMW-GS and finally the high molecular weight prolamins, which comprise the HMW-GS. In wheat, the interaction of both the gliadins and the glutenins facilitates formation of a dough.

Other related cereals from the family Graminae, or grasses, contain prolamins, as shown in Table 3.1.

Although both rye and barley have proteins equivalent to HMW glutenins, the ability of these two cereals to form dough is considerably more limited than wheat.

Other Graminae contain prolamins. These are present in their respective cereals in much lower proportions than the prolamins in wheat, rye and barley. Oats contain a prolamine fraction, known as the avenins, and in rice and maize the equivalent proteins are the oryzins and zeins. The subject is reviewed in more detail by Shrewry et al. (1992).

Cereals are polyploid in nature. Gluten is a storage protein; the genes encoding gluten have undergone repeated duplications and mutations, with little constraint on protein function. Thus, gluten from even a single wheat variety is a complex mixture. Differences in amounts of sunlight during germination, soil quality and fertilisation can also affect the proportions of different molecules, even within the same wheat variety (Wieser et al., 1994). Thus, each wheat variety expresses a large number of related proteins making biochemical studies and toxicity mapping very difficult.

3.3 WHICH CEREAL PROTEINS ARE TOXIC?

3.3.1 Wheat

The identification of wheat gluten as the noxious element in CD was established in 1953 (Dicke et al., 1953). While it was possible to obtain the gliadin fraction in sufficient purity to ascertain its toxicity, and the toxicity of each of the subfractions (Ciclitira et al., 1984), studies on glutenins were few, due to difficulties in extraction and purification.

A great deal is now known about toxic epitopes within the gliadins. The T-cell response is directed against gluten peptide epitopes that are resistant to enzymatic digestion in the GI tract and typically have a high content of proline and glutamine residues (Arentz-Hansen et al., 2002; Shan et al., 2002). Immunodominant epitopes have been defined (Anderson et al., 2000; Arentz-Hansen et al., 2000). A typical feature of several, if not most, of these epitopes is that the glutamine residues are converted to glutamic acid in a process of deamidation by the small intestinal enzyme tissue transglutaminase. The peptides, as a result, become far more antigenic to T-cells (Molberg et al., 1998). Another complication is that different epitopes affect different patients. This applies particularly to the tissue type of the patient. The majority (95%) of patients have the tissue antigen HLA-DQ2, while the minority (5%) are HLA-DQ8; the two groups respond to different gluten epitopes (Anderson et al., 2000; Arentz-Hansen et al., 2000; Mazzarella et al., 2003) (Table 3.2).

Other epitopes have been identified via T-cell studies, and whilst there is no doubt they are immunostimulatory in some CD patients, their importance in the majority of CD patients is not known.

As far as the glutenins are concerned, a great deal of information has emerged in the last few years. Molberg et al. (2003) demonstrated, in vitro, the T-cell immunogenicity of recombinant HMW-GS 1Dx5 and 1Dy10 in CD patients, both of the subunits being important for dough formation. We wished to confirm the in vivo toxicity of the HMW-GS. A mixture of HMW-GS (1Dx5, 1Dx4, 1Dy9 and 1Dy10) were separated chemically from Rektor variety wheat flour. Following repeated runs of preparative HPLC, the purity of the fraction was checked. This was found to be contaminated by less than 0.2% gliadins and LMW glutenins, a level which would not cause coeliac toxicity in vivo (Ciclitira et al., 1984). We obtained gluten-sensitive small intestinal T-cell lines from coeliac patients and showed that 11: 17 lines were stimulated by HMW-GS. We then instilled 500 mg of HMW-GS into the proximal duodenum of three further patients with known treated CD, from whom we took duodenal biopsies before and after commencing the infusions. All three patients challenged revealed significant in vivo toxicity to HMW-GS (Dewar et al., 2006). Later, in preliminary experiments, in vivo, we demonstrated the toxicity of the single recombinant 1Dx5 and 1Dy10 HMW-GS, in one patient each (Ellis 2006). Thus we have shown that the HMW-GS, as well as the gliadins, are toxic to coeliac patients. However, to date, only two HMW-GS epitopes have been identified, which have been shown, to stimulate DQ8 and DQ2 restricted T-cells respectively, from one patient each (Van de Wal et al., 1999; Vader et al., 2002). The evidence for toxicity of LMW-GS is less clear; however, a number of LMW-GS derived peptides have been shown to stimulate coeliac gluten sensitive small intestinal lymphocytes, the consensus sequence being PFSQQQQSPF (Vader et al., 2002).

Table 3.2 The well-characterised toxic wheat gliadin sequences.

Epitope	Level of evidence	Reference
A-gliadin 31—49	Toxic in vivo	Sturgess et al., 1994
Alpha gliadin 56–75	Immunodominant for T-cells in vitro (95% majority of CD patients)	Anderson et al., 2000; Arentz-Hansen et al., 2000
	Toxic in vivo	Fraser et al., 2003
Alpha gliadin 203–220	Toxic in organ cultures (5% minority of CD patients)	Mazzarella et al., 2003

3.3.2 Wheat Starch

The early experiments of Dicke et al. (1953) demonstrated that wheat starch did not exacerbate CD in vivo, although the measure of coeliac toxicity used was faecal fat analysis, rather than histological assessment. Wheat starch became a regular and useful addition to the diet. However, it became clear that the quality of wheat starches varied considerably with some containing significant amounts of residual gluten (Skerritt and Hill, 1992). Today, however, the quality of these products has improved considerably. A recent study showed that 57 newly diagnosed coeliac patients, randomly assigned to a naturally GFD or a diet containing wheat starch over 12 months showed no difference in abdominal symptoms. There were no differences between the groups in small intestinal mucosal morphology, the density of intra-epithelial lymphocytes or quality of life tests at the end of the study (Peräaho et al., 2003). We revisit the subject of wheat starch later in this chapter when we discuss levels of sensitivity.

3.3.3 Wheat Related Species

Triticale is a wheat/rye hybrid and therefore coeliac toxic. Ancient wheat varieties like kamut, spelt and einkorn are almost certainly harmful, as they are genetically similar to modern wheat and have similar amino acid compositions (Forsell and Wieser, 1995). Studies in our laboratory using monoclonal antibodies to toxic gluten peptides (Ellis et al., 1998, and unpublished data) show that spelt contains proportions of these epitopes comparable with normal wheat varieties. The wheat varieties of the type used for baking purposes (T. aestivum) have been refined since tetraploid and hexaploid wheat evolved approximately 9000 years ago. Then, wild einkorn (Triticum urartu) and goat grass (Aegilops speltoides) gave rise to the emmer wheat (T. turgidum), which later crossed with T. tauschii and gave the common bread wheat. A large panel of these wheat varieties was recently tested gluten sensitive with small intestinal T-cells from patients with CD (Molberg et al., 2005). The T. urartu did not express the most important T-cell epitopes. Thus, the possibility remains that these wheat varieties are less toxic for patients with CD, although this has not been tested clinically. These primitive wheat varieties give extremely small crop sizes, have low gluten loads and thus have very poor baking quality. Recent data has shown that there are toxic epitopes within the glutenin fraction of wheat, as discussed above, so it is unlikely that the lack of a set gliadin epitopes could lead to a non-toxic wheat.

3.3.4 Rye and Barley

Rye flour was shown to be toxic in vivo (Dicke et al., 1953) and similarly barley at a later date (Rubin et al., 1962). Little is known about toxic epitopes in these cereals, although it is clear that there are some T-cell cross-reactivities with toxic wheat gliadin epitopes (Vader et al., 2003).

3.3.5 Oats

Oats have traditionally been excluded from the GFD, based on early feeding experiments (Dicke et al., 1953). It has become apparent that many commercial oat products generally available are contaminated with wheat, rye or barley, and are therefore unsuitable for patients

with CD (Thompson, 2004). This fact may be behind the conflicting results gained in early experiments.

Recently, the problem has been addressed using pure oats samples. Ninety-two patients with CD were randomised to a standard GFD or a similar diet containing oats. No adverse effects of oats were seen. However, 11 patients withdrew during the study. After 5 years, 23:35 patients from the original oats-eating group were still eating oats with no harmful effect (Janatuinen et al., 2002), and with no evidence of immunological activation in the small intestine (Kemppainen et al., 2007). A Swedish study also suggested that oats are tolerated by coeliac patients. However, patients also withdrew from this study, raising some doubts concerning the conclusions (Størsrud et al., 2003). Similar results have been reported for patients with dermatitis herpetiformis, a skin disorder that normalises on a GFD (Hardman et al., 1997; Reunala et al., 1998). Only two studies with children have been published. A US study included 10 newly diagnosed children, all of whom received a GFD including oats (Hoffenberg et al., 2000). All improved clinically during the 6 months follow-up period. However, a control group was not included and the small intestinal morphology of 5 of the children only partially normalised during the study. A recent Swedish study enrolled 116 children at the time of diagnosis, which were randomised to a standard GFD with or without oats in a double blind design. No signs of toxicity was found, although 7 children in the control group and 16 in the oats group were withdrawn from the study (Högberg et al., 2004).

In Norway, oats consumed by patients with CD are produced by a small number of dedicated farmers that grow no harmful cereals. The oat grains are processed in a separate production line and repeated testing has shown that these oat products are free from contamination. In a study of 19 adult coeliac patients challenged with oats, one developed villous atrophy, dermatitis herpetiformis and clinically active CD during repeated challenges (Lundin et al., 2003). Later, two further patients were found to have deteriorated after eating oats at the nationally recommended level (Arentz-Hansen et al., 2004). Importantly, biopsies were taken and challenged with oats, ex vivo. The oat intolerant patients' biopsies contained oats reactive T-cells which multiplied in response to an oats peptide similar but not identical to wheat peptides which were also found to be stimulatory to these cells (Arentz-Hansen et al., 2004). Thus, the intestinal immune responses in these patients correlated well with their clinical intolerance. To date, only three definite oat intolerant patients have been reported. However, in the majority of the large clinical studies involving assessment of coeliac patients to oats, a number of patients withdrew, which leaves a question mark over the interpretation of the results. Our practice is to start patients off on a gluten-free, oat-free diet, and to check the small intestinal biopsy for normalisation of the histology. If the patient wishes, he or she may commence taking up to 50 g per day of oats, provided they come from a dedicated grower. The patient is monitored carefully for changes in coeliac antibody blood tests and clinical symptoms. In the event of an increase in the titre of coeliac serological antibodies or the development of symptoms, a repeat small intestinal biopsy is taken.

3.3.6 Enzymatic digests – beers, malt and glucose syrup

Foodstuffs based on enzymatically altered cereals, such as beers and malted barley were at one time considered safe for CD sufferers, on the basis that the gluten would be broken-down by the malting enzymes. However, this opinion was formed without any evidence that this was the case. It is now known that certain, significant T-cell-reactive areas of gliadin are highly resistant to enzymatic degradation (Shan et al., 2002; Arentz-Hansen et al., 2002). Other unsubstantiated claims were that keg beers, lacking sediment, could be considered safe. Again this ignores the known fact that enzymatically hydrolysed gluten is highly soluble

(Frazer et al., 1959). Gliadin-like epitopes have been detected in both beer and malt (Ferre et al., 2004; Ciclitira 2007). Coeliac UK does not recommend the consumption of beers and ales, nor indeed of any malted drink. Malt extract is used as flavouring in a large number of foods. In our practice, we find that certain individuals who consume large quantities of malted foods, for example malted breakfast cereals, may have severe symptoms. Challenge studies are lacking, but we recommend that patients taking an apparently strict GFD, but experiencing symptoms, should be questioned about consumption of malted breakfast cereals. Glucose syrups are widely used in the food industry and are made by hydrolysing wheat starch. Monoclonal antibody based ELISA tests have detected potentially toxic amounts of intact gliadin peptides in a minority of samples tested (Ferre et al., 2004). The levels of contamination presumably depend on the quality of the wheat starch used. The clinical significance of the products, when used as a minor ingredient, is not known.

3.3.7 Other 'Flours'

Gram flour is made from chickpeas and therefore coeliac safe. Buckwheat, despite its name is not a cereal, thus flour from this crop is also safe. Corn flour is made from maize and therefore safe.

3.3.8 Communion wafers

These are made from wheat flour. Communion wafers have been the subject of controversy for many years. A recent report showed that an adult coeliac patient consuming a milligram of gluten per day in the form of a fragment of communion wafer, showed failure of mucosal recovery after 18 months of an otherwise strict GFD (Biagi et al., 2004). Gluten-free communion wafers are available, based on wheat starch, but these are not universally considered acceptable, on theological grounds.

3.3.9 What level of gluten is toxic?

Clinical sensitivity differs considerably between patients. Some cannot tolerate trace amounts of gluten, whereas others appear to tolerate large quantities. In the standard Western European GFD, some gluten is accepted as a contaminant in wheat starch. This starch improves the baking quality and palatability of the GFD and, when purchased from a reputable manufacturer is tolerated by most CD patients. Patients with CD on such a wheat starch-containing diet generally show good histological and clinical response (Peräaho et al., 2003; Collin et al., 2004). In other countries, such as Australia, Canada, Italy and the US, wheat starch is not recommended, and a 'naturally' GFD is prescribed. The US National Food Authority has decided that the label 'gluten-free' can only be used for foods that contain no gluten. Foods that contain wheat starch, for example, must therefore be labelled as 'low-gluten' (Fasano and Catassi, 2001). The present standard operating in the UK has stood since 1981 (Codex Stan 118–1981) and states that the total nitrogen content from gluten-containing cereals does not exceed 0.05 g per 100 g of product on a dry matter basis. This applies to 'naturally gluten-free' products and those that have been rendered gluten-free, in particular, wheat starches from which the majority of the gluten has been removed. This method is, however, very non-specific, since the measurement of nitrogen in grains also includes the coeliac non-toxic albumins, globulins and starch granule proteins (Skerritt and Hill, 1992). A more precise measurement of the celiac toxic proteins was sought, accordingly the Codex Alimentarius Standard was revised in July 2008. It now states that foods labelled as

gluten-free must have no more than 20 ppm gluten, whilst foods with 21–100 ppm gluten may be labelled as low gluten. The recommended method for measurement is the R5 assay, as discussed below.

Delays in implementing new standards for gluten-free foods have been caused by both lack of a suitable method of measurement (see below) and differences in opinion regarding what the permissible level of gluten should be. In fact, the exact limit of gluten intake that is tolerated long term by patients with CD is still not known (Stern et al., 2001). A consensus suggests that the limit should be between 10–100 mg per day (Hischenhuber et al., 2006). Collin et al. (2004) assessed the gluten content of 83 gluten-free flour products and calculated the daily intake of gluten in 76 adult CD patients on a GFD. This intake was compared to mucosal histology. The gluten content was 20–200 ppm and the median flour consumption was 80 (range 10–300 g). In all patients, the long-term mucosal recovery was good. The authors concluded that provided the daily flour intake is no more than 300 g, a gluten contamination of 100 ppm results in 30 mg gluten/day, which has been shown to be safe. A recently published in vivo challenge study (Catassi et al., 2007) suggests that 50 mg gluten ingested daily causes damage to the small intestinal mucosa in CD patients. The patients showed no clinical symptoms and no changes in coeliac antibody levels. Thus, the debate continues. It is our opinion that wheat starch with up to 100 ppm gluten is acceptable for the majority of patients, provided that contamination is measured using a robust method which takes into account all the toxic gluten proteins.

3.4 METHODS FOR MEASUREMENT OF GLUTEN CONTAMINATION IN FOODS

It was recognised that an immunoassay was required for detection of gluten contamination at 20 ppm, the new standard for gluten-free foods, see above.

Assays for the determination of toxicity of gluten containing, or contaminated, products are required both for the food industry and for patients. If novel therapeutic methods such as detoxification by enzymes, selective breeding or genetic modification are to be developed, it will be essential to have robust methods for ensuring that the process results in a foodstuff that is indeed free of the immunostimulatory epitopes. The development of legislation on levels of gluten permissible in foods for patients with gluten intolerance has been hampered by lack of a suitable assay system. It is recognised that some type of immunoassay will be required for detection of gluten contamination at 20 ppm, the proposed new standard for naturally gluten-free foods, as discussed above.

This area has attracted a huge amount of literature in the last 30 years. While immunological techniques, such as ELISA offer sensitive, simple and robust solutions, the complex heterogeneity of gluten has frustrated attempts to produce a gold standard. Exactly what should we measure in order to assess coeliac toxicity? Workers have struggled to find ‘the toxic epitope’, concentrating on wheat gliadin. It now appears that there are many immunostimulatory epitopes and that they are distributed between both of the major groups within the wheat gluten complex (van Heel and West, 2006), that is, the gliadins and the glutenins, both of which contribute to the dough forming characteristics of wheat. Within rye and barley there appears to be cross-reactivities with toxic wheat gliadin epitopes (Vader et al., 2003); oats may be toxic in 5% of patients.

In an attempt to produce a standard gliadin, a mixture of 28 cultivars representative of the three main wheat producing European countries (France, Germany and the UK) were selected as a source for a preparation of a reference gliadin (Van Eckert et al., 2006).

Gliadins were extracted using 60% ethanol. Multiple analyses at 16 laboratories showed that the reference gliadin matched the gliadin composition of the source flours, and that no major gliadin component had been lost. The reference material showed good immunological sensitivity with various antibodies, in immunoassays. The material was suggested as a universal reference for gliadin. However, we have since shown that contamination of this material with HMW-GS causes spurious cross-reactivities with anti-HMW-GS antibodies (Ellis 2007). This will cause a problem should cocktail assays be used for the determination of gliadins and glutenins in foods, which is discussed further below.

Early assays used whole gliadin as the immunogen to produce polyclonal antisera and gave rise to antibodies that were insufficiently specific, for example giving spurious cross-reactivities with non-toxic maize (Troncone et al., 1986). Later, advantage was taken of monoclonal antibody technology to produce more precisely targeted reagents (Freedman et al., 1987). The first toxic sequence to be identified in vivo was A-gliadin 31–49 (Sturgess et al., 1994). We generated a monoclonal antibody to this epitope, and produced a highly sensitive sandwich assay (Ellis et al., 1998), and recently a competition format assay for wheat gliadin, using the same antibody, which allows the detection of partial hydrolysates of gliadin and cooked foods, see below (Bermudo-Redondo et al., 2005).

Use of a single monoclonal antibody may result in an antibody that is too specific for wheat, and misses other toxic cereals. As we have seen, some wheat varieties may be low or even completely devoid of certain epitopes; thus, use of a single monoclonal antibody could give false results.

In addition to the problem of specificity, there remains the problem of extraction. While monomeric prolamins are soluble in dilute ethanol, which once diluted to, say, 10% does not adversely affect immunoassays, polymeric or HMW prolamins need to be reduced. Cooked gliadins form polymers through the formation of inter-chain disulphide bonds and then themselves become insoluble in alcohol. While reducing agents can overcome the solubility problems in both these instances, they may be incompatible with the system, breaking inter-chain bonds within the antibodies used in immunoassays.

Partial hydrolysates, such as beers and malts, pose another problem. Gluten that has been enzymatically digested comprises a mix of polypeptides of various sizes. In conventional sandwich ELISA, comprising a capture antibody and a detection antibody, for example that of Ellis et al. (1998), small peptides may bind to the capture layer, but having no further appropriate epitopes to react with the detection antibody, effectively forms a blocking layer. This particular problem is overcome by using a competition assay format. In the latter, the sample or standard and the detection antibody are pre-incubated, and then the mix allowed to compete for binding with immobilised antigen on the plate. The problem of blocking is thus overcome.

Two assay formats are available commercially. The first is based on a monoclonal antibody to the heat stable omega gliadin fraction (Skerritt and Hill, 1991). This kit allows gliadin in cooked foods to be measured quantitatively. The lack of sulfur in this gliadin subfraction means that inter-chain disulfide bonds do not form during heating; thus, dilute ethanol can be used as the extractant without recourse to reducing agents. However, omega gliadins comprise a minor part of the gliadin fraction, and are most susceptible to variation of growing conditions of the wheat. Omega gliadins comprise between 6% and 20% of total gliadin, depending on the variety and environmental factors; extrapolating from omega to total gliadins can lead to errors of between −44% and +80% (Wieser et al., 1994). Additionally, the Skerritt system does not measure the glutenins. Valdes et al. (2003) described a patented extraction buffer suitable for use with cooked foods and an assay based on an anti-rye antibody (R5) which recognises wheat gliadin and related proteins in rye and barley, in equal proportions.

The assay is based on the epitope QQPFP, but has a number of cross-reactivities, such that it can also detect the immunodominant gliadin sequence, albeit rather weakly. This assay is clearly a useful step in the right direction but suffers from the following draw backs a) it does not detect high molecular weight glutenins, or oats b) it does not detect the immunodominant gliadin epitope for the 5% of patients who are HLA-DQ8 positive (Mazzarella et al., 2003), and hydrolysates are not quantitatively detected in the sandwich ELISA format of the commercially available kit, although a competitive ELISA, using the same antibody, has been described (Ferre et al., 2004). The assay therefore addresses some, but not all, of the problems.

It was previously thought that the total gluten content of a foodstuff could be estimated by multiplying the gliadin content by two. Indeed, this system is still universally applied. However, HPLC studies of wheat starches derived from different cultivars and differently processed revealed that the ratio of the amount of glutenin to gliadin varies between 0.2x and 5.8x (Wieser and Seilmeier, 2003). Thus, separate assays will be required for gliadin and glutenins, particularly now that the HMW glutenins are understood to be toxic (Dewar et al., 2006).

It appears likely that the way forward will be to raise antibodies to a number of defined toxic gluten epitopes, including the glutenins, and use them in a cocktail format. Spaenij-Dekking et al. (2005) has raised monoclonal antibodies to a number of gluten epitopes including the immunodominant epitope, in order to address this problem, and has developed separate ELISAs using some of them. We have used our own antibody to the immunodominant epitope and shown that beers and malt are heavily laden with a cross-reacting barley epitope (Ciclitira, 2007); we have also produced antibodies to HMW-GS (Ellis 2007). At present there is no universal standard glutenin that could be used in cocktail assays, and the gliadin standard is contaminated with HMW-GS. Should a cocktail ELISA be performed using this standard, incorrect results might be obtained.

The problem of oats remains. It may be necessary to devise methods for testing oats products for contamination with wheat rye or barley, whilst recognising that a minority of patients are sensitive to oats themselves. Separate assays may be required for detection of oats.

3.5 CONCLUSION

Gluten comprises a highly complex group of proteins. While great steps have been taken in the last 50 years in identifying the coeliac-toxic elements within this group, all available methods of measurement of toxicity fall short of the ideal. Appropriate assays and reference materials are required, together with a consensus view on toxicity thresholds.

ACKNOWLEDGEMENTS

The authors would like to thank the following for financial support:

The Commission of the European Communities specific RTD programme ‘Quality of Life and Management of Living Resources’ QLKI-CT-2002–02077 ‘Quantification of Coeliac Disease toxic gluten in foodstuffs using a chip system with integrated Extraction, Fluidics, and Biosensoric detection’. This paper does not necessarily reflect its views and in no way anticipates the Commission’s future policy in this area.

The German Federal Ministry of Education and Research (project no. 0312246 D).

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4 The Nutritional Quality of Gluten-Free Foods

Tricia Thompson

4.1 INTRODUCTION

Celiac disease is characterized by a permanent sensitivity to certain sequences of amino acids found in the prolamin fraction of the grains wheat, barley and rye. As a consequence, persons with celiac disease must adhere to what is commonly referred to as a gluten-free diet. This diet involves the strict avoidance of proteins from the offending grains.

A gluten-free diet primarily affects food consumption from the grain food group. In place of wheat, barley and rye-based foods, persons adhering to a gluten-free diet must consume foods made from gluten-free grains, including rice, corn, sorghum, millet, teff, amaranth, buckwheat, quinoa, wild rice and oats (in countries that allow their use). Because most mainstream breads, baked goods and pasta products are wheat based, and many ready-to-eat breakfast cereals are either wheat based or comprised of other gluten-containing ingredients (e.g. barley malt), persons with celiac disease must use gluten-free substitutes for these foods.

There are a wide variety of specially manufactured gluten-free cereal foods, including breads, pastas and breakfast cereals available to persons with celiac disease. However, questions have been raised about the nutritional quality of these foods (Sabry and Okada, 1992; Thompson, 1999, 2000), as well as the nutritional adequacy of the gluten-free diet (Sabry and Okada, 1992; Mariani et al., 1998; Bardella et al., 2000; Grehn et al., 2001; Thompson et al., 2005; Hopman et al., 2006). Gluten-free cereal foods generally are not enriched and frequently are made with refined flour and/or starch. As a result, they may not provide the same nutritional value as wheat-based foods, especially if the wheat-based foods are whole grain or enriched. In addition, because a gluten-free diet impacts food consumption from the grain food group, there are concerns about the effects of a gluten-free diet on the intake of carbohydrates and grain foods. Also, because grain foods are sources of fiber, B vitamins and iron, there are concerns about the effects of a gluten-free diet on intakes of these nutrients.

4.2 GLUTEN-FREE CEREAL FOODS

Only a few studies have been conducted on the nutritional quality of gluten-free foods. One study (Thompson, 1999) assessed the thiamin, riboflavin and niacin contents of gluten-free cereal foods to determine how they compared nutritionally to the enriched gluten-containing products they were intended to replace. Of the 64 gluten-free products assessed for nutrient content, 39 contained lower amounts of thiamin, riboflavin and niacin than their enriched wheat-containing counterparts. Fourteen gluten-free products contained lower amounts of two of the nutrients and six contained lower amounts of one nutrient. The enrichment status

of 368 gluten-free rice flours, breads (ready-made products and mixes), pastas and ready-to-eat breakfast cereals was also assessed. Only 35 of these products were enriched, including 5 of 95 bread mixes, 26 of 157 ready-made bread products and 4 of 21 ready-to-eat breakfast cereals. None of the 15 varieties of rice flour or 80 pasta products reviewed for enrichment status were enriched. The ingredients of 268 gluten-free breads, pastas and ready-to-eat breakfast cereals were also reviewed. Of these, 196 listed a refined grain or starch as the first ingredient. Based on these findings, it was concluded that many gluten-free cereal products do not provide the same levels of thiamin, riboflavin or niacin as their enriched wheat-based counterparts. It was recommended that persons with celiac disease should be advised to consume more nutrient dense gluten-free cereal foods in the form of whole grains or enriched products, as well as increase their intake of non-cereal foods that are good sources of thiamin, riboflavin and niacin. This chapter also pointed out the relative lack of nutritional information for gluten-free foods in nutrient databases, as well as the lack of data available from food manufacturers on the thiamin, riboflavin and niacin contents of their products.

Another study (Thompson, 2000) assessed the folate, iron and dietary fiber contents of gluten-free flours, breads, pastas and ready-to-eat breakfast cereals to determine how they compared nutritionally to the enriched gluten-containing products they were intended to replace. Of the 37 gluten-free cereal products assessed for folate, 30 contained lower amounts compared to their gluten-containing counterpart. Of the 83 gluten-free cereal products assessed for iron content, 64 contained lower amounts compared to their gluten-containing counterpart. Of the 85 gluten-free products assessed for dietary fiber, 26 contained lower amounts compared to their gluten-containing counterpart. The enrichment status of 58 gluten-free breads, pastas and ready-to-eat breakfast cereals was also assessed. Only three breakfast cereals were fortified with folic acid. None of the pastas or bread products was fortified with folic acid. Only nine products were enriched with iron, including seven bread products and two breakfast cereals. No pasta products were enriched with iron. Based on these findings, it was concluded that gluten-free cereal products generally provide lower amounts of folate and iron than their enriched gluten-containing counterparts. It was recommended that persons with celiac disease should be counseled on the nutritional adequacy of various gluten-free foods and encouraged to consume whole-grain or enriched products whenever available. In addition, it was recommended that manufacturers of gluten-free foods should be encouraged to improve the nutritional quality of their products. This chapter also pointed out the lack of information on the folate content of gluten-free foods. While dietary fiber and iron content are required to be included on the labels of foods sold in the US, folate content is not.

Findings from studies on the nutritional quality of gluten-free cereal foods suggest that many of these products are nutritionally inferior to the wheat-based foods they are intended to replace. This appears to be due in large part to the use of refined grains or starches and the relative lack of vitamin and mineral enrichment (for the nutritional content of various forms of gluten-free cereal foods, see Table 4.1). For example, ‘macaroni and cheese’ is a very popular food among children in the US. A popular mainstream brand made with the traditional wheat-based pasta is enriched with thiamin, riboflavin, niacin, folic acid and iron. A 70-g serving contains 15% of the Daily Value for iron (values for the other nutrients are not provided). Comparatively, a popular gluten-free brand is made with white rice pasta and is not enriched. An 85-g serving contains 0% of the Daily Value for iron.

According to the Codex Standard for Gluten-Free Foods, gluten-free foods that are dietary staples (e.g. flour, bread) should contain approximately the same amount of vitamins and minerals as the foods they are supposed to replace (Joint FAO/WHO Food Standards Program, Codex Alimentarius Commission, 1994). In reality however, this often is not the

Table 4.1 Nutritional content of various forms of gluten-free cereal foods.

Cereal food (100 g)	Dietary fiber (g)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Folate (DFE)	Iron (mg)
Whole-grain brown rice	3.5	0.40	0.09	5.09	20	1.47
White rice, enriched	1.3	0.58	0.05	4.19	387	4.31
White rice, unenriched	1.3	0.07	0.05	1.60	8	0.80
Rice flour, brown	4.6	0.44	0.08	6.34	16	1.98
Rice flour, white	2.4	0.14	0.02	2.59	4	0.35
Corn flour, masa, enriched	9.6	1.43	0.75	9.84	380	7.21
Corn flour, degermed, unenriched	1.9	0.07	0.06	2.66	48	0.91
Corn starch	0.9	0.00	0.00	0.00	0	0.47
Whole-grain cornmeal	7.3	0.39	0.20	3.63	25	3.45
Cornmeal, degermed, enriched	4.0	0.61	0.42	5.31	345	4.32
Cornmeal, degermed, unenriched	4.0	0.14	0.05	1.00	30	1.10

Source of nutritional information: US Department of Agriculture, Agricultural Research Service (2008).

case. Common wheat-based cereal foods – whole-grain and enriched – are good sources of carbohydrate, fiber (whole-grain products), B vitamins (e.g. thiamin, riboflavin, niacin, folate) and the mineral iron. In many countries, including the US, Canada and those in the UK, refined wheat flour (and in some cases refined wheat flour-based products) is enriched with B vitamins and iron. For example, in the UK, there is mandatory enrichment of refined wheat flour with iron, thiamin, niacin, and calcium and voluntary fortification of breakfast cereals with some nutrients, including iron and calcium (Food Standards Agency, Expert Group on Vitamins and Minerals, 2003). Similarly, in Canada, there is mandatory enrichment of refined wheat flour with thiamin, riboflavin, niacin, folic acid and iron (Health Canada, Food and Drug Regulations, 1998). In addition, there is voluntary enrichment of bread, pasta and breakfast cereal with B vitamins and iron. In the US, there is voluntary enrichment of refined wheat flour and refined wheat-based bread and pasta with thiamin, riboflavin, niacin, folate and iron (United States Food and Drug Administration, Code of Federal Regulations, 2006). In addition, most ready-to-eat breakfast cereal in the US is voluntarily fortified with vitamins and minerals. In contrast, while there are no government standards preventing the voluntary enrichment of gluten-free cereal foods, there are no standards mandating their enrichment either. Unfortunately, the vast majority of manufacturers of gluten-free cereal foods chose not to voluntarily enrich their products. As a consequence, most specially formulated gluten-free cereal foods are not enriched.

Manufacturers of gluten-free cereal foods have a wide variety of gluten-free whole grains to work with, including millet, teff, wild rice, sorghum, brown rice, whole-grain corn, buckwheat, amaranth, quinoa and oats (in countries that allow their use). For the most part, these grains are all good sources of fiber, iron and some B vitamins (for the nutritional content of gluten-free grains, see Table 4.2). While an increasing number of North American manufacturers of gluten-free cereal foods are incorporating these grains into their products, the availability of whole-grain gluten-free cereal products is relatively limited. Gluten-free breakfast cereals are one of the most likely places to find whole grains. Whole-grain gluten-free cereals made from buckwheat, brown rice and amaranth are available from several manufacturers, including Bob's Red Mill (Milwaukie, OR), Nu-World Amaranth (Naperville, IL), The Birkett Mills (Penn Yan, NY), US Mills (Needham, MA), Lundberg Family Farms (Richvale, CA), Barbara's Bakery (Petaluma, CA) and Nature's Path Foods (Richmond, BC, Canada). There are also a limited number of whole-grain gluten-free bread products.

Table 4.2 Nutritional content of gluten-free uncooked grains.

Grain	Amount	Fiber (g)	Iron (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Folate (DFE)
Amaranth[a]	$\frac{1}{4}$ c (48.0 g)	3.2	3.67	0.06	0.10	0.45	40
Brown rice, long-grain[a]	$\frac{1}{4}$ c (46.0 g)	1.6	0.68	0.19	0.04	2.36	9
Buckwheat[a]	$\frac{1}{4}$ c (43.0 g)	4.2	0.94	0.04	0.18	2.98	13
Millet[a]	$\frac{1}{4}$ c (50.0 g)	4.2	1.50	0.21	0.15	2.36	42
Oats[a]	$\frac{1}{4}$ c (39.0 g)	4.1	1.84	0.30	0.05	0.38	22
Quinoa[a]	$\frac{1}{4}$ c (43.0 g)	3.0	1.94	0.15	0.14	0.65	78
Sorghum[a]	$\frac{1}{4}$ c (48.0 g)	3.0	2.11	0.11	0.07	1.41	n/a
Teff[b]	$\frac{1}{4}$ c (45.0 g)	6.0	3.60	0.15	0.03	0.80	n/a
Wild rice[a]	$\frac{1}{4}$ c (40.0 g)	2.5	0.78	0.05	0.11	2.69	38

[a] US Department of Agriculture, Agricultural Research Service (2008).
[b] The Teff Company; available at: www.teffco.com.

Whole-grain bread products (both ready-to-eat and mixes) made from amaranth, brown rice and sorghum are available from a few manufacturers, including Nu-World Amaranth (Naperville, IL), Authentic Foods (Gardena, CA), Gluten-Free Pantry (Glastonbury, CT), Fearn Natural Foods (Mequon, WI), Van's International (Vernon, CA) and Sylvan Border Farm (Willits, CA). In addition, whole-grain gluten-free pasta products made from brown rice and quinoa are available from a few manufacturers, including Lundberg Family Farms (Richvale, CA), Quinoa Corporation (Gardena, CA), Eden Foods (Clinton, MI) and Food Directions (Scarborough, ON, Canada).

Some manufacturers of gluten-free cereal foods may simply prefer to make their products from refined flour and/or starches. If this is the case, it is important from the standpoint of nutritional quality that these products be enriched to the same extent that refined wheat-based products are enriched. Regulations regarding the enrichment of wheat-based cereal foods vary by country, and by extension, the enrichment of gluten-free cereal foods will vary as well. However, any enrichment of refined gluten-free cereal products is better than no enrichment. In North America, the number of manufacturers of gluten-free foods enriching their products is slowly increasing. There are at least four manufacturers of enriched gluten-free bread products (e.g. Ener-G Foods, Seattle, WA; Enjoy Life Natural Brands, Schiller Park, IL; Glutino, Canada; Kinnikinnick, Edmonton, Alberta, Canada) and two manufacturers of enriched gluten-free breakfast cereals (Enjoy Life Natural Brands, Schiller Park, IL; General Mills, Minneapolis, MN). Enriched gluten-free pasta products remain relatively rare with only one manufacturer (Maple Grove Gluten Free Foods, Fontana, California) currently producing this product.

4.3 THE GLUTEN-FREE DIET

There have been only a handful of studies conducted on the nutritional adequacy of the gluten-free diet. One study conducted in Canada (Sabry and Okada, 1992) assessed nutrient intakes using 7-day food records of 26 adults who had followed a gluten-free diet for at least 1 year. Researchers found that with the exception of iron intake by premenopausal

women, mean intakes by men and women of all vitamins and minerals assessed exceeded the Recommended Nutrient Intakes for Canadians. However, when compared to the diets of a group of healthy premenopausal women, women in the study group had lower mean intakes of calcium, iron, thiamin and riboflavin. Researchers also assessed energy contribution of the five food groups (i.e. grain products, fruits and vegetables, milk and milk products, meat and alternatives, and miscellaneous: fats, oils, sugars, sweets, condiments, beverages) to overall caloric intake. Among the study participants, grains contributed an average of 18% to overall energy intake compared to 23–31% among the general adult population. Fruits and vegetables contributed an average of 21% to overall energy compared to 11–17% for the general adult population. Amounts consumed by study participants of the other food groups were within the range consumed by the general adult population. The researchers concluded that a gluten-free diet can meet the nutrient recommendations for healthy adults with the exception of iron in premenopausal women. However, they reported that there is some risk of inadequate intakes of calcium, thiamin, iron and riboflavin among persons with celiac disease. Researchers also noted that an increase in fruits and vegetables rather than an increase in animal foods compensated for the decrease in grain foods. It was recommended that persons with celiac disease receive guidance on their diet particularly as it concerns sources of calcium and vitamin D if lactose intolerance is present, and thiamin, riboflavin and iron if energy intake is low.

A study (Mariani et al., 1998) conducted in Italy assessed nutrient intakes using 3-day food records of 25 adolescents who followed a strict gluten-free diet. Mean intakes of iron, calcium and fiber were less than the Recommended Daily Nutrients, Italy (LARN). Mean percent daily calories from carbohydrates were also below recommendations, while mean percent daily calories from fat and protein were above recommendations. Specifically, female study participants adhering to a strict gluten-free diet had a mean (standard deviation) intake of iron of 13.2 mg (+/− 4.7); for males it was 10.8 mg (+/− 3.8). The LARN for iron for females and males is 18 and 12 mg, respectively. Study participants adhering to a strict gluten-free diet had a mean (standard deviation) intake of calcium of 850 mg (+/− 439). The LARN for calcium is 1200 mg. Study participants adhering to a strict gluten-free diet had a mean (standard deviation) intake of fiber of 8.5 g (+/− 3.3). The LARN for fiber is 30 g. The mean percentage contribution of carbohydrates to total energy intake was 43.2%. The LARN for carbohydrates is 55–65%. Mean carbohydrate intake was also significantly lower among patients with celiac disease compared to the control group of healthy adolescents. Adolescents adhering to a strict gluten-free diet also consumed less pizza, bread and pasta and more protein foods. Researchers concluded that strict adherence to a gluten-free diet may be a nutritional risk factor for adolescents due to decreases in carbohydrate consumption and increases in protein and fat consumption. It was further stated that among this study group when certain carbohydrates were removed from the diet (e.g. bread), they were not replaced with other carbohydrates (e.g. rice) but instead were replaced with foods high in protein and/or fat. It was recommended that more attention be placed on the nutritional balance of the gluten-free diet.

In another study (Bardella et al., 2000) conducted in Italy, the diets of 71 persons aged 17–58 who strictly adhered to a gluten-free diet were assessed for calorie and macronutrient content using 3-day food records. Both male and female study participants consumed significantly less total calories, significantly lower percentages of calories from carbohydrates and significantly higher percentages of calories from fat as compared to control groups of healthy men and women. As with the study by Mariani and colleagues, persons with celiac disease ate less pasta, bread and pizza, and more eggs, meat and

cheese than control subjects. Researchers concluded that among their study participants, the gluten-free diet was unbalanced due to decreased carbohydrate consumption and increased fat consumption. It was recommended that persons with celiac disease receive help on proper food choices and information on the composition of foods to prevent malnutrition.

A study (Grehn et al., 2001) conducted in Sweden assessed the nutrient intakes using 4-day food records of 49 adults who had strictly adhered to a long-term gluten-free diet of 8–12 years. Female persons with celiac disease had significantly lower intakes of fiber, niacin equivalents, vitamin B12, calcium, phosphorus and zinc as compared to the control group from the general population. Male persons with celiac disease had significantly lower intakes of fiber, vitamin B12, folate and phosphorus as compared to the control group. Researchers also found that energy, protein, fat and carbohydrate intakes did not differ significantly between persons with celiac disease and controls. As compared to Nordic Nutrition Recommendations (NNR), 100% of participants with celiac disease had fiber intake levels below recommendations. The mean (standard deviation) fiber intake for female patients with celiac disease was 11.5 g (+/−4.3); for males it was 10.0 g (+/−3.3). The NNR level for fiber is 25–30 g. In addition, 97% of female patients with celiac disease and 100% of males had folate intake levels below recommendations. The mean (standard deviation) folate intake for female patients with celiac disease was 186 μg (+/− 54.9); for males, it was 172 μg (+/− 55.8). The NNR level for folate is 300 μg. In addition, the percentage of energy provided by carbohydrates was lower and for fat higher than NNR. Researchers concluded that the gluten-free diet as followed by adults in Sweden is nutritionally unbalanced. It was recommended that persons with celiac disease receive regular dietary counseling.

A study (Thompson et al., 2005) conducted in the US assessed the nutrient intakes of 47 adults with celiac disease who strictly adhered to a gluten-free diet using 3-day food records. The group mean (standard deviation) daily percent contribution of carbohydrates to overall energy intake was 55% for men and 52% for women. In the US, the Acceptable Macronutrient Distribution Range for carbohydrate is 45–65% of calories. Eighty-eight percent of men and 87% of women had carbohydrate intakes within the recommendations. The group mean (standard deviation) daily intake of dietary fiber was 24.3 g (5.3) for men and 20.2 g (6.9) for women. The recommended daily intake of dietary fiber in the US is 20–35 g. Eighty-eight percent of males and 46% of females had dietary fiber intakes that met or exceeded recommendations. The group mean (standard deviation) daily intake of iron was 14.7 mg (5.9) for men and 11.0 mg (3.5) for women. The US Daily Recommended Intake for iron is 8 and 18 mg depending upon age and gender. All men and 44% of women had iron intakes that met or exceeded recommendations. The group mean (standard deviation) daily intake of calcium was 1288.8 mg (670.2) for men and 884.7 mg (371.8) for women. The US Daily Recommended Intake for calcium is 1000 or 1200 mg depending upon gender and age. Sixty-three percent of men and 31% of females had calcium intakes that met or exceeded recommendations. The group mean (standard deviation) daily intake of grain food servings was 6.6 (2.5) for men and 4.6 (2.4) for women. Sixty-three percent of men and 21% of women consumed at least the minimum number of six servings from the grain food group (see Table 4.3 for a breakdown of the average daily consumption of grain foods by study participants over the 3-day recording period). Researchers concluded that emphasis should be placed on the nutritional quality of the gluten-free diet, particularly as it concerns the iron, calcium and fiber intake of women. It was recommended that persons with celiac disease increase the number of servings from the grains food group up to recommended levels, making sure that these foods were either whole grain or enriched. It was also advised that

Table 4.3 Average daily consumption of grain foods by study participants ($n = 47$) over 3-day recording period (Thompson et al., 2005).

Type of gluten-free grain food	Average daily number of servings
Sandwich breads, rolls, bagels, English muffins, pizza crust	46.5
Ready-to-eat breakfast cereal	35.8
Savory snacks	32.8
Quick breads, donuts, muffins	25.3
Rice	25.0
Corn tortillas	15.7
Sweet snacks	13.7
Waffles, pancakes	12.3
Pasta	10.7
Rice cakes	9.5
Hot cereal	8.3

the recommended number of servings from the milk food group (or non-dairy equivalents) should be consumed.

A study (Hopman et al., 2006) conducted in The Netherlands assessed the nutrient intakes of 111 persons with celiac disease aged 12–25 using 3-day food records and questionnaires. Mean fiber and iron intakes were significantly lower than Dutch recommendations (DRDA). Mean percentage energy intake from saturated fat was significantly higher than the DRDA. Mean intakes of calcium, thiamin, riboflavin and B6 met or exceeded the DRDAs. Compared to the general Dutch population, some age groups of persons with celiac disease had significantly lower intakes of fiber, iron, protein and saturated fat. Sixty-four percent of study participants reported using vitamin and mineral enriched gluten-free food products. Those participants who used enriched products had a significantly higher intake of thiamin, riboflavin, B6, calcium, iron and fiber than those who did not. Researchers concluded that nutrient intake among this group of persons with celiac disease was inadequate, although similar to the general Dutch population for this age group. It was recommended that attention should be focused on the saturated fat, fiber and iron intakes of young persons with celiac disease.

Findings from studies on the nutritional adequacy of the gluten-free diet suggest that adherence to a gluten-free diet may be a risk factor for inadequate intakes of certain nutrients, including fiber, folate and iron. These studies also suggest that carbohydrate intake and grain food consumption may be decreased in persons with celiac disease as compared to the population at large. This decreased consumption of carbohydrates and grain foods may be an important contributing factor toward inadequate intakes of fiber, folate and iron among persons with celiac disease. In the US, for example, cereal foods contribute a large percentage to an adult's daily intake of several nutrients, including fiber, folate and iron (Cotton et al., 2004). According to the United States Department of Agriculture's 1994 to 1996 Continuing Survey of Food Intakes by Individuals, yeast bread, ready-to-eat breakfast cereal, pasta, cakes/cookies/quick breads/doughnuts and flour/baking ingredients contribute 30.0% to the US adult daily intake of fiber. Ready-to-eat breakfast cereal, yeast bread, cakes/cookies/quick breads/doughnuts, pasta, flour/baking ingredients and hot breakfast cereal contribute 43.5% to the US adult daily intake of iron. In addition, ready-to-eat breakfast cereal and yeast bread contribute 25.6% to the US adult daily intake of folate. Similarly, in Great Britain, cereal and cereal products contribute significantly to an adult's daily intake of fiber, iron and folate (Hoare et al., 2004). According to the National Diet and Nutrition Survey, cereal and cereal

products were the main sources of dietary fiber for British adults aged 19–64 providing 42% of their daily intake, with bread products contributing 20% and breakfast cereal contributing 13%. Similarly, cereal and cereal products provide 44% of the British adult daily intake of iron, with breakfast cereal contributing 20% and white bread contributing 9%. Likewise, cereal and cereal products provide 33% of the British adult daily intake of folate, with breakfast cereal contributing 15%. Consequently, not consuming adequate amounts of grain foods could have a profound impact on intakes of fiber, iron and folate.

Little information is available as to why persons with celiac disease may consume lower amounts of grain foods in general or choose certain gluten-free cereal foods over others, although poor palatability of gluten-free foods is mentioned frequently by researchers (Björkman et al., 1985; Mariani et al., 1998; Bardella et al., 2000). This observation seems to be particularly true for bread products (Björkman et al., 1985; Bardella et al., 2000). Therefore, the challenge for manufacturers of gluten-free cereal foods is to develop bread, pasta and breakfast cereal that are both nutritious and palatable.

4.4 EDUCATIONAL NEEDS

Several steps should be taken to improve both the nutritional quality of gluten-free cereal foods and the nutritional quality of the gluten-free diet. Of primary importance is the education of healthcare professionals. Physicians and dietitians should be advised on the variation in nutritional quality that exists among gluten-free cereal foods depending upon whether they are whole grain, enriched, or refined and unenriched. They also should be advised on the nutrients that may be at risk due to adherence to a gluten-free diet, including iron, fiber, folate and grain foods.

Physicians and dietitians should educate their patients on how to choose nutrient dense gluten-free cereal foods. Patients should be advised to choose whole-grain gluten-free cereal foods whenever possible. They also should be advised to choose enriched gluten-free cereal foods over refined, unenriched products when available. In addition, they should be advised to consume the recommended number of servings from the grain food group.

Manufacturers of gluten-free cereal foods should be encouraged to improve the nutritional quality of the foods they produce. Improvements could be in the form of increased use of whole grains or through the enrichment of refined products. If a product is either whole grain or enriched, this fact should be highlighted on the product's label. Manufacturers also should promote the health benefits of their whole-grain and/or enriched products to gluten-free consumers. If consumers were made aware of the benefits of more nutrient dense foods both from manufacturers and healthcare providers, this increased knowledge hopefully would translate into greater market demand for such products.

4.5 RESEARCH NEEDS

Additional large-scale studies are needed on the nutritional adequacy of the gluten-free diet. In addition to country-specific studies, studies comparing the nutritional adequacy between countries are needed. This would help determine whether the nutritional quality of the diet varies depending on the specific type of gluten-free diet followed. For example, some countries allow the use of wheat–starch-based gluten-free products as well as oats; others do not.

To facilitate such research, it is also important to develop a nutrient database of gluten-free foods. In the US, for example, available nutrient databases contain information on only a very small number of gluten-free products. In addition, food manufacturers usually analyze their products only for those nutrients that must be listed on the food label. This lack of nutritional information severely restricts the nutrients that can be accurately assessed.

Market research is also needed on patient preferences regarding gluten-free products. For example, do persons with celiac disease choose whole-grain or enriched gluten-free products when they are available? Do they prefer naturally gluten-free foods such as rice, quinoa or corn tortillas to specially manufactured gluten-free products (e.g. pasta, bread, breakfast cereal)? If they consume specially manufactured gluten-free foods, which products and brands do they prefer and why? Having answers to these questions would help manufacturers in the development and improvement of gluten-free products.

4.6 SUMMARY

Gluten-free cereal foods and the gluten-free diet can and should be nutrient dense and healthy. Whole-grain and enriched breakfast cereals, bread products and pastas should be made readily available to persons with celiac disease in palatable, convenient forms. In the US, it is recommended that at least half of a person's grain food servings be in the form of whole grains (United States Department of Health and Human Services, United States Department of Agriculture, 2005). The remaining servings should be either whole grain or enriched. From a nutritional standpoint, there is no reason why persons with celiac disease should not be able to comply with these recommendations. Gluten-free grains include an abundance of healthy whole-grain options, including buckwheat, brown rice, corn, amaranth, quinoa, teff, millet, wild rice, sorghum and oats. In addition, there is nothing to prevent refined gluten-free cereal products from being enriched to the same extent as refined wheat-based foods. Increased availability of palatable whole and enriched gluten-free grain foods would go a long way toward ensuring that persons with celiac disease consumed a nutritionally adequate diet.

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5 Gluten-Free Doughs: Rheological Properties, Testing Procedures – Methods and Potential Problems

Athina Lazaridou and Costas G. Biliaderis

5.1 INTRODUCTION

The rheological behavior of dough is a significant determinant of its performance during mechanical handling (mixing and sheeting), fermentation and baking (Bloksma, 1990) as well as of the quality of the end product for baked items such as bread, cookies, biscuits, pasta and noodles. The main techniques used for measuring dough rheological properties are divided into descriptive or empirical techniques and basic or fundamental measurements (Weipert, 1992). Several rheological techniques, including oscillation, creep and creep recovery, lubricated compression, stress relaxation, adhesion, uniaxial and biaxial extension measurements have been used in many studies for probing the fundamental mechanical properties of gluten (Janssen et al., 1996a; Lee and Mulvaney, 2003) and wheat doughs (Launay, 1990; Weipert, 1990; Hoseney and Chen, 1994; Menjivar and Faridi, 1994; Janssen et al., 1996b; Morgenstern et al., 1996; Baltsavias et al., 1997; Dobraszczyk, 1997, 1999; Phan-Thien and Safari-Ardi, 1998; Safari-Ardi and Phan-Thien, 1998; Edwards et al., 1999, 2001, 2003; Bartolucci and Launay, 2000), as well as for establishing relations between those properties and quality attributes of the end product (Janssen et al., 1996b; Miller and Hoseney, 1997; Kieffer et al., 1998; Maache-Rezzoug et al., 1998a,b; Autio et al., 2001; Carson and Sun, 2001; Wang and Sun, 2002; Dobraszczyk and Morgenstern, 2003; Uthayakumaran and Lukow, 2003).

With the increasing incidence in celiac disease, or other allergic reactions/intolerances to gluten consumption, there is a rising demand for gluten-free products (Gallagher et al., 2004). The replacement of gluten presents a major technological challenge, as it is an essential structure-building protein, largely contributing to the appearance and crumb structure of many baked products. The gluten matrix is indeed a major determinant of the important rheological characteristics of flour doughs, such as elasticity, extensibility, resistance to stretch, mixing tolerance and gas holding ability (Schofield, 1986). In this respect, gluten-free formulations are made by incorporating ingredients such as starches, egg and dairy proteins and hydrocolloids into a gluten-free flour base (e.g. rice and corn flour) that could mimic the viscoelastic properties of gluten and result in improved structure, mouthfeel, acceptability and shelf life of these products (Ylimaki et al., 1991; Haque and Morris, 1994; Toufeili et al., 1994; Schober et al., 2003, 2005; Gallagher et al., 2003a,b, 2004; Gujral et al., 2003a; Kobylanski et al., 2004; Moore et al., 2004, 2006; Sivaramakrishnan et al., 2004; Ahlborn et al., 2005; McCarthy et al., 2005; Lazaridou et al., 2007). However, the use of empirical and fundamental mechanical measurements in studies of gluten-free dough properties has been limited (Defloor et al., 1991; Haque and Morris, 1994; Sanchez et al., 2002; Schober et al., 2003, 2005; Gujral et al., 2003a; Kobylanski et al., 2004; Moore et al., 2004; Sivaramakrishnan et al., 2004; Lazaridou et al., 2007).

5.2 DOUGH RHEOLOGICAL METHODS

The production of baked goods is a complex process involving several steps from mixing, molding, sheeting, proofing, to final baking. Dough rheological properties change considerably during each of these steps, which have a characteristic time scale, strain and strain rate (Walker and Hazelton, 1996; Dobraszczyk, 1997). Extreme shear deformation takes place during the initial mixing stage (shear rate 70 s^{-1}), and various additional degrees of deformation occur when the dough is molded (shear and extension, 30 s^{-1}) and sheeted (extension, 10 s^{-1}), proofed (biaxial extension, 10^{-3}–10^{-4} s^{-1}) and baked (biaxial extension, 10^{-2}–10^{-3} s^{-1}). Dough rheological tests are often designed to allow prediction of the quality of the finished product by the actual simulation of the manufacturing steps. Dough is a viscoelastic material with explicit shear thinning and thixotropic behavior (Faubion and Hoseney, 1990; Weipert, 1990; Walker and Hazelton, 1996). Moreover, the rheological behavior of dough is nonlinear, that is, the rheological parameters are strain and strain rate dependent (Bloksma, 1990). Therefore, the most accurate measurements of a baking process can be attained at levels of strain and strain rate similar to the actual conditions encountered during processing. The development of a rheological method may thus include prediction of the range of conditions the dough experiences, performance of rheological measurements under simple, well-defined laboratory conditions and processing of the data via mathematical modeling (Dobraszczyk and Morgenstern, 2003).

5.2.1 Empirical tests

Empirical or descriptive mechanical testing of doughs have had a rapid and widespread acceptance in baking industry as used for evaluation of flour quality and functionality (Menjivar, 1990; Weipert, 1992; Dobraszczyk and Morgenstern, 2003). Numerous advantages can be cited for these kinds of tests, such as they are relative easy and fast to perform, substantial experience and knowledge on their use and interpretation have been developed over many years, the instruments used are generally inexpensive, rugged and capable of withstanding demanding factory environments as well as not requiring highly skilled or technically trained personnel. However, these instruments do not fulfill the requirements of a fundamental rheological test, since the sample geometry is variable and not well defined, the stress and strain states are uncontrolled, complex and non-uniform.

The most commonly used empirical dough testing instruments can be divided into two groups, the continuous recording dough mixers, such as the Brabender farinograph and the mixograph, and the load-extension instruments, such as the Brabender extensograph and the Chopin alveograph.

The farinograph and mixograph are torque-measuring devices recording the power that is required to mix a dough at constant speed, but they differ slightly in their mixing action; the farinograph provides a gently kneading type of mixing, while the mixograph uses a harsher pin mixing method (Spies, 1990; Menjivar and Faridi, 1994; Walker and Hazelton, 1996). Both instruments are used to predict dough water absorption and the magnitude of work input for dough development by recording the resistance of the dough to the mixing blades during prolonged mixing. The area under the recorded curves is proportional to the energy required for mixing. The most common information obtained from these curves is the development time of flour, its tolerance to overmixing and optimum water absorption. The time for dough to reach the point of minimum mobility (optimum mix) is called the mixing

or peak time. In farinograph the height of the middle of the band, referred to as consistency, is expressed in Brabender units (BU). Useful parameters for a flour–water system derived from the farinograph curve are the optimum water absorption (the amount of water required to center the peak of the curve on the 500 BU line), the dough development time (DDT, time to reach 500 BU consistency) and the elasticity (bandwidth of the curve at the maximum consistency). Moreover, with a farinograph the tolerance of flour to overmixing is measured by the departure time (time at which the curve drops below the 500 BU), the mixing tolerance index (consistency difference between the height at peak and the value at 5 min later) and the stability or tolerance (time during which the dough consistency is at 500 BU). With a mixograph, the tolerance of flour can be seen in the weakening angle, the area under the curve, the height of the curve at a specific time after the peak, and the angle between the ascending and descending parts of the curve.

The two load-extension instruments, extensograph and alveograph, are widely used to describe the behavior of the dough and its gas retention capacity during fermentation, proofing and oven-rise stages by measuring the resistance and extensibility of a uniformly shaped piece of dough (Spies, 1990; Weipert, 1992; Menjivar and Faridi, 1994; Walker and Hazelton, 1996). In extensograph, extension is applied to the dough piece in only one direction (uniaxial), whereas in alveograph expansion is performed in two directions (biaxial), radial and tangential to the surface of a blown bubble. For the extensograph test, flour-water-salt doughs are mixed first in a farinograph at a slightly higher than normal water absorption and then a piece of dough is molded into a cylinder, clamped into a saddle and after a suitable rest period, a hook stretches the dough until it ruptures. For the alveograph, a stiff dough is prepared with the alveograph's integral mixer at a constant hydration level using a sigmoidal blade to both mix and extrude the dough into a uniform sheet. Five individual disks are cut from the dough sheet and after relaxation each disk is clamped above a valve mechanism, and air is blown under the disk at a constant rate, thus creating a bubble; the pressure inside the bubble is recorded until rupture occurs. In both tests, a curve of the force required for extension or expansion versus extension is recorded during the stretching process; the maximum height of the curve is a measure of the dough resistance and its length a measure of the dough extensibility. The resistance to extensibility ratio is an indicator of the balance of elastic to viscous components of the dough. The surface area under the extensograph or alveograph is proportional to the energy that is required to rupture the dough and is used for the characterization of the strength of the flour, since flour strength is associated with both considerable resistance and sizable extensibility; a large surface area supposedly indicates high dough strength and suggests a strong ability of the dough to hold gasses, and usually results in high bread volume.

The rheology of dough during fermentation can be determined empirically using the rheofermentometer (Czuchajowska and Pomeranz, 1993) which simultaneously measures and records the parameters related to dough development, gas production and gas retention, such as height and time at which the dough reaches maximum development, loss in dough height at the end of the test, as well as time of maximum gas formation and gas retention (volume of the gas retained in the dough at the end of the test). Other empirical instruments used for testing of dough rheological properties are penetrometers, which measure the distance moved by a cone or a needle through the material in a particular time. A modified penetrometer is used for measuring the biscuit dough compliance and elasticity by introducing a circular metallic plate into a cylindrical dough piece (Manohar and Rao, 1992, 2002). The initial height of the dough (h_1) and its height after compression for 10 s (h_2) by the plate are noted. The compression plate is removed, and after 1 min allowance for recovery, the height of the

dough (h_3) is measured and the following parameters are calculated:

$$\text{Percentage compliance} = \frac{h_1 - h_2}{h_1} \times 100 \tag{5.1}$$

$$\text{Elastic recovery} = (h_3 - h_2) \times 10 \tag{5.2}$$

The Simon Research Water Absorption Meter (SRWAM) is also an empirical method used in the biscuit industry for determination of dough consistency as extrusion time (Manohar and Rao, 1992, 2002; Charun et al., 2000). A dough sample is introduced into the cylindrical die of SRWAM regulated at 28°C with a water jacket. After 5 min resting, a plunger (3.83 g) is placed on top of the dough and the time taken for the plunger to travel 4 mm is recorded. The flow in the system undergoes shear and elongational deformations and is strongly influenced by dough and die wall contact conditions.

The pasting properties of flours and starches from several sources (wheat, corn, tapioca, oats and amaranth) can be evaluated using the Brabender viscograph (amylograph) (Yamamoto et al., 1996; Rojas et al., 1999) or the rapid viscoanalyzer (RVA) (Xu et al., 1992; Bahnassey and Breene, 1994; Zhou et al., 2000). These techniques determine the effect of α-amylase and starch gelatinization on flour viscosity during the baking procedure by measuring the viscosity of a flour slurry submitted to a heating–cooling cycle at a certain rate and stirring speed (heating up to 95°C at a constant rate, holding at 95°C for a certain time period, cooling at a constant rate to 50°C or 40°C and then holding at 50°C or 40°C for a certain time). The parameters that can be determined by the amylograph and RVA are pasting temperature (the temperature at which the curve leaves the baseline), peak viscosity (highest viscosity during heating), time to peak viscosity, trough (lowest viscosity after cooling started), breakdown (peak viscosity minus trough), bump peak (highest viscosity during the cooling cycle), bump area (the area under the curve connecting the starting point of the bump peak to the end of this peak), final viscosity (highest viscosity during the second holding period) and setback (final viscosity minus trough). During the heating stage, the increase of paste viscosity is attributed to starch gelatinization and the maximum viscosity reflects the ability of the starch granules to swell freely before their physical breakdown due to shear. Trough represents the cooking or hot paste stability of the already broken starch at the cooking temperature. During the cooling stage retrogradation that occurs due to association of linear amylose molecules gives rise to setback viscosity; retrogradation is largely responsible for the firming of breadcrumb. Thus, setback as well as bump area, which is related with the extent of the amylose-lipid complex formation, are useful indicators of bread staling.

5.2.2 Fundamental tests

With fundamental or basic methods the mechanical properties of doughs can be described over a wide range of strains and strain rates (Weipert, 1990). By careful control of the geometry of the measured sample and exact measurements of the stress and strain rate, the results obtained in absolute physical units allow direct comparison of results attained by various testing instruments and researchers. Moreover, increased computerization in fundamental approaches to dough rheology has opened unexpected possibilities for gaining new information and knowledge of the mechanical properties of dough. However, fundamental tests are expensive because of complex instrumentation, time consuming, difficult in interpretation of results and in simulation of an industrial environment, require high levels of technical skills,

often employ inappropriate deformation conditions, and may encounter slip and edge effects during testing (Dobraszczyk and Morgenstern, 2003).

5.2.2.1 *Small deformation oscillatory testing*

Measurements with the two most widely used empirical instruments (extensograph and alveograph) for the study of extensional processes can hardly be used to gain a true understanding of bread dough fermentation and oven rise because they operate at higher deformation rates than the rates of deformation employed under actual baking situations (Bloksma, 1990; Faubion and Hoseney, 1990). Thus, small deformation testing methods have been introduced for the study of dough mechanical properties.

Dynamic oscillatory measurements are non-destructive tests that measure the elastic (G') and viscous (G'') moduli by the application of sinusoidally oscillating shear stress or strain with time, temperature, strain and frequency (Dobraszczyk and Morgenstern, 2003). The instrumentation that is utilized in these small deformation tests is rheometers equipped with parallel plate, cone and plate or coaxial cylinder geometries (Faubion and Hoseney, 1990). Wheat flour-water doughs exhibit linear viscoelasticity at strain levels lower than 1% (Weipert, 1990; Menjivar and Faridi, 1994; Phan-Thien and Safari-Ardi, 1998; Safari-Ardi and Phan-Thien, 1998; Autio et al., 2001). In general, frequency sweep experiments performed under dynamic conditions (i.e. in the linear viscoelastic region) have shown that for wheat flour dough formulations, the elastic (or storage) modulus, G', is greater than the viscous (or loss) modulus, G'', over the whole range of frequencies, and both moduli slightly increase with frequency, implying a predominant solid elastic-like behavior for the doughs; therefore, tan δ ($=G''/G'$) values are lower than 1 (Weipert, 1990; Menjivar and Faridi, 1994; Baltsavias et al., 1997; Dobraszczyk and Morgenstern, 2003; Edwards et al., 2003). Recently, Edwards et al. (2001) suggested that wheat dough seems to be more analogous to polymer gels with reversible physical cross-links than to entanglement networks. Moreover, changes in viscoelastic moduli can be monitored under dynamic oscillating conditions upon heating and cooling stages of a simulated baking test (Dreese et al., 1988; Weipert, 1990, 1992). These changes occurring throughout the whole temperature sweep are caused by various heat-induced physicochemical changes in the tested system, such as starch gelatinization or protein denaturation events.

In breadmaking, flours producing doughs with balanced tensile and elastic properties are required to ensure optimal baking performance. Weipert (1990) attempted to relate the elastic and tensile properties of wheat doughs determined by extensograph and alveograph, as well as sensorial analysis with the dynamic rheological parameters of dough. This author demonstrated that a dough with small tan δ reflects a rigid and stiff material, and doughs characterized as moist and slack possessed higher tan δ values than those described as having a short texture and dry surface appearance. On the other hand, Edwards et al. (1999) found no significant correlation between the tan δ values and the dough strength of durum wheat cultivars as measured by empirical methods, while the G' values strongly correlated with the dough strength; for stronger and least extensible samples, higher G' values were found than for their counterparts from weaker cultivars. Although oscillatory measurements in the linear viscoelastic region seem to segregate wheat doughs differing in strength, the dynamic rheological parameters of dough often show little or no relationship with its functionality during processing and end-use performance. In previous studies, neither dynamic modulus nor tan δ values of wheat flour doughs showed a clear relationship with bread loaf volumes (Janssen et al., 1996b; Safari-Ardi and Phan-Thien, 1998; Autio et al., 2001; Wang and Sun,

2002). Moreover, the low deformation conditions used for these measurements are often inappropriate to practical processing situations, because they are carried out at rates and conditions very different from those usually experienced by the dough during processing or baking expansion. Nevertheless, low strains, which allow measurements but do not disturb or destroy the inherent structure, are of great value in studying the influence and action of water and additives such as hydrocolloids in dough systems (Weipert, 1990; Berland and Launay, 1995; Yu and Ngadi, 2006) because the dynamic mechanical parameters of the hydrated network are highly sensitive to changes in polymer type and concentration as well as water content (Ferry, 1980). For example, the decrease of elastic modulus with increasing water level in dough is a well-documented response for wheat flour doughs (Edwards et al., 1996, 1999; Masi et al., 1998; Phan-Thien and Safari-Ardi, 1998; Autio et al., 2001), and is mostly attributed to plasticization and constituent dilution effects.

5.2.2.2 *Creep and creep recovery*

In a creep test, a constant stress ($\sigma = \sigma_0$) is applied to the sample and the change in strain is measured over time (Steffe, 1992). When the stress is released, some recovery is observed as the material attempts a return to the original shape. In creep-recovery tests, the creep strain as well as the strain recovery after removal of load is measured over time. Creep-recovery curves of wheat doughs have exhibited a typical viscoelastic behavior combining both viscous fluid and elastic components (Janssen et al., 1996a; Edwards et al., 1999; Wang and Sun, 2002; Sivaramakrishnan et al., 2004; Rouille et al., 2005). Creep data can also be expressed in terms of creep compliance, J:

$$J = f(t) = \gamma/\sigma$$

where γ is the strain and σ is the constant applied stress during the creep test (Steffe, 1992). A typical creep-recovery test performed on doughs is illustrated in Fig. 5.1.

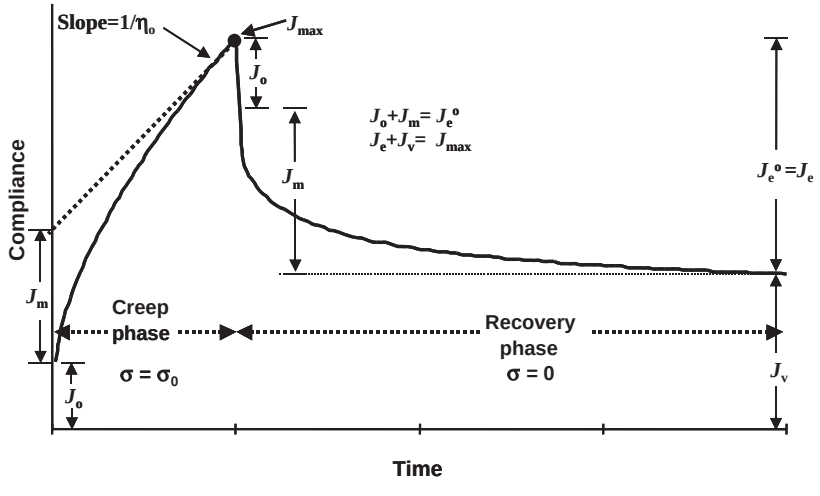

Fig. 5.1 Typical curve of creep-recovery test of a dough and calculation of parameters obtained from Burgers model fitting.

The compliance curve data of doughs can be fitted to the Burgers model (Edwards et al., 2001, 2003; Lazaridou et al., 2007):

$$J(t) = J_0 + J_m \cdot (1 - \exp(-t/\lambda)) + t/\eta_0 \text{ for the creep phase} \quad (5.3)$$

and

$$J(t) = J_{max} - J_0 - J_m \cdot (1 - \exp(-t/\lambda)) \text{ for the recovery phase} \quad (5.4)$$

where J_0 is the instantaneous compliance, J_m is the viscoelastic compliance, λ is the mean retardation time, η_0 is the zero shear viscosity and J_{max} is the maximum creep compliance (Fig. 5.1). The steady state compliance J_e^0 is also calculated by subtracting the compliance value at the terminal region of curve where dough recovery has reached equilibrium from the J_{max}. The steady state compliance J_e^0, can be attributed to the elastic components of the material, subsequently referred to as elastic compliance, J_e, whereas the compliance, where dough recovery reached equilibrium at the terminal region of the curve, is caused by the viscous components of dough and referred to as viscous compliance, J_v.

Flour doughs exhibit apparent yield stress and shear thinning behavior when tested in shear flow mode (Rouille et al., 2005). The shear viscosity η exhibits a Newtonian plateau at low shear rate $\dot{\gamma}$ ($<10^{-5}$ s^{-1}) and a shear thinning response at higher shear rates. For creep stresses $\sigma_0 \geq 80$ Pa, the dough begins to exhibit time-dependent flow behavior. Creep measurements are conducted both under small (Janssen et al., 1996a; Edwards et al., 2001, 2002, 2003; Wang and Sun, 2002; Rouille et al., 2005) or large deformation (Edwards et al., 1999; Rouille et al., 2005) conditions.

Edwards et al. (1999) using large deformation creep tests found an increase in maximum strain with increasing water absorption as determined by a micromixograph. These researchers also correlated the strength of durum wheat doughs, as measured by the alveograph, to maximum strain. In their measurements, maximum strain ranged from <5% for the stronger least extensible cultivar to >25% for the weakest most extensible samples. Moreover, Wang and Sun (2002) reported that the maximum creep strain might be used to describe dough rigidity; strong doughs, such as typical bread flour doughs, were found to have greater resistance to deformation than softer doughs, like pastry flour dough. Edwards and coworkers, analyzing the creep compliances in terms of a Burgers model, found that for the durum wheat doughs, the entire elastic compliance curve was shifted to higher values as the strength of the dough (measured by extensograph) decreased (Edwards et al., 2001, 2003), while the steady state viscosity increased with dough strength (Edwards et al., 2001). They interpreted the differences in creep behavior by the differences in strength of the associative network established by non-covalent intermolecular associations among gluten protein chains.

5.2.2.3 *Extensional measurements*

In dough systems, the shear viscosity decreases with stress and strain rate (shear thinning behavior), whilst the extensional viscosity increases with stress and strain rate, a phenomenon known as strain hardening (Dobraszczyk, 1999). Large deformation extensional flow is often experienced during dough molding, sheeting, extrusion and bubble expansion during fermentation and baking. The types of extensional flow measurements used for dough testing include simple uniaxial tension, lubricated compression, capillary extrusion and biaxial extension using inflation (Dobraszczyk and Morgenstern, 2003).

The stable micro systems (SMS) Kieffer dough and gluten extensibility rig is a test method used to measure the uniaxial extensional properties of doughs (Kieffer et al., 1998; Collar et al., 1999; Suchy et al., 2000; Sharadanant and Khan, 2003; Uthayakumaran and Lukow, 2003). Dough is rounded into a ball and placed over three or four channels (20 mm × 60 mm) in a Teflon-coated block to yield dough strips of uniform geometry; the channels are coated with mineral oil to avoid sample adhesion. The upper half of the Teflon block is placed in position and clamped tightly. The dough strips are then separated from the Teflon channels, positioned across the Kieffer rig dough holder, and centrally extended by a hook probe. The maximum force (resistance to extension) and distance to break (extensibility) are measured by this test. This extension test that can be performed easily and reproducibly, allows a reliable prediction of the loaf volume (Kieffer et al., 1998); dough extensibility was found to be significantly correlated with loaf volume of wheat bread (Suchy et al., 2000; Uthayakumaran and Lukow, 2003). Moreover, resistance to extension of wheat flour dough showed significant correlations with some functional properties of the end products, such as tortilla firmness, cooked noodle hardness and pan bread composite fineness (cell size and shape) (Uthayakumaran and Lukow, 2003).

The dominating deformation of the dough matrix between expanding gas cells during fermentation of a dough system is a biaxial stretching flow. Theoretical considerations by van Vliet et al. (1992) on strain hardening of dough as a requirement for gas retention have underlined the interest of this type of deformation for breadmaking. Biaxial extension can be achieved by performing a lubricated uniaxial compression test (Chatraei et al., 1981). Cylindrical dough discs are subjected to uniaxial compression between two lubricated circular plates at constant stress and up to a certain deformation. During uniaxial compression at a constant speed v, the strain rate, $\dot{\varepsilon}_h$, will increase due to the decreasing height of the piece:

$$\dot{\varepsilon}_h = d\varepsilon_h/dt = d(\ln(h_t/h_0))/dt = 1/h_t(dh/dt) = v/h_t \tag{5.5}$$

where ε_h is the Hencky strain, h_0 is the initial height of the test piece, and h_t is the height at time t.

The biaxial strain rate is defined as:

$$\dot{\varepsilon}_b = \dot{\varepsilon}_h/2 \tag{5.6}$$

The compression stress, σ, can be calculated according to:

$$\sigma = F_t/\pi r^2 \tag{5.7}$$

where F_t is the compression force at time t, and r is the radius of the moving plate. Then the biaxial extensional viscosity, η_{BE}, can be calculated by the following equation:

$$\eta_{BE} = \sigma/\dot{\varepsilon}_b = 2(F_t/\pi r^2)/(v/h_t) \tag{5.8}$$

The slope of the curve of $\ln(\sigma)$ versus $\dot{\varepsilon}_h$ defines the strain hardening index (SHI) (van Vliet et al., 1992).

Uniaxial compression in the lubricated squeezing flow test (LSF) has been performed for wheat gluten (Janssen et al., 1996a) as well as for wheat bread (Wikstrom et al., 1994; Janssen et al., 1996b; Rouille et al., 2005) biscuit (Manohar and Rao, 1997, 2002), cookie (Miller and Hoseney, 1997) and cracker (Lin et al., 1993) doughs. Janssen et al. (1996a) related

the biaxial viscosity of gluten from two wheat cultivars to their different baking potentials, considering the importance of strain hardening. Later, these researchers (Janssen et al., 1996b) suggested that in order to obtain a high loaf volume and fine crumb structure, wheat flour dough has to exhibit biaxial strain hardening and extensibility exceeding a minimum level; it is likely that the resistance to deformation may vary within a certain range. Recently, the specific volume of bread was found to be inversely related to bi-extensional viscosity, whereas crumb fineness could be related to the SHI, in agreement with gas retention and bubble growth phenomena during proofing (Rouille et al., 2005). Moreover, dough biaxial extensional viscosity determined by lubricated uniaxial compression tests is found to be a useful tool for predicting cookie (Miller and Hoseney, 1997) and biscuit (Manohar and Rao, 2002) spread rate and hence their diameter and other quality attributes of the end products; biaxial extensional viscosity of doughs was negatively correlated to biscuit and cookie diameter and positively correlated to density of biscuits.

In addition to the lubricated compression tests, the biaxial extensional rheological properties of wheat doughs are studied by an inflation method developed by Dobraszczyk and Roberts (1994). Later, these researchers improved the dough inflation method using a texture analyzer (Dobraszczyk, 1997). The developed apparatus called the Dobraszczyk/Roberts dough inflation system (D/R system) determines dough rheological attributes under conditions of strain similar to those of baking expansion. The device inflates a sheet of dough by volume displacement of air. The pressure during inflation and the volume of the inflating dough sheet are measured. The D/R system allows determination of stress, strain, biaxial viscosity and extensional strain hardening which have been related to bubble failure strain and are important indicators of baked loaf volume (Dobraszczyk, 1997, 1999). Doughs with good strain hardening characteristics expand to greater volumes, give thinner cell walls, and have a more even distribution of bubble sizes than do doughs with poor strain hardening behavior.

Extensional properties of dough sheets are measured under large deformation using the SMS tortilla/pastry burst rig (http://www.stablemicrosystems.com) and a sheet-deforming device attached in the Instron universal testing machine (UTM) (Morgenstern et al., 1996). The SMS fixture has been developed to perform extension and elasticity measurements on laminated pastry and tortilla dough (http://www.stablemicrosystems.com). The rig consists of two plates that can be bolted together with the samples sandwiched between. The plates have holes through their centers, which expose a circular section of the sample allowing a spherical probe to be pushed through. The sheet-deforming device of Instron (UTM) consists of two Perspex plates with a circular aperture in the middle (Morgenstern et al., 1996). A sheet of dough is placed between the plates and is deformed by a flat probe with rounded edges, which is moved vertically down the center of the aperture; during deformation, the shape of the dough sheet resembles a cone with a flat top. The experimental data derived from utilization of this device showed qualitative agreement with data obtained from other compression methods. Measurements on sheeted wheat flour dough at a water level suitable for pastry showed that stress increases with elongational rate, in accordance with the strain hardening effect. Moreover, elongational viscosity decreased with addition of water.

Compression–extrusion dough tests can be performed by capillary rheometers, which provide reliable information on shear and extensional flow properties for dough systems (Steffe, 1992). A set of capillaries is screwed at the bottom of a cylindrical cell (Faridi, 1990; Cuq et al., 2002). Small pieces of dough are introduced into the cell and pushed down into the cell using a plunger. The pressure needed to extrude the dough through the capillaries is calculated from the measured values of forces acting on the plunger. A method used for

testing of cookie and cracker doughs is back extrusion, which is performed by an Instron instrument (Faridi, 1990). Dough is placed in a cylindrical cell and then compressed by a cylindrical plunger to flow upward through a concentric annular space.

5.2.2.4 *Stress relaxation*

The viscoelastic behavior of wheat flour doughs is of great significance in the baking industry, since the rate at which internal stresses induced by mechanical treatment relax during a rest period depends on both viscosity and dough elasticity (Launay, 1990). Further to the creep and recovery measurements, elasticity of doughs has been characterized by stress relaxation tests. In stress relaxation measurements, an instantaneous strain (γ_0) is applied to the material and the stress (σ) required to maintain the deformation is measured as a function of time (t) (Steffe, 1992). The stress relaxation modulus, $G(t)$, is obtained from the following equation:

$$G(t) = \sigma(t)/\gamma_0 \tag{5.9}$$

Stress relaxation dough testing can be performed after application of shear forces in a rheometer (Launay, 1990; Phan-Thien and Safari-Ardi, 1998; Safari-Ardi and Phan-Thien, 1998; Wikstrom and Eliasson, 1998) or after biaxial extensional deformation following a LSF (Maache-Rezzoug et al., 1998a,b; Bartolucci and Launay, 2000; Fustier et al., 2007) or bubble inflation tests (Launay, 1990; Dobraszczyk, 1997; Bartolucci and Launay, 2000). A typical compression–relaxation curve after LSF is illustrated in Fig. 5.2.

A slower relaxation time is associated with good baking quality (Bloksma, 1990; Launay, 1990). Measurements of large deformation shear stress relaxation properties were found to be useful in discriminating between different wheat varieties of varying quality, and were found to be closely associated with baking volume (Safari-Ardi and Phan-Thien, 1998; Wikstrom and Eliasson, 1998). At small deformation (0.1%), doughs obtained from cultivars with different baking strength (weak, medium, strong and extra strong) showed no differences in relaxation behavior (Safari-Ardi and Phan-Thien, 1998). However, at a range of intermediate and large strains (up to 29%) the magnitude of the relaxation modulus, which indicates the level of elasticity, followed the order extra strong > strong > medium > weak, allowing the discrimination between wheat cultivars varying in baking quality.

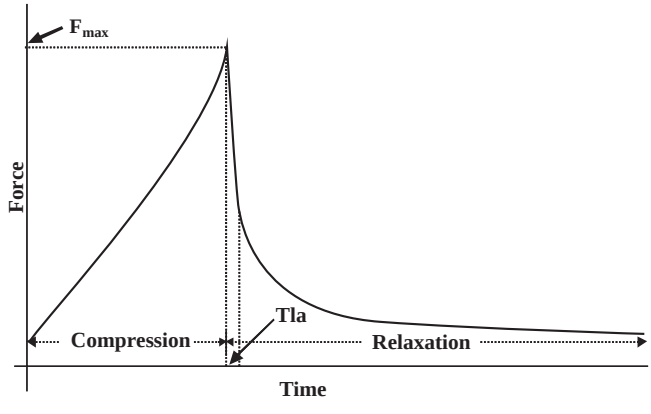

Fig. 5.2 Typical compression–relaxation curve after lubricated squeezing flow of a dough.

Rheological data obtained from stress relaxation following LSF of biscuit doughs are often related with quality parameters of end products, such as biscuit dimensions (Maache-Rezzoug et al., 1998a; Bartolucci and Launay, 2000; Fustier et al., 2007). The relaxation curve can fit to the following equation proposed by Bartolucci and Launay (2000) that is based on a nonlinear Maxwell model:

$$\sigma(t) = \sigma_0 \left[1 + k\left(\frac{1}{n} - 1\right)t\right]^{\frac{n}{n-1}} \tag{5.10}$$

where σ_0 is σ at zero time, k is the rate of relaxation and n is the flow behavior index. From the relaxation curve, F_{max} can also be calculated, which is considered as equivalent to dough hardness, and the half relaxation time, denoted as Tla, is measured as the time required for the force to decrease to a value $F_{max}/2$, as shown in Fig. 5.2. The Tla and k responses can be explained in terms of elastic recovery; compression of dough during the sheeting process stores mechanical energy, inducing partial strain recovery. A lower Tla value is translated into a higher speed of recovery of the dough once laminated and cut and thus reduced length of the biscuits in the laminating direction. High k means that the energy stored in the dough is dissipated more rapidly, and as a result, the dough recovery is smaller and the dough retraction will be minimized. Thus, the length of biscuits has been found to correlate negatively with Tla and positively with k values obtained from stress relaxation testing of biscuit doughs (Bartolucci and Launay, 2000; Fustier et al., 2007). Moreover, density, an important quality parameter for predicting biscuits crispiness has been found to positively correlate with F_{max} and Tla values of biscuit doughs (Bartolucci and Launay, 2000).

5.2.2.5 *Other large deformation tests*

Other large deformation tests performed by Instron or texture analyzers on doughs are texture profile analysis (TPA), simple penetration tests and adhesion tests for determination of dough stickiness.

For bread (Armero and Collar, 1997; Collar et al., 1999) and biscuit (Manohar and Rao, 2002) doughs made of wheat flour, texture parameters, such as hardness, cohesiveness, springiness, resilience, gumminess, chewiness and adhesiveness can be determined by the TPA test as described by Bourne (1978). The breadmaking process and the addition of hydrocolloids to bread doughs have a strong impact on TPA parameters (Armero and Collar, 1997). Dough cohesiveness seems to be a good predictive parameter of bread and biscuits quality. More cohesive doughs resulted in a higher specific volume and softer breads. Biscuit dough cohesiveness was also reported to positively correlate with the density of biscuits (Manohar and Rao, 2002).

For measurement of dough stickiness the SMS Chen–Hoseney dough stickiness ring is often used according to a method developed by Chen and Hoseney (Hoseney and Chen, 1994; Chen and Hoseney, 1995). This apparatus includes an extrusion-type sample holding device together with a plexiglass plunger; an extrusion screen is also supported on the device base. A dough sample is placed on the base of the device and extruded through the screen in order to expose a certain portion above the screen. The dough is then compressed by the probe and the tensile force required to separate the plunger from the dough is recorded as a measure of adhesion. The procedure is reproducible and appears to correlate well with subjective measurements of dough stickiness. Several factors were shown to affect the stickiness of wheat flour doughs (Chen and Hoseney, 1995; Armero and Collar,

1997). Overmixing, increasing amount of water and addition of hydrocolloids and enzymes (α-amylase or protease) resulted in increase of dough stickiness.

A compression–penetration method for measurement of the consistency of short doughs using a texture analyzer was developed by Miller (1985). The method includes a simple device of dough preparation for the removal of air while keeping dough handling to a minimum. One hundred and five grams of dough are weighed into a sample cup, compressed with a spiked lid to remove all the pockets of air and then, the surface of the dough is flattened by a smooth-surfaced lid. Dough consistency is expressed by the maximum force required for a cylindrical probe to insert into the dough sample at a specified rate and depth.

5.3 DOUGH RHEOLOGY OF GLUTEN-FREE FORMULATIONS

In the literature, studies on the rheological properties of gluten-free doughs as well as reports addressing the possible relations between those properties and quality attributes of the end products are limited. Regarding empirical methods used for the study of dough rheology, the farinograph has been used for the determination of parameters such as water absorption, DDT, consistency and elasticity of gluten-free doughs based on rice flour with or without hydrocolloid addition (Gujral and Rosell, 2004a, b; Sivaramakrishnan et al., 2004; Lazaridou et al., 2007). Moreover, the consistency of gluten-free dough prepared from cassava flour with defatted soy flour has been measured using a mixograph (Defloor et al., 1991). The RVA and Brabender viscoamylograph have been employed for determining the pasting properties of gluten-free bread doughs based on rice flour (Nishita and Bean, 1979; Gujral et al., 2003a; Baxter et al., 2004; Marco and Rosell, 2008), blends of corn and cassava starches (Kobylanski et al., 2004) and cassava flour fortified with defatted soy flour (Defloor et al., 1991), as well as for studying the pasting characteristics of flours from sorghum, buckwheat and rice used for gluten-free pasta production (Suhendro et al., 2000; Alamprese et al., 2007). The setback parameter that is related to the retrogradation behavior of starch, and in turn to amylose gelation–crystallization, was found to be one of the most important parameters in predicting rice bread characteristics (Nishita and Bean, 1979). In fact, soft bread crumb was obtained from rice flour with low viscosities at 50°C and low setback values, whereas high viscosities at 50°C and high setback values gave harsher textures. Lately, RVA was used for measurements of apparent viscosity of fresh and ripened gluten-free sourdoughs added at 20% level to a gluten-free formulation based on brown rice flour, cornstarch, buckwheat soy flour and xanthan gum (Moore et al., 2007). An empirical cone penetrometer has been utilized for adjustment of water content in a gluten-free bread formulation consisting of cornstarch, rice flour and cassava starch; water was adjusted to a content at which the dough reached 250 units of the penetrometer (Sanchez et al., 2002).

The SMS texture analyzer (SMS-TA) has been used widely for studying large deformation mechanical properties of gluten-free doughs. For example, it has been utilized for determining the force at 25 mm penetration of a spherical probe into a rice dough; this was selected as a convenient index of dough consistency (Haque and Morris, 1994). Similarly, for penetration measurements of gluten-free batters, a 20 mm cylindrical probe of SMS-TA has been employed (Moore et al., 2004). The hardness of gluten-free biscuit dough has been measured using the dough preparation set of SMS-TA with a 6 mm cylindrical probe (Schober et al., 2003). The set comprises of a test cell, a spiked aeration plunger (to remove pockets of

randomly distributed air) and a flattening plunger (to achieve an even surface); the force in compression at 20 mm distance depth was considered as the dough hardness. Furthermore, the forward extrusion cell with the 10 mm nozzle of SMS-TA has been utilized for extrusion tests of gluten-free doughs based on sorghum flour (Schober et al., 2005) and on several gluten-free ingredients, such as brown rice, soy and buckwheat flour, potato and cornstarch, skim milk powder and whole egg (Moore et al., 2004); the maximum extrusion force (at 8–18 mm distance) was used as an indicator of batter firmness or consistency. Moreover, this test was used for standardizing the amount of water added to the formulation when various sorghum hybrids were compared for their breadmaking potential; the water was adjusted to obtain a constant batter consistency during extrusion (Schober et al., 2005). Recently, the consistency in fresh and ripened gluten-free sourdoughs added at 20% level to a gluten-free formulation was also determined by this extrusion method (Moore et al., 2007). The stickiness of gluten-free biscuit doughs has been measured using the SMS Chen–Hoseney dough stickiness cell (A/DSC) and a 25 mm Perspex cylinder probe (Schober et al., 2003).

Only a small number of reports exist in the literature which deal with the mechanical properties of gluten-free doughs measured by fundamental rheological methods; these measurements include oscillatory tests, such as strain and frequency sweeps as well as creep-recovery tests on gluten-free dough formulations based on rice flour (Gujral et al., 2003a; Gujral and Rosell, 2004a,b; Sivaramakrishnan et al., 2004; Lazaridou et al., 2007; Marco and Rosell, 2008).

5.3.1 Effects of gluten-free ingredients on dough rheological properties

Gluten-free bakery products based on pure starches have dry, sandy mouth feel with a flat 'starchy' aroma and therefore, in these products wheat flour is usually replaced by a mixture of gluten-free flours and starches rather than pure gluten-free starches. Flours recommended for celiac patients are rice, corn, sorghum and buckwheat, with rice flour being the preferable raw material for this type of products. Rice flour has bland taste, white color, low sodium content, easily digested carbohydrates and hypoallergenic properties (Juliano, 1985). However, breads produced from rice flour can have lower loaf volume, harder texture and are more prone to retrogradation during storage than whole wheat breads (Kadan et al., 2001). The aforementioned gluten-free flours alone or in combination with starches such as corn, potato and cassava starch have been utilized to formulate gluten-free baked products (Nishita et al., 1976; Defloor et al., 1991; Ylimaki et al., 1991; Eggleston et al., 1992; Haque and Morris, 1994; Toufeili et al., 1994; Kadan et al., 2001; Sanchez et al., 2002; Gujral et al., 2003a; Gallagher et al., 2003a,b; Schober et al., 2003, 2005; Kobylanski et al., 2004; Moore et al., 2004, 2006; Sivaramakrishnan et al., 2004; Ahlborn et al., 2005; McCarthy et al., 2005; Lazaridou et al., 2007). Starch acts as a filler, raising the dynamic elastic modulus, G', of the dough (Uthayakumaran and Lukow, 2003). However, these formulations require a gluten replacement to provide structure and a proper network necessary to retain carbon dioxide in the dough. In this respect, non-gluten proteins, such as egg and milk protein or soybean protein or soy flour with or without hydrocolloid additions, such as xanthan, pectin, agarose, oat β-glucan, carboxylmethylcellulose (CMC), hydroxypropylmethylcellulose (HPMC), psyllium, gum arabic and konjac, locust bean and guar gums are incorporated into gluten-free formulations. Addition of fat from vegetable or dairy sources (Eggleston et al., 1992; Gujral et al., 2003a; Schober et al., 2003) as well as of emulsifiers (Defloor et al., 1991) to gluten-free formulations has been studied to a lesser extent. Recently, the actions

of several enzymes on improvement of loaf volume and texture as well as on retarding of staling of rice-based bread have been investigated (Gujral et al., 2003a,b; Gujral and Rosell, 2004a,b; Moore et al., 2006). A different approach has been lately introduced by utilization of gluten-free sourdough into a gluten-free bread formulation in order to enhance crumb texture, flavor and nutritional value of the end products (Moore et al., 2007).

5.3.1.1 *Flour–starches*

The standard farinograph curve obtained for pure rice flours has shown a water absorption of 42.9–60.7% (based on the dough weight) and a long DDT, 20–28 min, to reach the consistency of 500 BU (Gujral et al., 2003a; Sivaramakrishnan et al., 2004; Lazaridou et al., 2007). Moreover, the signal of the curve was noisy and the consistency increased with measuring time showing a poor dough for baking. However, this curve was improved by the addition of cornstarch and milk protein (sodium caseinate) to rice flour (Lazaridou et al., 2007). The water absorption was similar (~60.5%) for both formulations, pure rice flour and rice flour fortified with starch and proteins, but the DDT of 20 min for the pure rice flour was reduced to 4 min for the fortified flour and the consistency remained relatively constant throughout the measuring time for the latter formulation. Gujral et al. (2003a) found that when 42.9% water was added to a rice dough, a 500 BU consistency was achieved; however, at this consistency level, the amount of dough rise during proofing and the specific volume of the resultant bread were very low. These researchers found that with further water additions increased dough rising during proofing and the loaf volume, whereas the dough consistency was reduced. A water absorption of 47.4% was found to be the optimum in this study.

Similarly, the consistency of doughs obtained from sorghum flour and measured as extrusion force using a SMS-TA nozzle was negatively correlated with the water content of the dough (Schober et al., 2005). Larger bread volumes were reached when cornstarch (up to 30%) was added to the sorghum flour and a soft (low consistency) dough was produced using high water levels; loaf specific volume and crumb texture of sorghum breads were improved by increasing water content in the dough. Haque and Morris (1994) studied the consistency of dough from rice flour using an SMS-TA spherical probe. They found that the resistance to penetration increases sharply as the water content of the dough is decreased; a 50% increase in resistance corresponded roughly to a 3% reduction in water content.

Defloor et al. (1991) found that the incorporation of prehydrated extruded cornstarch (12% db) into bread products prepared from cassava flour fortified with soy flour (20% db) resulted in improved gas retention during fermentation, and had a positive impact on the volume and crumb texture; addition of 9–12% extruded starch led to 12% loaf volume increase. Mixographs showed that extruded cornstarch considerably increased the consistency of gluten-free dough. The action of pregelatinized starch can be attributed to an increase in the viscosity of the fermenting dough which facilitates gas retention. Additional experimental data indicated that only incorporation of an extruded starch into the gluten-free formulation resulted in this delay; it did not happen if native cornstarch granules were added. Moreover, the pregelatinized starch influenced the pasting properties of the cassava starch by decreasing the intensity of the peak of maximum viscosity obtained from dough amylographs; however, whether or not this fact can be related to the favoring effects exerted by this material is not clear.

Cultivar, particle size and processing conditions affected the pasting properties of sorghum flour used for noodle manufacturing as revealed by RVA (Suhendro et al., 2000). Sorghum cultivars with harder endosperm produced finer flours, which generally have higher pasting viscosities and earlier viscosity development; development of pasting viscosity occurs about 10°C earlier for finer flours than for their coarser counterparts. The combination of roller

and hammer milling methods produced sorghum flours with smaller particle size than flours produced by roller milling methods alone. In general, sorghum flours with smaller particle size yielded better noodles. In gluten-free pasta, starch gelatinization and retrogradation enhance its structure, therefore the sequence and timing of these physicochemical processes significantly affect the characteristics of noodles. Thus, optimized processing conditions yielded sorghum noodles with good quality attributes when properly cooked. Fine flour that was preheated using a microwave oven and dried using a fast-high humidity drying method gave the best dry or cooked noodles; that is, the strongest and firmest as well as the least sticky and chewy noodles with moderate (10%) dry matter loss during cooking. Similarly, the pasting properties of flours used for pasta (*lasagna* sheets) containing buckwheat with rice flour, precooked rice flour or pregelatized rice starch were found to affect the end-product quality (Alamprese et al., 2007). The gelatinized rice materials showed an early development of high initial viscosity due to their high water absorption capacity, and exhibited low setback values indicating a limited starch retrogradation during cooling. Pregelatinization of rice starch also allows a better dough workability during rolling – sheeting of the pasta dough. Furthermore, the inclusion of precooked rice flour in the buckwheat pasta resulted in a better product, in terms of break strain for the cooked product and the weight increase during cooking. Thermal processing, such as pasteurization, causes denaturation of proteins and therefore, contributes to a better structure of the product, which is further characterized by lower losses of water solubles during cooking.

The viscoelasticity of rice flour doughs used for bread production has been examined by oscillatory and creep measurements (Sivaramakrishnan et al., 2004; Lazaridou et al., 2007). The region of linear viscoelasticity for the gluten-free dough samples, established by strain sweep experiments (Fig. 5.3), was limited up to a strain of 0.1%; that is, the decrease in elastic

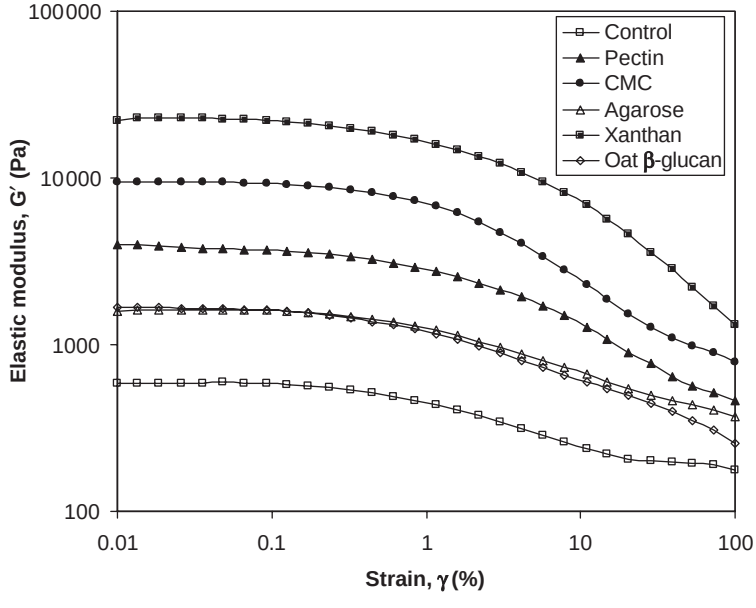

Fig. 5.3 Effect of hydrocolloid addition at 2% level (rice flour basis) on strain sweep curves (1 Hz frequency, 25°C) of gluten-free dough based on rice flour, cornstarch and milk proteins (adapted from Lazaridou et al., 2007).

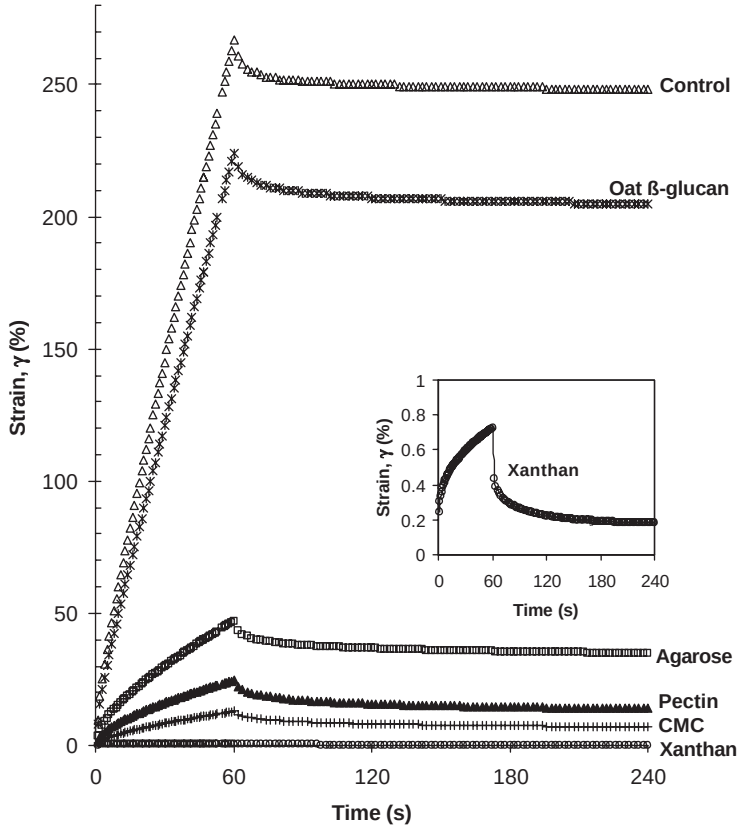

Fig. 5.4 Effect of hydrocolloid addition at 2% level (rice flour basis) on creep-recovery test curves (50 Pa stress, 25°C) of gluten-free dough based on rice flour, cornstarch and milk proteins; inset shows the creep-recovery data for xanthan at a larger scale (adopted from Lazaridou et al., 2007).

modulus, G' started to occur above 0.1% strain and became large above 1% strain, indicating the breakdown of the gluten-free dough structure beyond this deformation level (Lazaridou et al., 2007). Dynamic oscillatory rheological measurements of rice flour doughs showed that the elastic modulus, G', was higher than the viscous modulus, G'', over the entire frequency range examined; this suggests a solid-like behavior of the rice dough (Gujral et al., 2003a; Gujral and Rosell, 2004a,b; Sivaramakrishnan et al., 2004; Lazaridou et al., 2007; Marco and Rosell, 2008). Moreover, the elastic modulus was found to decrease with increasing water level in the rice dough (Lazaridou et al., 2007). The creep-recovery curves of gluten-free doughs based on rice flour exhibited a typical viscoelastic behavior (Fig. 5.4) combining both viscous fluid and elastic responses (Sivaramakrishnan et al., 2004; Lazaridou et al., 2007). Sivaramakrishnan et al. (2004) found that rice doughs appeared to be stronger than wheat doughs as indicated by the higher elastic modulus values found for doughs from pure rice flour compared to that from pure wheat when measured at similar water contents, 57.5% for rice and 58% for wheat doughs. Similarly, creep-recovery curves of rice doughs shifted to lower deformation values compared to the control wheat doughs. Moreover, the rice dough exhibited lower compliance values and higher zero shear viscosity, η_0, than doughs made of pure wheat flour. However, calculation of the elastic portion of maximum creep compliance

(% J_e/J_{max}) showed approximately 65% elastic recovery for both doughs, indicating similar elasticity. Lazaridou et al. (2007) studied the creep-recovery behavior of gluten-free doughs based on rice flour, cornstarch and milk proteins. They found that this creep parameter is largely dependent on moisture content; increasing dough water content from 56.5% to 60%, the elastic portion of maximum creep compliance decreased from 33% to 7%. These researchers also showed that the maximum creep strain as well as all creep compliance parameters (J_0, J_m, J_{max} and J_e^0) calculated from the Burgers model (equations 5.3 and 5.4) increased and the n_0 decreased with rising water content in the gluten-free doughs.

5.3.1.2 *Proteins*

Milk proteins have previously been shown to improve gluten-free breads by increasing their acceptability/preference and loaf volume of the breads in comparison with controls which contained only rice flour (Gallagher et al., 2003a,b). However, Schober et al. (2005) found that except for improved crust browning, increasing of skim milk powder levels (1.2–4.8% fwb) in gluten-free formulations, based on sorghum flour and cornstarch, resulted in reduced loaf height, a collapsed top, increased bake loss and reduced crumb cohesiveness, indicating a weakened bread structure. In sorghum bread, milk proteins and lactose seem to interfere with the starch gel, probably by competition for water or by disrupting the continuity of the starch gel network.

On the other hand, Moore et al. (2004) using confocal laser scanning microscopy showed that when egg proteins were added to gluten-free breads the crumb contained network-like structures resembling the gluten network in wheat bread crumb. The formation of a continuous protein phase and film-like structures by added egg proteins appears to be critical for the keeping quality of gluten-free breads because they can mask the changes caused by starch retrogradation. Kobylanski et al. (2004) studying by differential scanning calorimetry the thermal transitions of gluten-free doughs based on corn and cassava starches (75:25) found that the peak and conclusion temperatures of starch gelatinization are affected in opposite way by water and added egg white when their contents varied in the range of 80–110% and 0–10%, respectively; thus, low water content (80%) in combination with a high egg white level (10%) would elevate the peak and conclusion temperatures of gelatinization. Furthermore, Eggleston et al. (1992) found that egg white reduced the extent of starch gelatinization and solubilization in the breads made from cassava flour fortified with 20% soy flour. This behavior was attributed to the emulsification properties of the egg white proteins or more likely to the protein network created by coagulation of the whisked egg white proteins (albumin) in the early stages of baking, acting as a barrier to the water reaching the starch and thus, hindering starch swelling and gelatinization. Moreover, addition of whisked egg white increased the amount of air entrapped in the cassava doughs at the mixing stage, as indicated by the decrease of dough density; in this study a direct linear relationship ($r = -0.94$) was observed between the dough densities and the maximum gas retention volumes of the gluten-free doughs. However, final loaf volumes also depended on the stability of the dough, with the egg white acting as a stabilizer as well. The denaturation of albumins upon whisking forms a relatively stable, aerated foam structure, which can stabilize other dough ingredients. This functional property, along with the coagulation of the egg protein network on baking in the oven, appeared to hinder the collapse of baked loaves.

Different protein isolates used to modify rice flour functionality were found to affect differently the pasting properties of flour (Baxter et al., 2004; Marco and Rosell, 2008). RVA measurements showed that pea, soybean and whey proteins significantly decreased the final

viscosity; in addition, whey protein and rice prolamins promoted a significant decrease in the peak viscosity. The breakdown that relates to the ability of the starches to withstand heating at high temperature and shear stresses showed a considerable decrease by the presence of egg albumin and whey protein in the rice flour. The pea protein decreased setback viscosity, whereas the whey proteins added in the rice flour increased this parameter. The G' values, recorded in oscillatory tests were also affected by the protein isolates (Marco and Rosell, 2008); the extent was dependent on the protein source.

The influence of gluten-free flour mixes on biscuit dough characteristics and their relationship with gluten-free biscuit quality have been examined (Schober et al., 2003). Three different mixes with varying proportions of ingredients, such as brown rice flour, potato starch, soy flour, buckwheat flour and millet flakes, were tested. Hardness and stickiness of the biscuit doughs expressed as compression and tension force, respectively, were measured using a SMS-TA system. Significant correlations ($r > 0.8$) showed that firm and non-sticky doughs yielded firm, thin and round biscuits, whereas soft and sticky biscuit doughs resulted in soft, thick and oval biscuits. Moreover, in this study it was shown that among the various flour mixes, the gluten-free formulation fortified with soy flour, and thus enriched in protein, gave the firmest and less sticky dough, resulting in the firmest, thinnest and most round biscuits. In contrast, gluten-free formulations with high starch and low protein contents exhibited weak structural stability and high dough stickiness.

5.3.1.3 *Hydrocolloids*

The effect of supplementation of rice doughs with hydrocolloids on standard farinograph curves has been investigated (Gujral et al., 2003a; Sivaramakrishnan et al., 2004; Lazaridou et al., 2007). In their studies, Lazaridou et al. (2007) found that the water absorption of gluten-free doughs based on rice flour, cornstarch and milk proteins increased following the addition of various hydrocolloids, such as pectin, agarose, CMC and xanthan due to the hydrophilic nature of these biopolymers; water absorption of the formulations containing hydrocolloids at 2% level (rice flour basis) varied in the range 63.4–67%. Furthermore, the addition of hydrocolloids increased the DDT farinograph parameter from 4 min for the control formulation to the range of 7.5–26.5 min, with the exception of xanthan, which decreased the DDT to 2.0 min. The width of the farinograph curves, which is a measure of dough cohesiveness and elasticity, showed that the elasticity of dough, when 500 BU of consistency is reached, was differently affected by each hydrocolloid. The addition of xanthan resulted in highest elasticity values (100 BU) and in a farinograph curve which resembled that of a standard farinograph curve obtained typically by wheat flour; that is, the development time was short, the consistency and elasticity had a maximum and then reduced with the mixing time. Rice doughs supplemented with oat β-glucan were also tested in this study but the farinograph parameters were not accessible because the curves were noisy. A strong negative effect on the farinograph curve was exhibited by CMC; its inclusion increased the DDT to 26.5 min, with the consistency continuously increasing with time, and the width of the curve showing high cohesiveness. Similar characteristics had been reported by Sivaramakrishnan et al. (2004) in their studies with rice flour containing 4.5% HPMC. Gujral and his colleagues (Gujral et al., 2003a; Gujral and Rosell, 2004a,b) found that dough consistency measured with the farinograph increased with increasing HPMC concentration in rice flour doughs; the dough consistency reported after 5 min mixing increased by 120% with an increase in HPMC from 2% to 6%. Haque and Morris (1994), using an SMS-TA spherical probe for measuring rice dough consistency, found that the water content required

to maintain constant dough consistency increased with increasing concentration of added HPMC and psyllium gum.

Fundamental rheometry revealed an improvement in the viscoelastic properties of gluten-free doughs after supplementing the formulations with hydrocolloids. Lazaridou et al. (2007) found that addition of various hydrocolloids (pectin, CMC, agarose, xanthan and oat β-glucan) at 1% and 2% levels (rice flour basis) into a gluten-free dough resulted in rise of the elastic modulus, G', measured in the linear viscoelastic region (Fig. 5.3) as well as in an increase in the resistance to deformation as revealed by the reduction of maximum creep% strain (Fig. 5.4). In this study, frequency sweep tests showed that G' remained higher than G'' over all frequency ranges and for both concentration levels of hydrocolloids, that is, a solid-like rheological behavior. The effect of increasing hydrocolloid level from 1% to 2% on G' values was not as clear, because in the preparations with increasing hydrocolloid levels, water added to the dough also increased from 58.3% (at 1% hydrocolloid addition) to 60.0% (at 2% hydrocolloid addition). In the case of xanthan, β-glucan and pectin addition, the doughs became firmer (higher G' values) with increasing hydrocolloid concentration, indicating that the rise of biopolymer level affected the rheological properties of the dough more than the increasing content of water; the opposite effect was observed for CMC and agarose. However, Gujral et al. (2003a) and Sivaramakrishnan et al. (2004), maintaining a constant water content for rice doughs fortified with HPMC, at 47.4% and 38.0%, respectively, showed an increase of G' values of the dough with increasing hydrocolloid concentration in the gluten-free formulations. In the former study, for all HPMC concentrations (2–6%) employed, the rice doughs exhibited a solid-like rheological behavior. In contrast, in the study by Sivaramakrishnan et al. (2004), rice doughs supplemented with HPMC (1.5–4.5%) showed the typical viscoelastic behavior of random coil polymeric dispersions with chain entanglements; that is, in the latter case the rheological impact of the additive was more dominant than those of the other flour components. Moreover, according to these researchers, creep tests showed that the dough flowability (zero shear viscosity) of rice flour supplemented with 3% HPMC was similar to that of a typical dough made of wheat flour.

Lazaridou et al. (2007) employed oscillatory and creep measurements for different types of hydrocolloids added to gluten-free doughs and found that the elasticity and resistance to deformation of doughs followed the order of xanthan > CMC > pectin > agarose > oat β-glucan (Fig. 5.4). Thus, in the gluten-free doughs fortified with these hydrocolloids, the dynamic elastic modulus and the η_0 followed this order, while the maximum creep strain as well as all creep compliance parameters (J_0, J_m, J_{max} and J_e^0) calculated from the Burgers model (equations 5.3 and 5.4) followed the reverse order. The elastic portion of maximum creep compliance (% J_e/J_{max}) of gluten-free doughs depended on water and hydrocolloid content and increased by approximately 65–75%, 45–50%, 35–40%, 25% and 8–15% when xanthan, CMC, pectin, agarose and oat β-glucan, respectively, were added. Despite the concentration effect, the magnitude of influence of hydrocolloids on rheological properties of gluten-free dough also seems to be related to the molecular structure and chain conformation of the polysaccharide that determine the physical intermolecular associations (cross-links or entanglements) of the polymeric chains. Amongst the polysaccharides added, xanthan exhibited the most enhanced elastic properties. The high elasticity of dough formulations supplemented by xanthan could be explained by the well-known weak gel properties and high viscosity values at low shear rates of aqueous xanthan dispersions due to its rigid, ordered chain conformation (Doublier and Cuvelier, 1996; Rodd et al., 2000). Among the other hydrocolloids, β-glucan showed the less pronounced elastic properties, as revealed by the creep and oscillation tests. The weak elastic properties of β-glucan compared to the rest

of the polysaccharides could be attributed to the relatively low molecular size (apparent peak molecular weight $\sim 110 \times 10^3$) of the β-glucan used in this study. The rheological behavior of β-glucan aqueous dispersions has been extensively studied and shown to be strongly dependent on the molecular size of the polymeric chains and their fine structure (Lazaridou et al., 2003; Vaikousi et al., 2004).

The type and extent of influence on bread quality attributes, such as loaf volume and crumb texture, produced from gluten-free doughs fortified with hydrocolloids seems to be dependent on the specific hydrocolloid used and its supplementation level (Lazaridou et al., 2007). The improving effect of several hydrocolloids, such as HPMC, CMC, locust bean gum, guar gum, κ-carrageenan, gum tragacanth, xanthan, β-glucan and psyllium gum, on volume of bread based on gluten-free formulations (Ylimaki et al., 1988; Haque and Morris, 1994; Gujral et al., 2003a; Gallagher et al., 2004; Sivaramakrishnan et al., 2004), as well as on conventional wheat flour breads (Bell, 1990; Delcour et al., 1991; Biliaderis et al., 1995; Rosell et al., 2001; Sharadanant and Khan, 2003; Guarda et al., 2004; Barcenas and Rosell, 2005) has also been reported by various authors. Hydrocolloids are added as gluten substitutes in the formulations of gluten-free breads because they can mimic the viscoelastic properties of gluten in wheat bread dough (Toufeili et al., 1994). A possible explanation is that hydrocolloids can improve dough development and gas retention (Rosell et al., 2001) by increasing dough viscosity (Delcour et al., 1991) or by formation of a weak gel network structure classified as cold-set gel, such as in the case of xanthan (Rodd et al., 2000) and psyllium gum (Haque et al., 1993a). Eggleston et al. (1992) found that xanthan increased the air entrapped in doughs made from cassava flour fortified with 20% soy flour during the mixing stage and contributed to the preservation of the gas retention volume during fermentation. However, the final loaf volume also depends on the dough stability upon baking. Some modified cellulose derivatives such as CMC, HPMC and methylcellulose (MC) have high water retention properties due to their hydrophilic nature, but they also contain hydrophobic groups which induce additional properties including increased interfacial activity within the dough system during proofing, and forming gel networks on heating during the breadmaking process (Bell, 1990; Haque et al., 1993b), similar to those of thermosetting proteins (e.g. egg white proteins). Such network structures serve to increase viscosity and to further strengthen the boundaries of the expanding cells in the dough, thus increasing gas retention through baking, and consequently leading to a better loaf volume. Furthermore, a combination of two different hydrocolloids that stabilize the gas-cell structure over complementary ranges of temperatures can be used in order to optimize their favorable effects on loaf volume. Haque and Morris (1994) added both HPMC and psyllium gum to rice flour dough and produced breads with loaf volumes 2–3 times larger than those obtained by incorporating each hydrocolloid alone. Psyllium gum forms a weak gel network which traps carbon dioxide generated during proofing and is stable up to $\sim 75^{\circ}$C (Haque et al., 1993a; Haque and Morris, 1994). This temperature coincides with the onset of hydrophobic gelation of HPMC, which enhances the stability of gas cells at oven temperatures (Haque et al., 1993b; Haque and Morris, 1994). However, it seems that the loaf volume can be increased up to a certain hydrocolloid concentration, whereas with further increases of the polymer level the loaf volume decreases (Haque and Morris, 1994; Lazaridou et al., 2007). In addition to the polysaccharide concentration, the molecular weight also seems to affect the breadmaking performance of hydrocolloids, as shown in studies on wheat flour breads. Thus, the concentration of wheat arabinoxylans (Biliaderis et al., 1995) and barley β-glucans (Skendi et al., 2006) added to wheat flour doughs, resulting in maximum loaf volumes, was dependant on polysaccharide molecular weight and the baking quality of the wheat flour base; generally, the addition of polysaccharides with high molecular weight

resulted in higher loaf volumes than incorporation of their low molecular weight counterparts. It is also worth noting that in some cases, hydrocolloids may have detrimental effects on bread quality characteristics (e.g. loaf volume and crumb structure), as revealed with incorporation of xanthan into gluten-free formulations based on rice (Haque and Morris, 1994; Lazaridou et al., 2007) and sorghum (Schober et al., 2005) flours; increasing xanthan levels in the dough resulted in a decrease in loaf volume of the gluten-free breads. This behavior of xanthan seems to be in contrast with the results of the mechanical tests that showed the highest strength and elasticity of doughs supplemented with this hydrocolloid (Lazaridou et al., 2007); in fact, the high rigidity of doughs containing xanthan resulted in breads with low loaf volumes and high crumb firmness. There is certainly an optimum value for the resistance of dough to deformation; too high resistance can cause a limited and slow expansion of the gas cells during proofing (van Vliet et al., 1992). Uthayakumaran and Lukow (2003) used reconstituted wheat dough systems and found that storage modulus and dynamic viscosity are negatively correlated with dough extensibility and loaf volume and positively correlated with bread firmness.

In previous studies, it has been found that addition of some hydrocolloids, such as HPMC, MC, CMC, κ-carrageenan and alginate, causes crumb softening of wheat bread and these polysaccharides act as antistaling agents, while inclusion of xanthan can result in an increase of crumb hardness (Bell, 1990; Collar et al., 2001; Rosell et al., 2001; Guarda et al., 2004; Barcenas and Rosell, 2005). Similarly, an increase in crumb hardness with xanthan gum concentration in gluten-free breads from sorghum flour has been reported (Schober et al., 2005). Incorporation of xanthan and oat β-glucan into rice doughs was found to increase the crumb hardness, while inclusion of other hydrocolloids, such as pectin, CMC and agarose did not seem to significantly alter the crumb structure (Lazaridou et al., 2007). Biliaderis et al. (1997) proposed that the effects of the hydrocolloids on starch gel structure and mechanical properties result from two opposite phenomena; first an increase in the rigidity as a consequence of the decrease in the swelling of the starch granules and the reduced amylose leaching from the granules, and second a weakening effect on the composite starch network structure due to the inhibition of interparticle contacts among swollen granules. It is perhaps a combination of these factors that determine the overall effect on mechanical properties of the bread structure, an effect that is dependent on the specific hydrocolloid used and also the processing conditions (temperature and shear affects). Thus, the influence of added hydrocolloids on melting, gelatinization and starch retrogradation processes is reflected on the pasting properties and rheological behavior of doughs as well as the mechanical properties of the end products (Bahnassey and Breene, 1994; Rojas et al., 1999; Zhou et al., 2000). It is well known that phase transition-related phenomena of aqueous starch dispersions extensively depend on water content (Gudmundsson et al., 1991; Kobylanski et al. 2004), type and concentration of starch (Gudmundsson and Eliasson, 1991; Bahnassey and Breene, 1994) and added hydrocolloids (Rojas et al., 1999; Kobylanski et al., 2004) as well as interactions with other components (Biliaderis, 1998; Collar et al., 1999). Kobylanski et al. (2004) studied gluten-free doughs based on corn and cassava starches (75:25) and found that HPMC–water interactions mainly controlled the onset of starch gelatinization; at low water contents and HPMC ($<0.5\%$) the onset temperature of starch gelatinization increased.

5.3.1.4 *Fat, emulsifiers, enzymes and sourdough*

Studies on the role of fat in the stabilization of gas cells in cake batters and in bread doughs have emphasized the importance of the adsorption of a number of fat crystals at

the interface of gas bubbles in doughs (Brooker, 1993, 1996). According to the proposed mechanism when crystals melt during baking, the fat–liquid interface of the absorbed crystals provides a source of extra interfacial material for the bubble surface. This allows expansion without rupture of the bubble. Thus, fats, by their melting phase transitions, may play a role in the stabilization of gas cells in doughs and influence the dough rise during baking (Chevallier et al., 2000). Eggleston et al. (1992) found that margarine increased the air entrapped in gluten-free doughs based on cassava flour fortified with 20% soy flour during the mixing stage, and delayed the critical buoyant size (maximum gas retention volume) over the 60 min fermentation period. These researchers found that margarine reduces the extent of starch gelatinization and solubilization in the cassava bread.

The effect of vegetable seed oil on the rheological properties of rice dough and bread quality has been studied (Gujral et al., 2003a). The dough consistency reported after 5 min of mixing in the farinograph decreased by 20% with increasing oil concentration from 2% to 10% in rice flour doughs showing that oil acts as a lubricant between the particles in the dough and decreases resistance to mixing, that is, lowering dough consistency. These authors also studied the pasting properties of rice dough with the RVA, and revealed that addition of oil to rice flour slightly increased the peak and final viscosities and also the setback, whereas a considerable decrease was observed in the breakdown, probably due to the inhibition of the amylose leaching by complexation of the linear starch component with monoacyl lipids. Dynamic rheometry also showed that the oil increased both the elastic and viscous moduli compared to those of the control formulation. In general, inclusion of oil resulted in improvement of rice bread quality by decreasing crumb firmness and increasing specific volume; bread specific volumes increased by almost three times with an increase in oil content from 2% to 10%.

Fat powders have been used in gluten-free biscuit formulations based on brown rice flour, soy flour and corn and potato flour instead of conventional shortening (solid palm oil), thus making use of the advantages of these powders, such as convenient handling (Schober et al., 2003). In this study, three different fat powders were tested for use instead of palm oil (PO); a high fat dairy powder (HFP) based on cream, a low fat dairy powder (LFP) and a microencapsulated high fat powder (ME) based on vegetable fat (palm and coconut oil). Hardness and stickiness of the biscuit doughs were measured by a SMS-TA system using compression test and the Chen–Hoseney cell, respectively. The pure PO acting as a lubricant resulted in a softer dough, whereas the high amount of protein in LFP contributed to the formation of a stronger structure. Additionally, the sugars present in high amounts may bind water and thus result in doughs with the highest stiffness. Dough stickiness showed an opposite trend in comparison with dough hardness; inclusion of PO gave the stickiest doughs, while LFP the least sticky. Structure-forming substances in the fat powders, mainly in LFP, may bind water and thus account for the lower stickiness of the surface, whereas the PO itself or the excess of moisture in the PO dough may have contributed to the higher stickiness. The lack of fat and the surplus in protein and sugars (high lactose content) in LFP could be responsible for the much firmer biscuits obtained with this formulation, compared to those made from the other fat sources; on the other hand, HFP and ME yielded biscuits of comparable texture to PO.

In addition to hydrocolloids, bread structure improvement, softening effects and antistaling properties have been ascribed to emulsifiers as well. The effects of emulsifiers, also known as surfactants, on retardation of bread staling have been often attributed to their interactions with starch, resulting in retardation of starch chain recrystallization events, and their blocking of moisture migration between gluten and starch, thus preventing starch from taking up water

(Rao et al., 1992; Collar et al., 1998; Stauffer, 2000). The use of emulsifiers also results in better air uptake at the mixing stage, which can be explained by reduction of the surface tension in gas bubbles (Hoseney, 1984). A larger number of smaller air bubbles are mixed in, and thus gas is retained better during the fermentation phase because the gas cells take longer to attain the critical buoyant size. Similar observations have been reported for cassava–soy composite doughs when the emulsifier glycerol monostearate (GMS) was added (Defloor et al., 1991). Incorporation of 4% GMS into a gluten-free formulation doubled the dough volume and enhanced dough consistency, as tested by the mixograph. Viscoamylographs of fermented cassava–soy bread doughs showed that the emulsifier delayed the appearance of maximum viscosity peak and increased dough viscosity during the cooking and cooling phases. With regard to the end products, inclusion of 4% GMS led to a loaf volume increase of 30%, while lower amounts of the emulsifier (2%) were sufficient to ensure a much lower bread firmness after 72 h of storage.

Among the additives used for retarding wheat bread firming are enzymes, such as different α-amylases, hemicellulases and lipases (Martinez-Anaya et al., 1999; Haros et al., 2002; Leon et al., 2002). Rice flour has been shown to be less responsive to the presence of dough conditioners and enzymes (Nishita et al., 1976), most likely due to the hydrophobic nature of the rice proteins compared to the more hydrophilic nature of wheat gluten (Lumdubwong and Seib, 2000). Eggleston et al. (1992) found that cassava flour with a high diastatic activity had a detrimental effect on loaf volume. Gujral et al. (2003a,b) tested two different starch hydrolyzing enzymes, α-amylase of intermediate thermostability and cyclodextrin glycosyl transferase (CGTase) or cyclodextrinase, for their effect on fresh rice bread quality and staling during storage. The addition of α-amylase improved bread specific volume and crumb firmness but resulted in a sticky crumb. The addition of CGTase produced even higher specific volume and better texture; the addition of CGTase to the rice flour increased the specific volume of bread up to 73% and reduced crumb firmness up to 53% (Gujral et al., 2003a). The firming kinetics revealed that CGTase is a better antistaling agent because of its starch hydrolyzing and cyclizing activity (Gujral et al., 2003b). CGTase (EC 2.4.1.19) cleaves α-1,4 glycosidic linkages in starch molecules, and concomitantly links the reducing and non-reducing ends (cyclization reaction) to produce cyclic doughnut-shape molecules of six, seven and eight glucose units, referred to as α-, β- and γ-cyclodextrin, respectively (Ohnishi et al., 1997). These compounds have a polar surface responsible for the aqueous solubility and a hydrophobic inner core. Apart from the cyclization reaction, CGTase is also involved in coupling, disproportionation and hydrolysis reactions, yielding along with cyclodextrins a mixture of small and long oligosaccharides. Therefore, starch hydrolysis by cyclodextrinase yields fermentable sugars, which are metabolized by yeast, and consequently results in high loaf volume and soft crumb (Gujral et al., 2003a). The cyclodextrins produced can also act as emulsifiers, as they can form complexes with triglyceride molecules and monoacyl lipids, lowering interfacial tension. In addition, the cyclodextrins can form complexes with the hydrophobic proteins (globulin and glutelin) of rice, increasing their solubility; such complexes may be further involved in film forming and better entrapment of the CO_2.

Gujral et al. (2003a) studied the rheological properties of rice doughs fortified with CGTase and found that the dough consistency reported after 5 min of mixing in the farinograph decreased with increasing enzyme level from 0 to 40 μL/100 g of rice flour, implying starch breakdown during the mixing process by the cyclodextrinase. Similarly, incorporation of CGTase to the dough lowered the elastic modulus and complex viscosity and increased tan δ. Thus, although the optimum activity of the CGTase is $\sim$70°C, it seems that this

enzyme can act also during the mixing process and proofing period (30°C), resulting in some hydrolysis, which affects the dough rheology. These researchers also investigated the pasting properties of rice flour as affected by CGTase using the RVA. Addition of CGTase lowered the peak viscosity, slightly affected the final viscosity and increased the viscosity breakdown, indicating a strong hydrolytic impact of the enzyme on starch. The setback parameter, which is related to starch retrogradation, was reduced only when the highest enzyme dosage was added, most likely because at high enzyme concentration a high amount of cyclodextrins is produced, which could physically interfere with amylose gelation events. Moreover, the addition of CGTase in the presence of vegetable seed oil resulted in a pronounced decrease in the final viscosity and setback values of rice flour, suggesting interaction between lipids and cyclodextrins and other hydrolysis products obtained from the enzyme action.

Protein cross-linking enzymes, such as transglutaminase and glucose oxidase, are known to induce protein network formation, necessary for gas holding during fermentation (Gujral and Rosell, 2004a,b). With increasing levels of these enzymes added into rice flour the dough consistency increased, as measured by farinography and dynamic rheometry. Improvement in rice protein functionality during breadmaking was thus feasible, yielding rice breads with increased specific volume and a softer crumb.

Recently, the utilization of sourdough technology has been tested for quality improvement and delay of staling of gluten-free breads (Moore et al., 2007). A flour mixture based on brown rice flour, cornstarch, buckwheat, soy flour and xanthan gum was fermented by three different strains of lactic acid bacteria; this sourdough was added at 30% level to a batter made from the same flour mixture. Extrusion measurements conducted by the SMS-TA showed that the sourdoughs were softer during 24 h of fermentation. Instead, the bread batters containing the sourdoughs were notably firmer than the non-acidified and the chemically acidified control batters. Two of the strains tested gave significantly softer breads, after 5 days of storage, than those of the control formulations. Overall, the addition of sourdough seems to reduce the staling of gluten-free breads, although such effects are not as pronounced as those observed with conventional wheat sourdough breads.

5.3.2 Potential problems in gluten-free dough handling

Doughs produced from gluten-free formulations do not have the cohesive and elastic character obtained from wheat flour, because of the absence of gluten. Gluten-free doughs are more fluid than wheat doughs and, due to the lack of a gluten network, are closer to cake batters in viscosity and rheological behavior (Cauvain, 1998). Therefore, many researchers use the term batter to characterize gluten-free doughs used for breadmaking (Defloor et al., 1991; Eggleston et al., 1992; Moore et al., 2004; Schober et al., 2005). These batter-type doughs have to be handled like cake batters rather than typical bread doughs and thus hand-kneading is no longer appropriate (Haque and Morris, 1994; Schober et al., 2005). Instead, mixing is done mechanically, often in a kitchen mixer or in a mixer using a batter attachment (Defloor et al., 1991; Haque and Morris, 1994; Kobylanski et al., 2004; Moore et al., 2004; Lazaridou et al., 2007). In contrast to bread, the gluten network in biscuit doughs needs only to be slightly developed in order for the dough to be cohesive without being too elastic (Contamine et al., 1995). From that point of view, there is an advantage in the case of development of gluten-free biscuit formulations because they allow successful handling with different combinations of ingredients involved (Schober et al., 2005).

As already mentioned, the standard farinograph curve for rice flour fortified or not with hydrocolloids shows a very long time for dough to reach the consistency of 500 BU and

a continuous increase in consistency during mixing time (Sivaramakrishnan et al., 2004; Lazaridou et al., 2007). This shows a poor dough for baking with relaxing stretchable properties and with limited machinability, which creates handling problems during mixing. Sivaramakrishnan et al. (2004) observed that the rice flour particles were inside the gap between the mixing bowl and the mixer blade of the farinograph, resulting in increased torque values toward the end of the measurements. Because of the above problems, researchers often do not use the farinograph parameters for breadmaking. Instead the water content required to give acceptable loaf volume and structure is determined by empirical trial-and-error testing (Haque and Morris, 1994; Gujral et al., 2003a). Haque and Morris (1994) determined the appropriate mixing time of the rice dough also arbitrarily. The dough was mixed until the development of a firm and cohesive dough, as judged by eye and by touch; shorter or longer mixing times gave lower specific volumes for the dough and for the final loaves, and an inferior crumb structure in the bread.

Other potential problems of dough handling could be created by addition of components such as hydrocolloids, because of their hydrophilic nature and their pronounced viscoelastic behavior. Defloor et al. (1991) found that addition of pregelatinized cornstarch to a cassava–soy flour mixture creates problems when this material was added to the formula in a dry form. The results of such experiments were found to lack reproducibility, as indicated by the high degree of variation in the crumb structure of breads obtained from these doughs; prehydration of the starch by conditioning with 20% water (w/w) at room temperature before mixing the batter solved this problem. Moreover, the incorporation of hydrocolloids contributes to dough stickiness (Armero and Collar, 1997), which is an important part of the handling difficulties of these materials (Bloksma, 1990). Sivaramakrishnan et al. (2004) reported that addition of HPMC at 1.5% in rice flour dough resulted in a sticky mass during mixing, while mixing at 3% and 4.5% HPMC supplementation was extremely difficult, as the obtained dough was quite sticky. Furthermore, Schober et al. (2003) developing gluten-free biscuits formulations, found that stickiness as well as rigidity of the dough can create dough-handling problems, which are also reflected to the quality of the end products. Soft and sticky doughs obviously adhere more strongly to the rollers of the sheeter and are then extended more intensely uniaxially during sheeting, resulting in uniaxial tensions in the dough pieces and consequently yielding oval biscuits.

On the other hand, in the development of gluten-free pasta analogs, based on mixtures of buckwheat with rice flour, precooked rice flour or pregelatinized rice starch, an improvement in dough workability was noted with the use of pregelatinized rice materials (Alamprese et al., 2007). With the inclusion of MC, further enhancement in dough workability was also observed during rolling at an industrial plant scale. Similarly to bread batters, the dough hydration level in gluten-free pasta manufacturing has to be adjusted to achieve a proper consistency.

5.4 CONCLUDING REMARKS

A survey of the rather scarce literature information on the rheological properties of gluten-free doughs, as presented in this chapter, points to some interesting relations between data derived from either empirical approaches or mechanical tests based on fundamental rheological principles and end-product quality characteristics. However, the complex interplay between different hydrocolloids and other ingredients used in gluten-free formulations (emulsifiers, sugars, water, etc.) as well as their effects on the physicochemical properties of the dough

during processing are not fully understood. As more research findings become available on the relationships between dough viscoelasticity, baking performance and sensory attributes of the final products, there will be new directions in the development of optimized formulations and the establishment of appropriate manufacturing protocols to improve the sensorial quality and extend the shelf life of such gluten-free bakery items.

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6 Enzymatic Manipulation of Gluten-Free Breads

Cristina M. Rosell

6.1 INTRODUCTION

Currently, enzymes are widely used as technological aids in numerous processes of food technology, as they are considered clean label compounds. In particular, the baking industry has paid special attention to the replacement of several chemical compounds by enzymes. They are considered the best and safest alternative to chemical compounds because enzymes are proteins which have the ability to catalyze chemical reactions, can be labeled as GRAS (generally recognized as safe), and do not remain active after breadmaking due to their protein structure being denatured during baking. Enzymes have been used extensively in the production of cereal-based products with different purposes: improving dough handling properties, improving fresh product quality or extending shelf life. Ingredients such as starch degrading enzymes, non-starch degrading enzymes, lipases, proteases, transglutaminase, glucose oxidase (GO), phytases, etc. are currently present in the daily practice of the bakery industry. The enzymes most frequently used in breadmaking are the α-amylases from different origins (cereal, fungal and microbial) (Rosell et al., 2001) for increasing bread volume, improving crumb grain, crust and crumb color, and for their contribution to flavor development. The hydrolases of non-starch polysaccharides usually bring positive effects on dough and bread characteristics (Haros et al., 2002). Hemicellulase activity is reported to improve gluten elasticity and final bread quality giving better bread volume and crumb porosity in whole wheat bread (Jimenez and Martinez-Anaya, 2000, 2001).

However, in the production of gluten-free products the use of enzymes has been scarcely explored, despite their success in the production of conventional gluten-containing cereal products. The limited use of the enzymes in the production of gluten-free bread may be ascribed to the fact that many commercially-produced enzyme preparations may also contain wheat flour or wheat starch.

Enzymes can be grouped into three different categories according to their main action or effect on the breadmaking process:

- dough structuring agents
- fresh bread quality improvers
- shelf life extension

In this chapter, the role of these different groups of enzymes within the context of gluten-free bread will be discussed.

6.2 ENZYMES FOR IMPROVING GLUTEN-FREE DOUGH STRUCTURE

The majority of fermented cereal-based products are obtained by using wheat flour or other cereal flours containing gluten. Wheat dough behaves as a viscoelastic material that exhibits an intermediate rheological behaviour between a viscous liquid and elastic solid. Schofield and Scott Blair (1983) discussed the main contribution of gluten to the unique properties of wheat dough properties, with it having a predominant role determining both dough machinability and textural characteristics of the finished bread (Collar and Armero, 1996; Uthayakumaran et al., 2000). Gluten is a non-pure protein system that contains 75–86% protein and the remainder is made up of carbohydrates and lipids that are held strongly within the gluten-protein matrix. Although the non-protein components have significant effects, the rheological properties of gluten are derived from the properties and interactions among the proteins. Gluten proteins comprise two main subfractions, glutenins that confer strength and elasticity and gliadins that impart the viscous properties to the gluten dough (Kokini et al., 1994; Khatkar et al., 1995). After mixing and full hydration, gliadins and glutenins interact, giving a cohesive, elastic and viscous network that combines the properties of the two components. The gluten matrix is a major determinant of the important properties of dough, such as extensibility, resistance to stretch, mixing tolerance and carbon dioxide-holding ability during proofing (Rosell and Foegeding, 2006).

Gluten can be considered as the structural component in bread, and provides structure and texture in other bakery products also. Its alteration by protease degradation or its complete absence often results in a viscous system or even a liquid bread batter rather than dough (Rosell et al., 2002). Baking without gluten results in many problems, including a weak or poorly developed dough structure, a crumbling bread texture, poor color and other quality defects.

Rice and corn are safe for a gluten-free diet; wheat, spelt, kamut, rye, triticale, barley and possibly oats are never safe, and must be eliminated from the celiac diet. Most gluten-sensitive individuals can tolerate buckwheat, millet, amaranth, quinoa, teff and/or sorghum. Rice flour is a very suitable cereal flour for the production of gluten-free bread due to its bland taste, white color, easily digested carbohydrates and hypoallergenic properties (Neumann and Bruemmer 1997). Additionally, other attributes such as the low content of protein and sodium and the low levels of prolamins are also of benefit.

However, despite the numerous advantages of rice flour, rice proteins have relatively poor functional properties for food processing in comparison with other proteins. Rice proteins are extremely insoluble due to their hydrophobic nature (Lumdubwong and Seib, 2000), and are unable to form the viscoelastic matrix necessary to retain the carbon dioxide produced during proofing of the yeast-leavened bread-like products. The low content of prolamins in the rice flours are unable to form a dense gluten-like protein network when rice flour is kneaded with water. As a consequence, the carbon dioxide produced during fermentation cannot be retained thus yielding a product with low specific volume that does not resemble the soft and open structure of wheat bread. In the absence of gluten, different strategies have been used in order to mimic its viscoelastic properties. Different polymeric materials, mainly falling within the category of gums and starches, have been proposed (Nishita et al., 1976; Kang et al., 1997) for providing the necessary network for the gluten-free bread.

Hydroxypropylmethylcellulose (HPMC), locust bean gum, guar gum, carrageenan, xanthan gum and agar have been tested on rice bread (Kang et al., 1997), yielding products

with a good specific volume (Gallagher et al., 2004; Gujral et al., 2003a; Gujral et al., 2004; Sivaramakrishnan et al., 2004; McCarthy et al., 2005). Starches from corn, cassava and potato have been widely used in the formulation of gluten-free bread (Sánchez et al., 2002; López et al., 2004; Schober et al., 2005), and sometimes, composite flours are obtained by mixing diverse flours with a high proportion of different starches (Osella et al., 2005).

Another area which has been scarcely explored is the use of enzymes for creating a protein network through the formation of covalent cross-links between the polypeptide chains. Protein functionality can be modified through the formation of intramolecular or intermolecular cross-links (Gerrard, 2002; Jong and Koppelman, 2002), obtaining certain bridges among the polypeptide chains and resembling a gluten-like network.

6.3 USE OF TRANSGLUTAMINASE IN THE PRODUCTION OF GLUTEN-FREE BREAD

Transglutaminase (protein-glutamine γ-glutamyltransferase) (EC.2.3.2.13) catalyzes an acyl-transfer reaction between the γ-carboxyamide group of peptide-bond glutamine residues (acyl donors) and a variety of primary amines (acyl-acceptors) including the ε-amino group of lysil residues in certain proteins (Motoki and Seguro, 1998; Gerrard, 2002) (Fig. 6.1). Primary amino groups in a variety of compounds may act as acyl acceptors with the subsequent formation of monosubstituted γ-amides of peptide-bound glutamic acid. ε-Amino groups of lysyl residues in proteins can also serve as substrates, generating intra- or intermolecular ε-(γ-glutamyl)lysyl cross-links, which are isopeptide bonds (Zhu et al., 1995; Jong and Koppelman, 2002). This is the most dominant reaction, but transglutaminase also catalyzes two other reactions: the incorporation of amine groups when primary amines are linked to a γ-carboxyamide group on protein-bound glutamine residues. In the absence of amine substrates, water is used as an acyl acceptor leading to the glutamine deamination (Fig. 6.1). Transglutaminase catalyzes the cross-linking of a wide amount of proteins, including those from milk, soy, casein, conalbumin, lactalbumin, gelatin, myosin, pea legumin or oat globulin (Ikura et al., 1980; Larre et al., 1993; Babiker et al., 1996; Siu et al., 2002; Rosell et al., 2003;

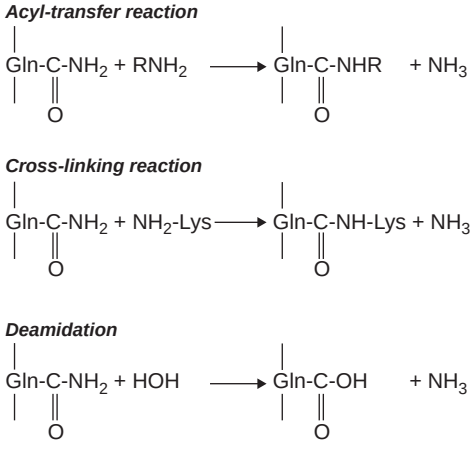

Fig. 6.1 Reactions catalyzed by the transglutaminase.

Kolodziejska et al., 2004). The cross-linking of wheat proteins has been widely investigated in numerous studies: these have reported both the biochemical and rheological effects of the transglutaminase on wheat dough (Gerrard et al., 1998; Larré et al., 1998; Bauer et al., 2003; Mujoo and Ng, 2003; Rosell et al., 2003; Collar and Bollain, 2004, 2005; Collar et al., 2005). Transglutaminase is also able to restore the functional and biochemical properties of damaged wheat or wheat that has undergone hydrolysis by proteases (Bonet et al., 2005; Caballero et al., 2005). Lately, it has been suggested that transglutaminase in wheat-baked products may act upon the gliadin proteins to generate the epitope associated with the celiac response; however, this hypothesis has yet to be confirmed (Gerrard and Sutton, 2005).

One of the main problems, associated with gluten-free bread is obtaining a good crumb structure. Gujral and Rosell (2004a) used transglutaminase with rice flour to create a protein network through the formation of covalent cross-links between the polypeptide chains of the rice flour. The enzyme reaction among the rice proteins was confirmed by a progressive decrease of the amount of free amine groups when increasing the enzyme concentration. In fact, the addition of 1.0 U of transglutaminase/gram of rice flour resulted in a 36% reduction of the free amine groups of the rice flour. Further increases in the enzyme concentration did not promote any significant decrease in the free amine group amount (Gujral and Rosell, 2004a). However, the transglutaminase level required for network formation is greatly dependent on the particular protein structure and the disposition of the lysil and glutamine residues (Han and Damodaran, 1996). A concomitant decrease in the amount of free thiol groups was also reported as a side-effect of the transglutaminase reaction. It is most likely that the cross-linking reaction catalyzed by transglutaminase may draw together some sulfur-containing amino acids leading to the formation of disulfide bonds by spontaneous reaction (Gujral and Rosell, 2004a; Bonet et al., 2005).

The addition of transglutaminase to rice flour has a strong influence on the small deformation properties of the resulting dough (Gujral and Rosell, 2004a, Table 6.1). The values of the elastic and viscous moduli (G' and G'', respectively) indicate that rice dough behaves as elastic solid ($G' > G''$), and the presence of increasing amounts of transglutaminase resulted in a steady increase of both the storage (G') and loss (G'') moduli. Therefore, the addition of transglutaminase modified the elastic and viscous components of the rice dough, inducing a strengthening effect (increase in G' and G'') on the dough due to cross-linking (Gujral and Rosell, 2004a). The complex modulus G^* that combines the elastic and viscous components $[(G^*)^2 = (G')^2 + (G'')^2]$ also increased with increasing transglutaminase levels. Despite the low lysine content of the rice flour, the cross-linking activity of transglutaminase was sufficient to improve the viscoelastic (increase the elastic and viscous moduli) properties of the dough.

Table 6.1 Viscoelastic parameters of dough made from rice flour in the presence of varying transglutaminase levels at 1 Hz of frequency.

Transglutaminase (U/g of flour)	G' (Pa)	G'' (Pa)	G^* (Pa)
0	3789	527	3825
0.25	4602	634	4645
0.50	4681	654	4726
1.00	5291	894	5366
1.50	6289	1026	6372

Source: Data adapted from Gujral and Rosell (2004a).

Table 6.2 Effect of increasing levels of transglutaminase on the technological quality of rice bread.

Transglutaminase (U/g of flour)	Specific volume (cm^3/g)	Crumb hardness (N)
0	1.47 ± 0.06	13.8 ± 0.6
0.5	1.57 ± 0.09	10.7 ± 1.0
1.0	1.70 ± 0.04	8.9 ± 0.9
1.5	1.36 ± 0.06	16.2 ± 0.7

Source: Data adapted from Gujral and Rosell (2004a).
Data are the mean of at least three replicates ± standard deviation.

Protein cross-linking, catalyzed by transglutaminase, results in the formation of a protein network that is able to retain the dough structure during proofing and baking. Gujral and Rosell (2004a) found an increase in the specific volume of the rice bread in the presence of transglutaminase (Table 6.2). The highest specific volume (1.7 cm^3/g) was obtained by adding 1.0 U of transglutaminase/g of rice flour (this is in comparison to 1.47 cm^3/g for the sample with no transglutaminase addition). Higher levels of transglutaminase did not promote any further improvement, but in fact had a deteriorating effect. Simultaneously, a decrease in the crumb hardness of the rice bread was observed (Table 6.2), reaching the maximum softness with 1.0 U of transglutaminase/g of rice flour; this can be explained by the inverse relationship between the crumb hardness and the specific volume. The negative effect observed with excessive levels of transglutaminase has been ascribed to extremely firm dough resulting from the numerous cross-links formed (Gujral and Rosell, 2004a).

The cross-linking action can be applied to the glutamines and lysines of two different types of proteins (Jong and Koppelman, 2002). Several authors have described indirect evidence of the formation of heteropolymers by transglutaminase, such as between casein and gelatine (Nonaka et al., 1997) or soybean and whey protein isolate (Yildirim and Hettiarachchy, 1997).

The addition of external sources of protein has been suggested for increasing the number of lysine residues, which are the limiting factor of the cross-linking reaction (Moore et al., 2006). Exogenous protein sources like soya flour, skim milk powder or egg powder were added (12.5% composite flour basis) to a gluten-free bread formulation (containing rice flour, potato starch, corn flour, xanthan gum) in the presence of increasing levels of transglutaminase. Confocal laser scanning micrographs of the bread crumb confirmed cross-linking of dairy proteins, although very high amounts of transglutaminase (10 U of transglutaminase/g of protein) were needed, most likely due to the thermodynamic incompatibility between polar and apolar surfaces of the milk proteins (Moore et al., 2006). The gluten-free breads containing skim milk powder and high level of transglutaminase had a compact crumb characteristic, with high number of cells/cm^2, determined by image digital analysis. In addition, egg powder in the presence of transglutaminase gave a protein network similar to that of wheat bread, and an improvement in the specific volume of gluten-free breads was also found (Moore et al., 2006). No significant interactions were obtained between the soya flour and transglutaminase in the gluten-free breads.

The compatibility between rice flour proteins and different protein isolates (pea, soybean, egg albumen and whey proteins) in the cross-linking reaction catalyzed by transglutaminase has been evaluated by studying rice dough behaviour when subjected to small deformations (Marco and Rosell, 2008). The elastic modulus (G') recorded in the oscillatory tests was significantly affected by both the protein isolates and the transglutaminase. The extent of

the effect was dependent on the protein source; pea and soybean proteins increased the elastic modulus, whereas egg albumen and whey protein decreased it. A deeper evaluation of possible cross-links in the presence of soybean proteins, by using gel electrophoresis, indicated that the main protein fractions involved in those interactions were β-conglycinin and glycinin of soybean and the glutelins of the rice flour, although albumins and globulins were also cross-linked (Marco et al., 2008). The interaction between rice proteins and soybean proteins was strengthened by the formation of new intermolecular covalent bonds catalyzed by transglutaminase and also the indirect formation of disulfide bonds. With regard to pea proteins, the presence of transglutaminase resulted in the disappearance of numerous protein bands, with reduced solubility. The main protein fractions involved in those interactions were the albumins and globulins from the pea protein isolate and rice flour, but also the glutelins were cross-linked (Marco et al., 2007). Initial studies carried out with soybean and pea proteins revealed that their combination with the transglutaminase is a good approach for creating a protein network in the gluten-free doughs.

6.4 USE OF GLUCOSE OXIDASE AS STRUCTURING AGENT IN THE PRODUCTION OF GLUTEN-FREE BREAD

Glucose oxidase (GO) [EC 1.1.3.4] is one of the most interesting oxidative enzymes in the food industry. It catalyzes the oxidation of β-D-glucose in the presence of O_2 (Fig. 6.2), producing D-gluconic acid and a molecule of hydrogen peroxide that can cause the oxidation of free sulfhydryl units from gluten protein giving disulfide linkages, or the gelation of water-soluble pentosans, changing the rheological properties of wheat dough (Hoseney and Faubion, 1981; Primo-Martin et al., 2003). This hypothesis was confirmed by Vemulapalli et al. (1998), who found that free thiol groups of the water-soluble proteins of flour or dough decreased in the presence of GO. Recently, the simultaneous formation of dityrosine cross-links by treating proteins with hydrogen peroxide or peroxidase has been described (Singh, 1991; Oudgenoeg et al., 2001; Tilley et al., 2001). Rasiah et al. (2005) stated that

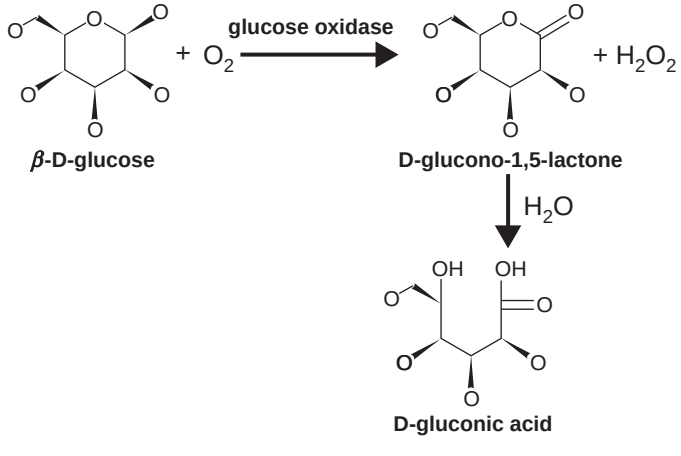

Fig. 6.2 Reaction catalyzed by the glucose oxidase.

Table 6.3 Viscoelastic parameters of dough made from rice flour in the presence of varying glucose oxidase levels at 1 Hz of frequency.

Glucose oxidase (U/g of flour)	G' (Pa)	G'' (Pa)	G^* (Pa)
0	3789	527	3825
1	4238	591	4279
2	4455	683	4507
3	5510	805	5569

Source: Data adapted from Gujral and Rosell (2004b).

the treatment of wheat flour with GO resulted in the cross-linking of water-soluble protein (albumin and globulin) fractions, involving both disulfide and non-disulfide linkages. Protein characterization by high performance capillary electrophoresis and cryo-scanning electron micrographs of the resulting doughs revealed that the GO treatment also modified the gluten proteins (gliadins and glutenins) through the formation of disulfide and non-disulfide cross-links (Bonet et al., 2006). The addition of GO increases the wheat dough stress, the strain hardening (Dunnewind et al., 2002) and the loaf volume, and improves the crumb grain of wheat bread (Vemulapalli et al., 1998; Xia et al., 1999).

Gujral and Rosell (2004b) demonstrated the ability of GO to modify rice flour proteins. Their studies showed that the amount of sulfhydryl groups in the rice flour dough decreased by almost 41.3% when GO was added at 1.0 enzyme units/g of flour as a consequence of the formation of disulfide bridges, and this decrease was in parallel to the increase of the level of GO added. Hydrogen peroxide released from the enzyme reaction causes the oxidation of the free sulfhydryl units from the rice protein giving disulfide linkages; this may result in stronger dough being obtained. In the case of wheat dough, such an effect results in greater resistance to mechanical shock, better oven spring and larger loaf volume (Vemulapalli et al., 1998). It is likely that pentosans are also responsible for this effect, as because they are able to absorb high amounts of water through inter-chain associations involving oxidative coupling and chain entanglements. The decrease in sulfhydryl groups could also be attributed to their reaction with the activated double bond of the ferulic acid resulting in a linkage between arabinoxylans and adjacent protein molecules (Hoseney and Faubion, 1981).

Dynamic oscillatory studies of rice flour dough containing increasing levels of GO showed that the elastic modulus (G') was higher than the viscous modulus (G''), describing a solid elastic-like behaviour of the rice flour dough (Table 6.3) (Gujral and Rosell, 2004b). These moduli were frequency dependent, and increased with increasing frequency. This effect was more pronounced at higher frequencies (10 Hz). The viscous and elastic moduli increased with increasing levels of enzyme addition, thus more work was needed to deform the doughs containing GO (Table 6.3). Changes in dough rheological properties may be attributed to the ability of hydrogen peroxide to induce the gelation of water-soluble pentosans, and the formation of disulfide bonds in the rice flour. This has previously been suggested for wheat dough (Hoseney and Faubion, 1981; Crowe and Rasper, 1988). Other authors have also observed an increase in the elastic and viscous moduli of wheat dough following the addition of GO (Vemulapalli et al., 1998; Dunnewind et al., 2002).

In their studies, Gujral and Rosell (2004b) found that the specific volume of rice flour breads was positively affected with the addition of GO (Fig. 6.3), and an increase in the specific volume was obtained by raising the enzyme concentration in the rice bread formulation. At a high level of GO addition (3 U/g rice flour), a rice flour bread with a specific

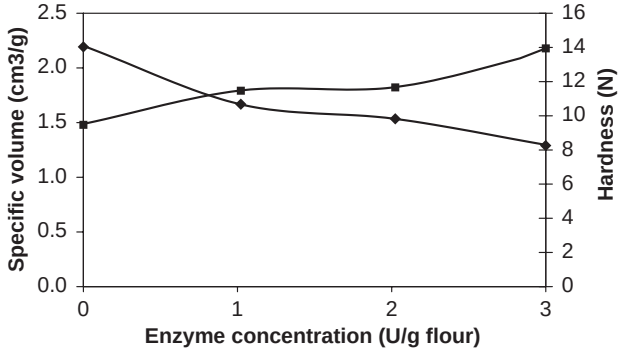

Fig. 6.3 Effect of varying levels of glucose oxidase on the specific volume (■) and crumb hardness (♦) of the rice flour breads. (Data collated from Gujral and Rosell, 2004b).

volume of 2.12 cm^3/g was obtained. In wheat bread, there is a good relationship between the specific volume and the crumb hardness, and the same trend has been observed in gluten-free bread. The crumb hardness of the rice flour bread decreased with increasing levels of GO addition. In fact, a decrease in hardness of approximately 42% was observed when 3 U/g flour of GO was added (Fig. 6.3). This study revealed that the addition of GO to rice flour resulted in the protein cross-linking, evidenced by a decrease in the amount of free thiol and amino groups, and the change of the rice glutelin electrophoregrams (Gujral and Rosell, 2004b). The increase observed in the elastic and viscous moduli of the rice flour dough also supports the idea of the formation of an artificial protein network, which combined with the strengthening effect resulting from the gelation of the pentosans might give a structure with similar functionality to the gluten network.

In unpublished data by Rosell and Marco, no synergistic effect was observed with the combined addition of GO (1 U/g rice flour) and transglutaminase (1 U/rice flour) on bread characteristics. The specific volume of the rice flour bread containing both enzymes was lower than that obtained by the individual addition of the proteins, indicating a possible antagonistic effect. The same trend was observed with the crumb texture of the rice bread.

6.5 LACCASE UTILIZATION IN THE PRODUCTION OF GLUTEN-FREE BREAD

Laccase (p-diphenol oxygen oxidoreductase) (EC 1.10.3.2) is another oxidative enzyme, which has attracted considerable interest in breadmaking. Laccase catalyzes the oxidative gelation of feruloylated arabinoxylans by dimerization of their ferulic esters (Figueroa-Espinoza et al., 1998; Labat et al., 2001). A model system has been proposed for explaining the attachment of arabinoxylans to proteins via tyrosine-ferulic acid linkages (Oudgenoeg et al., 2001; Piber and Koehler, 2005); however, the involvement of laccase on the protein linkages to water-extractable arabinoxylans has yet to be clearly proven (Figueroa-Espinoza et al., 1998; Labat et al., 2001). However, the formation of a network structure through the activity of laccase could be another alternative in gluten-free research. Studies by Gujral and Rosell (unpublished data) have shown that the addition of small levels of laccase (1.5 enzyme units/g rice flour) resulted in an improvement of the bread specific volume,

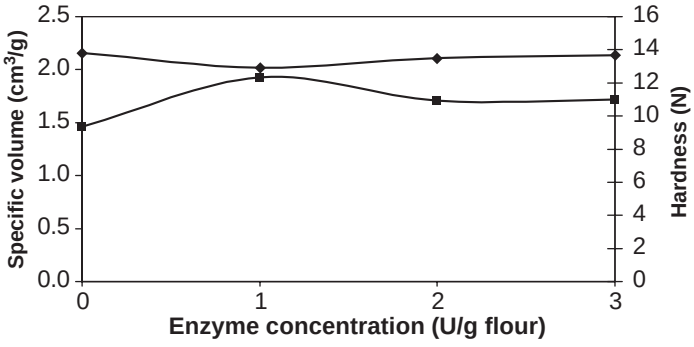

Fig. 6.4 Effect of increasing levels of laccase on the specific volume (■) and crumb hardness (♦) of the rice flour breads. (Data collated from Gujral and Rosell).

but further increases of enzyme levels had a detrimental effect on the quality of the bread. This was reflected by an increase in the crumb hardness (Fig. 6.4).

6.6 ENZYMES FOR IMPROVING FRESH GLUTEN-FREE BREAD CHARACTERISTICS

Gluten-free breads are usually characterized by deficient quality characteristics in comparison with wheat breads. Problems related to volume and crumb texture are associated with gluten-free breads when suitable ingredients, such as rice flour, are used. Nishita et al. (1976) reported that the hydrophobic nature of rice flour proteins may result in the flour being unresponsive to the presence of dough conditioners and enzymes. However, recent research has highlighted the potential of some enzymes in the manufacture of rice-based breads (Gujral et al., 2003a; Gujral and Rosell, 2004a, b). Sections 6.3 and 6.4 have already described the improving effects obtained with the addition of GO or transglutaminase on the specific volume and crumb hardness of rice flour breads.

The cyclodextrin glycosyltransferase (EC 2.4.1.19) is able to catalyze four different reactions: (i) cyclization, (ii) coupling, (iii) disproportionation and (iv) hydrolysis (Ohnishi et al., 1997). This enzyme is active at pH 5.0 and temperature up to 95°C (Starnes, 1990; Wind et al., 1995; Slominska and Sobkowiak, 1997). Cyclodextrin glycosyltransferase cleaves α-1,4 glycosidic linkages in starch molecules while at the same time linking the reducing and non-reducing ends (cyclization reaction) to produce cyclic molecules (Ohnishi et al., 1997). These molecules, called cyclodextrins are closed circular molecules of six, seven or eight glucose units, typically referred as α-, β- or γ-cyclodextrins, respectively. Their most characteristic property is that they have a hydrophilic exterior, which can dissolve in water and a hydrophobic cavity that can form inclusion complexes with a wide variety of hydrophobic guest molecules. The cyclodextrins may form complexes with fatty acids and emulsifiers affecting the starch rheological properties and functionality of the resultant starch. β-cyclodextrin is the most easily produced and purified of the cyclodextrins and it is used for reducing the cholesterol in a wide variety of foods (Cully and Vollbrecht, 1994; Smith et al., 1995) and modifying the pasting properties of different starches (Kim and Hill, 1984a, b; Li et al., 2000; Liang et al., 2002). Cyclodextrins behave as surface-active

agents and form complexes with oil, providing greater emulsion stability. The pasted starch-containing cyclodextrins exhibits enhanced emulsifying properties, and lower elastic and viscous behaviour (Gujral and Rosell, 2004c).

Apart from the cycling reaction, cyclodextrin glycosyltransferase is also involved in coupling, disproportionation and hydrolysis yielding a mixture of small and long oligosaccharides along with cyclodextrins (Ohnishi et al., 1997). Due to the special properties of the cyclodextrins, cyclodextrin glycosyltransferase is currently used for the production of different foods and other materials (Starnes, 1990; Mutsaers and Van Eijk, 1995). Cyclodextrin glycosyltransferase has found application in high fructose corn syrup production and the reduction in viscosity achieved is similar to that obtained with α-amylase (Starnes, 1990). In the case of rice bread, due to the hydrophobic nature of the rice proteins, the use of cyclodextrin glycosyl transferase could reduce the hydrophilic environment by hydrolyzing and cycling the starch and also through the hydrolysis products that can form complexes with a variety of solid, liquid and gaseous compounds.

An experimental design was developed by Gujral et al. (2003a) to optimize the performance of cyclodextrin glycosyltransferase, HPMC and oil in rice flour bread formulation. Optimum specific volume, shape index and crumb texture in gluten-free breads was obtained by using 0.066 U cyclodextrin glycosyltransferase/100 g flour and 4% and 6% hydrocolloid and oil, respectively (Gujral et al., 2003a; Rosell and Solís, 2005). A further improvement (softer crumb and higher bread volume) was obtained by increasing the enzyme level in the rice flour dough to 0.122 U/100 g flour (Table 6.4). The presence of cyclodextrins was chromatographically detected on the bread crumb, confirming the cycling activity along the breadmaking process. Therefore, the improving effect of the cyclodextrin glycosyltransferase in the gluten-free breads results from a combination of its hydrolyzing and cycling activities. The hydrolysis is responsible for the release of cyclodextrins, which have the ability to form complexes with lipids and proteins; whereas cyclization provides the necessary substrates for the complex formation between lipids and proteins with the cyclodextrins.

The α-amylases are the enzymes which are most frequently utilized in commercial bakeries, due to their positive effect on bread volume, improvement of crumb grain, crust and crumb color and flavor development. (Armero and Collar, 1996; Qi Si, 1996; Sahlstrom and Brathen, 1997; Martínez et al., 1999; Rosell et al., 2001). The α-amylase is an endo-enzyme that randomly hydrolyzes the α -1,4 glucosidic linkages in polysaccharides, resulting in short chains which are further fermented by yeast. However, they can hydrolyze only damaged or gelatinized starch, as these are susceptible to enzyme attack. Besides being necessary for fermentation, the polysaccharides obtained from the hydrolytic activity also participate in the Maillard reactions that take place during baking.

Table 6.4 Effect of cyclodextrin glycoxyltransferase on the rice flour bread characteristics.

Sample	Enzyme levels (U/100 g flour)	Specific volume (cm^3/g)	Crumb hardness (N)
Control	0	2.5	3.1
Cyclodextrin glycoxyltransferase	0.066	4.3	2.4
	0.122	3.8	2.1
α-amylase	15	3.2	2.2
	30	3.3	2.2

Source: Data adapted from Gujral *et al.* (2004).

When α-amylase was added to a rice bread recipe, the resulting bread had a specific volume of 3.2 mL/g (Table 6.4), which was higher than that obtained without the use of this enzyme (2.5 mL/g), but was still lower than that obtained with the cyclodextrin glycosyltransferase. In relation to the crumb texture, the presence of α-amylase reduced crumb hardness by almost the same extent as that promoted by the cyclodextrin glycosyltransferase (Table 6.4) (Gujral, Rosell, unpublished data). However, gluten-free breads containing α-amylase were still found to have quite a sticky crumb texture.

6.7 ENZYMES USED FOR EXTENDING THE SHELF LIFE OF GLUTEN-FREE BREADS

Firming of bread crumb during storage is a common phenomenon, leading to a crumbly texture and a reduced consumer acceptance. Staling of baked products is generally defined as an increase in crumb firmness and a parallel loss in product freshness (Hebeda et al., 1990). In spite of the numerous attempts to explain the mechanism responsible for bread staling, several aspects remain unclear. Staling is a complex process, and since starch is the major component of the system, a predominant factor relating to bread staling must also relate to starch retrogradation, which involves the progressive association of gelatinized starch segments into a more ordered structure (Seow and Teo, 1996; Zobel and Kulp, 1996). Retrogradation of rice starch has been reported to be higher than that of wheat starch (Baker and Rayas-Duarte, 1998). Rice bread has a drier and more crumbly texture and is more prone to retrograde during storage than wheat bread (Kadan et al., 2001). Different approaches have shown that it has been possible to obtain fresher gluten-free rice bread of almost comparable quality to wheat bread; nevertheless, rice bread still has the problem of a short shelf life due to its tendency to retrograde (Nishita et al., 1976; Kadan et al., 2001).

Several additives have been used for retarding wheat bread firming. Some examples are enzymes (different α-amylases, hemicellulases, lipases) (Martínez et al., 1999; Rosell et al., 2001; Haros et al., 2002). However, as already mentioned, rice flour is less responsive to the presence of dough conditioners and enzymes (Nishita et al., 1976), probably due to the hydrophobic nature of its proteins. Therefore the need to explore new aids to prevent staling in rice bread is important.

Two different starch-hydrolyzing enzymes (α-amylase of intermediate thermostability, and cyclodextrin glycoxyltransferase) have been used for extending the shelf life of gluten-free breads. Both enzymes decreased the ability of amylopectin to retrograde during storage. α-amylase of intermediate thermostability is the enzyme most widely used as an anti-staling agent. Its anti-staling effect has been attributed to the low molecular weight dextrins produced as a result of starch hydrolysis. These dextrins interfere with the ability of the amylopectin to retrograde (Lin and Lineback, 1990; Defloor and Delcour, 1999; Rojas et al., 2001; Leon et al., 2002), or with other interactions also, namely starch –protein or protein-protein entanglement involved in firming (Lin and Lineback, 1990; Martin and Hoseney, 1991). α-amylase of intermediate thermostability improves the shelf life of gluten-free bread. An enzyme dosage of 600 MANU/kg gluten-free premix has shown significant improvement in the crumb softness and elasticity of a gluten-free bread (Novozymes, 2004). Dough characteristics remained unchanged, so no alternation in the mixing process was required. The crumb hardness of the gluten-free bread increased with the time of storage, but in the presence of α-amylase the increase was significantly lower than that observed for the control which contained no enzyme (Fig. 6.5).

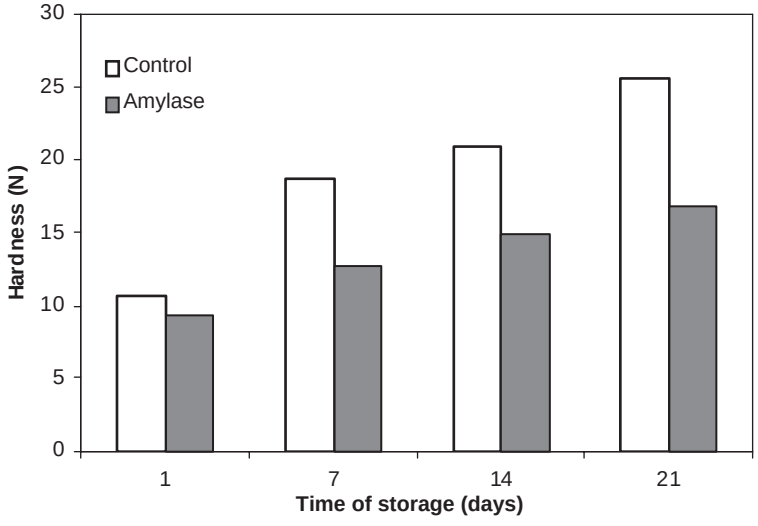

Fig. 6.5 Effect of α-amylase of intermediated thermostability on the crumb hardness of gluten-free breads during storage at 25°C. (Data collated from Novozymes, 2004).

The addition of cyclodextrin glycosyltransferase also contributed to extending the shelf life of rice bread, acting as an anti-staling agent through its hydrolyzing and cycling activity (Gujral et al., 2003b). It has been reported that when the kinetics of the hardening process during staling are analyzed by using regression equations and applying the Avrami equation (Collar and Armero, 1996), both enzymes (α-amylase of intermediate thermostability and cyclodextrin glycoxyltransferase) decrease the rate of the crumb hardening during storage (Gujral et al., 2003b). However, the α-amylase did not decrease the limiting hardness, while the rice crumb in the presence of cyclodextrin glycoxyltransferase firmed quickly having a very short range of firmness increase. Gujral et al. (2003b) established that the starch hydrolysis induced by the α-amylase is not sufficient to retard staling, instead the cyclodextrin glycoxyltransferase has a pronounced anti-firming effect on the rice crumb by decreasing the total hardness increase. The hydrophobic nature of the rice proteins might require the special characteristics of the cyclodextrins released from the cyclization reaction catalyzed by cyclodextrin glycoxyltransferase. The cyclodextrins ability to form inclusion complexes with proteins and lipids, which decrease the interfacial tension acting in consequence as emulsifiers (Shimada et al., 1992; Liang et al., 2002) may be partially responsible for the anti-staling effect induced by the cyclodextrin glycoxyltransferase.

Some specialty breads have been adapted to obtain gluten-free products which would address people with gluten intolerance. This is the case of chapatti, unleavened bread made from whole wheat in India. The use of different hydrocolloids (HPMC, guar gum, xanthan gum, locust bean gum) and α-amylase in the formulation of rice flour chapati improved the texture and keeping properties during storage (Gujral et al., 2004). In addition, hydrocolloids and α-amylase delayed the amylopectin retrogradation, keeping the freshness of the chapattis for longer period.

All the enzymes that are currently used in the bakery industry can be used for gluten-free products, if they are free of gluten. Therefore, the enzymes preparation must also be free of gluten-containing ingredients. The companies which produce bread improvers have solved

that problem by producing concentrated enzymes preparations that do not contain any other compound.

Recent scientific studies have proven that enzymes can be used as processing aids in gluten-free breadmaking, improving dough handling properties, fresh bread quality and also extending the shelf life. Nevertheless, prior to their addition to a bread formulation, preliminary studies to optimize the quantity of enzyme to be added to the formulation are necessary.

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7 Commercial Aspects of Gluten-Free Products

Lorraine Heller

The market for gluten-free foods has exploded in recent years, and today's consumer can find more gluten-free products on supermarket shelves than ever before.

The growth of the category can be put down to three major factors:

1. An increase in the number of people diagnosed with coeliac disease, and corresponding increased consumer demand.
2. A more developed and responsive food industry eager to tap into any new niche that represents a market opportunity.
3. Developments in science and technology that allow for the production of tasty and marketable gluten-free foods.

This chapter will focus on how gluten-free foods have developed from a niche category to a mainstream food group. Topics to be examined include the incidence of gluten intolerance, the importance of gluten in the formulation of baked goods, industry response to the challenges of removing gluten, regulations for the labelling of gluten-free foods, market growth for gluten-free products, the gluten-free consumer, challenges and opportunities in marketing gluten-free products, examples of products in the category currently available on the marketplace, retail channels for gluten-free products and forecast category growth.

7.1 GLUTEN AND INTOLERANCE

Gluten is a protein found in a number of cereal grains, including wheat, rye and barley. It is essentially what is responsible for the structure of baked goods, and is often described as the 'glue' that keeps baked goods from crumbling and provides them with their familiar and desirable texture.

However, gluten has also been identified as the trigger for coeliac disease. This disease is an auto-immune condition, not an allergy, and although it is not a life-threatening condition, it can severely compromise the quality of life of a sufferer. Some symptoms of coeliac disease include abdominal cramping, bloating, gas and constipation. The condition has also been linked to depression, infertility and osteoporosis (National Institutes of Health, 2004). Coeliac disease is a lifelong condition, and can only be managed through complete avoidance of gluten in the diet.

7.2 PEOPLE AFFECTED GLOBALLY

The prevalence of coeliac disease in Europe ranges from 1 in 50 in Finland to 1 in 232 in the Netherlands. (Lohi et al., 2007). In the UK, although the diagnosed sufferers of coeliac disease are numbered at 125 000, the charity Coeliac UK, which provides information and support for people suffering from the condition, estimates that around 500 000 – or one in 100 – people are affected, when including those undiagnosed sufferers. In the US too, current research shows that around one in 133 Americans are affected by coeliac disease (NIH Consensus Development Conference on Coeliac Disease, 2004).

However, it is also thought that around 97% of the coeliacs in the US remain undiagnosed. The National Institutes of Health Consensus Development Conference Statement on Coeliac Disease (2004) estimates that three million Americans, a little less than 1% of the population, may have coeliac disease.

7.3 THE IMPORTANCE OF GLUTEN

A gluten network is formed during the kneading process of dough, creating a three-dimensional structure that brings elasticity to the mix. This network is also what traps carbon dioxide bubbles that occur during fermentation, which ultimately causes the dough to rise. When the dough is baked, the network sets/solidifies, and this contributes to the final stability of the product.

These unique properties make gluten almost indispensable to the formulation of baked goods (in particular, breads). Until quite recently, 'gluten-free' items were found only in specialty food stores, and were associated with bland, crumbly, colourless products.

7.4 CHALLENGES

As demand for gluten-free increased, the food industry has stepped up to the formulation challenges associated with removing the protein from dough mixes. Alternative ingredients were tested, bulking agents were examined and different processing methods were developed.

In general, popular substitutes for wheat flour have included starchy flours, such as rice, corn, potato and tapioca. The starch in these can be used to confer the bulk of a gluten-free bread, but additional gums and celluloses are generally needed in order to create the necessary structural network and obtain a relatively soft texture.

However, using these alternatives presents new challenges that need to be dealt with, for example the high starch content in bread can also reduce its shelf life. Additionally, the need for more ingredients – such as gums – would significantly increase manufacturer costs. Another negative side effect would be that the additional ingredients would necessarily lengthen a product's ingredient list, which could put off the discerning consumer who may equate a short ingredient list with a more 'natural' and therefore a 'better' product.

One solution found by manufacturers and food technologists was to develop the 'right' mixture of different alternative flours in order to achieve the desired effect without the need for additional ingredients. The German company Hanneforth Food For You, for example, has developed a number of bakery pre-mixes derived from different mixtures of rice flours, potato flours, buckwheat, chestnut and even plantain flour.

Food scientists at the US government's Agricultural Research Service (ARS), the research branch of the US Department of Agriculture (USDA), developed a wholegrain rice bread mix and a rice flour bread (Kadan et al., 2001; Kadan and Schluckebier, 2007), which were designed specifically to meet the needs of the gluten-free market. The development involved experimenting with different rice cultivars and flour particle sizes until the ideal combination was found.

Scientists in Ireland's Ashtown Food Research Centre also experimented with potato starch and rice flour combinations in order to find the right blend to improve the taste, texture and volume of gluten-free bread. In this case, xanthan gum and a cellulose gum were added to the formulation for their water-binding properties (McCarthy et al., 2005). The project was paralleled by similar research at Ireland's University College Cork, where formulations for gluten-free goods such as pizza bases were developed (O'Brien, von Lehmden and Arendt, 2002).

In addition, scientists both in Europe and the US have been experimenting with sorghum. This gluten-free grain is not commonly used in food formulations, but the researchers have been testing kernels of food-grade sorghum in order to introduce the grain into mainstream products such as bread, pastry, pizza crusts, biscuits and noodles.

As more and more food manufacturers add gluten-free to their product portfolios, they have also had to adapt their production facilities to ensure there is no cross-contamination. As well as manufacturing gluten-free products in completely separate areas, companies are also investing in viable testing methods, to ensure product quality and safety. Whereas large-scale manufacturers are often able to invest in laboratory analyses, this is not a viable solution for smaller producers, who often do not have the budget or the product quantities to justify complex testing methods. However, a number of simple on-site tests have been developed recently in order to meet the needs of these small-scale producers. For example, UK firm Hallmark Analytical Ventures has developed its credit-card-sized Gluten FlowThrough Test. This consists of a test card, onto which a sample solution is applied. If gluten is present, a coloured spot will appear on the card.

7.5 REGULATIONS FOR GLUTEN-FREE LABELLING

In November 2005, the European Union implemented new allergen labelling regulations, which required packaged foods to label all of the 12 most common food allergen ingredients. These include gluten, fish, egg, peanut, soyabeans and milk. These new regulations abolished a previous '25% rule', which meant that a compound ingredient in a food, making up less than 25% of the finished product, would not have to have its individual ingredients – or allergens – listed.

The UK's Food Standards Agency (FSA) in January 2008 published guidance for the food industry, to help marketers of foods that are not pre-packed – such as sandwich bars and bakeries – to provide clear allergen information to their customers.

The US also implemented new allergen labelling regulations in 2006, which require food companies to label 'in simple language' the eight major food allergens identified by the country. Although wheat constitutes one of these allergens and must be labelled, there is currently no specification for gluten, which could be present in products that do not contain wheat – either found naturally in rye or barley products, or added into formulations for its stabilising properties.

However, in January 2007, the US regulatory agency Food and Drug Administration (FDA) published a proposed rule for the definition of gluten and the labelling of gluten-free products. The rule recommends that a 'gluten-free' statement can be made on any products that do not contain wheat, rye and barley, and their crossbred derivatives. In addition, derivatives of these ingredients, for example wheat starch, would not be allowed in gluten-free foods, unless they are processed to remove the gluten to levels of less than 20 mg/kg of food.

In addition, the Codex Alimentarius Commission sets out standards for what constitutes gluten-free. The commission was created in 1963 by the United Nation's Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) to develop internationally recognised food standards and guidelines relating to food production and food safety. Under the new European Union regulations (2009) only foods that contain less than 20 mg gluten/kg can be labelled as gluten-free, and foods containing less than 100 mg/kg can be claimed as 'very low gluten'.

7.6 MARKET GROWTH

In the past, food manufacturers would avoid the gluten-free segment for a number of reasons, including the relatively low demand, the technical and cost challenges involved in developing gluten-free products, and lacking or inconsistent regulations governing the definition or labelling of these foods.

However, with the increased diagnosis of coeliac disease and the corresponding surge in demand for food products to meet the needs of this consumer group, gluten-free products have started spilling onto the market around the world. In fact, it would be fair to say that today the category has edged its way into the mainstream food supply.

In the UK, the market for gluten-free products in 2007 was valued at £74 million, according to the market analyst Mintel (October 2007). This marked a 12% increase on retail sales in 2006, and a 57% increase compared to 2004, when the market was valued at £42 million. The gluten-free category in the UK currently makes up 41% of the total 'free-from' food and beverage market, marginally lagging the dairy-free market, which holds a 48% share.

On the other side of the Atlantic, gluten-free is thought to be the dominant player in the 'free-from' marketplace. The US market for this category was valued in 2006 at $696 million, a 13% increase from $617 million in 2004 (Mintel, April 2007).

According to the most recent figures available from Euromonitor International (personal communication, 2008), Western Europe was the largest market for gluten-free goods in 2005, followed by North America. Other markets lagged far behind, with Eastern Europe coming in a distant third, followed by Australasia, Asia Pacific and Latin America. However, sales growth rates between 2005 and 2006 revealed double-digit growth levels in dollar terms in most of these markets, clearly indicating the emerging markets for these items. The highest growth was seen in North America, which posted a 22% growth in the year, followed by Latin America at 21%, Asia Pacific at 19% and Eastern Europe at 11%. Western Europe, which has a more developed market for the products, showed a slower growth rate of 9%.

7.7 GLUTEN-FREE CONSUMERS

Although the majority of gluten-free products are bought by consumers with some degree of sensitivity, there is also a growing segment of the population that seeks out these products because of a wider notion that gluten-free constitutes a healthier option. Accordingly, the

popularity of gluten-free products was particularly boosted in 2003 and 2004, paralleling the low-carb diet boom. According to Mintel (October 2007), some consumers seeking low-carb or low-GI products go 'one step further' and seek out gluten-free products. Indeed, growth in the market is expected to be boosted by new consumers who self-diagnose or who simply choose to avoid certain foods for reasons other than medically proven allergies or intolerance.

In addition, many gluten-free products also have other 'free-from' benefits, which are already publicised for diets. Consequently, the sector is expected to grow further on the back of its dieting appeal, particularly in light of the growing global obesity rates. However, one potential obstacle to growth in this field is the addition of other ingredients – such as sugar compounds – which are used as binding agents in gluten-free products, and which may not be considered suitable in dieting items.

7.8 MARKETING GLUTEN-FREE PRODUCTS: CHALLENGES AND OPPORTUNITIES

In the UK, a range of gluten-free products are available to coeliac sufferers on prescription, which has had an impact on retail sales of these goods. For example, because consumers can get pasta and bread products at a reduced price through prescription, this has historically resulted in lower sales of these as snacking items. However, brands in the pasta and bread sectors are growing, and manufacturers are enticing the consumer beyond the realm of prescription goods, by offering added-value products. These include organic, low fat or 'super-food' alternatives.

However, overall, the challenge facing the gluten-free sector is that it needs to lose the image of being 'better than nothing', and to offer a credible range of products with improved choice, taste and quality, according to Mintel (October 2007).

The market researcher points out that manufacturers will endeavour to differentiate their brands and retain their status in the market by offering high-quality alternatives that meet all consumer expectations. High consumer demands and a growing industry response to meet these demands mean that people will simply no longer accept to compromise on taste or quality when purchasing free-from foods.

Differentiation is, therefore, crucial to product success in a highly competitive market; and innovation in products and in marketing methods is key to differentiation. At the same time, because the market for gluten-free foods is still immature, brands have the opportunity to own a sector. Producers that are able to back up their brands with the necessary marketing spend could well drive the sector forward, and position themselves as leading players within the category.

Nevertheless, despite the room in the marketplace, most gluten-free products – particularly in the US – are manufactured by smaller players, with the big multinationals steering clear of the segment for the time being. The reason for this is simple: as long as there is no clear legislation in place, these big firms remain reluctant to take the risk of launching gluten-free product ranges. According to Packaged Facts (2006), a publisher of market research, it is these mega-marketers that have in recent years become the target of consumer activist groups, and for the most part these companies have learned to proceed more cautiously in such areas. As a result, they are unwilling to take the chance of using the term 'gluten-free' until a clear definition has been established. Another reason that keeps many players out of the market is a reluctance to make the commitment to establish gluten-free product lines and invest in the extensive research needed to develop high-quality, appealing products – particularly in the

knowledge that the risk for coeliac sufferers can be extremely high, even when consuming a very small amount of gluten.

For those companies that do take the leap, one significant area of opportunity is in the development of gluten-free products specifically targeting certain segments of the population. For example, children are a key consumer group, increasingly affected by food intolerances. Manufacturers can therefore try to develop gluten-free products that respond to the particular needs of children, including nutritional needs, taste preference and the importance of convenience.

In addition, younger consumers aged between 18 and 34 are most likely to suffer from some form of food intolerance and purchase free-from foods, although a large portion of them remain undiagnosed. Older consumers, aged 55 and above, are the most likely to have received a formal diagnosis, and are therefore also the most likely to completely avoid the problematic products. In addition, this population segment tends to have more disposable income and is able to pay the premium for specialty goods.

According to Mintel (April 2007), it is essential to target key demographics in order to tap market growth. These include Asians, Hispanics and black people. In the US market, Asian consumers tend to report high incidences of food intolerance, and also show a strong interest in free-from foods. Hispanics report an average food intolerance level, while black people are less likely to be aware of food allergies or intolerance.

7.9 GLUTEN-FREE PRODUCT LAUNCHES

In 2007, there were 3950 new products launched around the world that carried a gluten-free claim. This represents a huge leap from the 330 new products recorded in 2002. The figures, taken from Mintel's Global New Products Database (GNPD) – which tracks new product introductions around the world – reveal an explosion in the marketplace for gluten-free products over the 5-year period.

Most new gluten-free products are in the bakery and snacks segments, which are the food categories most likely to use grain ingredients that contain gluten. Other popular categories for gluten-free foods include sauces and seasonings; processed fish, meat and egg products; beverages; confectionery; and dessert or ice cream products. Overall, the GNPD data reveal an extremely broad range of categories with gluten-free products, indicating how far reaching the impact of gluten can be. For example, some of the smaller gluten-free categories include sweeteners, pet food, fruit and vegetable products, and even personal hygiene items. Mintel suggests that this highlights the opportunity that exists to promote the gluten-free status of nearly anything that could be in question as a way to appeal to, and simplify life for, those following a strict gluten-free regimen.

Examples of global gluten-free product introductions in 2007 drawn from GNPD include:

- **UK**: Tesco private label Free From Caramel Shortcake Slices, which contain maize starch, rice flakes, millet flakes, modified potato starch and soya flour, as well as cellulose, emulsifier and stabiliser as replacements for wheat.
- **USA**: Think Products' Think Thin Pink energy bar, which is also marketed as sugar-free and trans fat-free. This contains a protein blend – including whey protein isolate, calcium caseinate and soya protein isolate – as well as a soya crisp – including tapioca starch, soya protein isolate, cocoa powder and calcium carbonate.

- **Germany**: C W Cocoflour, marketed in the baking ingredients and mixes category. It is 100% coconut flour, and is made of the pulverised fibres of coconut flesh left over after pressing virgin coconut oil. It is marketed as a flour replacement (up to 25%) and also for addition to finished goods, such as cereal, yoghurt and smoothies.
- **Australia**: Organ Natural Foods' Gluten Free Alternative Grain Wholemeal Bread Mix contains no gluten, wheat, dairy, egg, yeast, GMOs or soya. Ingredients include sorghum flour, rice flour and maize starch, as well as raising agents (such as dextrose from maize) and gums (such as guar gum).
- **China**: Jin Wang Da Food's Rui Ping France crackers contain wheat but are marketed as gluten-free. They are made with refined flour, sugar, vegetable oil, cream, milk powder, eggs and raising agents.
- **Brazil**: Cultivar Brazil's Biscoito de Milho com Melado Organico are cookies made with corn flour and manioc flour. Other ingredients are molasses, palm oil, eggs, salt and anise.

7.10 WHERE GLUTEN-FREE PRODUCTS ARE SOLD

Overall, although gluten-free products are increasingly seen in mainstream supermarket aisles, research published by Packaged Facts (2006) indicates that – in the US at least – most sales still occur in health and natural food stores. The market researcher estimates that in 2006, some 40% of US sales in the gluten-free category occurred through these outlets, while 20% occurred through Internet or catalogue sales. Mainstream supermarkets took a 14% share in sales, followed by mass merchandisers at 12%. Club stores, dollar stores and drug stores account for less than 10% of all gluten-free food sales.

According to Packaged Facts (2006), many retailers are starting to recognise the opportunity in carrying gluten-free products, and specifically merchandising them in a special aisle or section of the store. The gluten-free category is particularly appealing for retailers because these products are not responding to a fad, but to a lifelong condition, and demand is therefore not slated to decrease.

7.11 MARKET GROWTH FORECASTS

In the UK, the overall market for 'free-from' products is expected to show continued strong growth in the near future. Within the next 5 years, it is expected to almost double, growing by 97% from 2007–2012, reaching an estimated £354 million. 'Free-from' foods as a rough proportion of the total market are expected to increase from 1.7% in 2007 to 2% in 2008, increasing further to 3.1% by the end of 2012. According to Mintel (October 2007), this indicates that more people will swap over some of their usual food consumption into free-from alternatives. In addition, as the proportion remains relatively low, there remains a vast market for the category to potentially tap into. US forecasts are specific to the gluten-free category, and point to annual growth of between 15% and 25%. Sales of gluten-free foods are expected to reach around $1.3 billion by 2010 (Mintel, April 2007).

The overall extent of growth in the market will be largely dependent on the level of awareness of coeliac disease and the overall benefits of 'free-from' products. Other drivers for growth will include people shifting to gluten-free because of a belief that certain allergens or components in food also have an effect on other health conditions, ranging form migraine to menstruation; a switch to 'free-from' foods from a growing health-concerned

ageing population; and dieters turning to gluten-free because of a general perception that these products fit into a slimming food category.

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8 Dough Microstructure and Textural Aspects of Gluten-Free Yeast Bread and Biscuits

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8.1 INTRODUCTION

Gluten is a fundamental component for the overall quality and structure of wheat bakery products, particularly yeast leavened ones. The gluten complex is composed of two main protein groups: gliadin (a prolamin) and glutenin (a glutelin). The gliadins are mechanically characterised by little or no resistance to extension, therefore appearing to be responsible for dough's cohesiveness, while the glutenins are apparently responsible for the dough's resistance to extension (Hoseney, 1994). The combination of these two proteins, which results in the gluten complex, confers the dough unique viscoelastic properties. The properties of gluten become apparent when flour is hydrated, giving an extensible dough, with good gas holding ability. This unique structure contributes to the texture and crumb characteristics of the final baked product (Faubion and Hoseney, 1990; Stear, 1990). For these reasons, the replacement of gluten network in the development of gluten-free cereal products is a challenging task for the cereal technologist. This is particularly the case for yeast-leavened products such as breads. In fact, the absence of gluten results in a liquid batter rather than a dough, and is responsible for the deficient quality characteristics as compared to wheat breads. A marketing review by Arendt et al. (2002) found that most of the gluten-free products were of low quality, exhibiting poor mouthfeel and very often showing off-flavours. The structure of the products was mostly crumbly and very dry. In wheat breads, the solid matrix of the crumbs consists of a continuous phase of gelatinised starch (Moss, 1975; Pomeranz et al., 1984; Durrenberger et al., 2001) and a continuous gluten network which encloses the starch granules and fibre fragments (Fig. 8.1). In gluten-free breads such a continuous protein network able to embed the starch granules is missing. On the other hand, biscuits differ from yeast-leavened products as gluten network formation is not necessary in most of them. In fact, the texture of baked biscuits is primarily attributable to starch gelatinisation and supercooled sugar rather than a protein/starch structure (Gallagher, 2002). Nevertheless, commercial gluten-free biscuits are generally based on pure starch, thus having poor organoleptic quality (Schober et al., 2003).

There are a limited number of studies published on gluten-free bakery products, which reflects both the difficulty of the technological challenge and the lack of awareness of the large incidence of the coeliac disease (CD). In recent years, several studies have been conducted, which investigated the suitability of particular flours such as rice and sorghum flours (Gujral et al., 2003b; Schober et al., 2003; Gujral and Rosell, 2004 a,b), dairy products (Gallagher et al., 2002, 2003, 2004), protein supplementation that is, surimi (Gormley et al., 2003), soyabean (Ranhorta et al., 1975; Sanchez et al., 2002), egg proteins (Moore et al., 2004) or the use of gums and hydrocolloids (Toufeili et al., 1994; Guarda et al.,

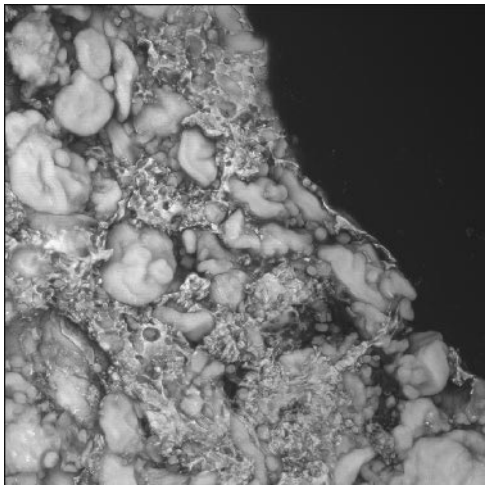


Fig. 8.1 CLSM image of wheat bread crumb showing the gluten-starch matrix: gelatinised starch granules embedded in the gluten network (40× magnification). (*Source*: S. Renzetti and E.K. Arendt (2006) unpublished data.)

2004; Lazaridou et al., 2007) to improve volume, crumb texture and the overall quality and acceptability of gluten-free products. Such investigations focused on fundamental rheology, standard baking tests and texture profile analysis (TPA) in order to understand the impact of different ingredients on the physical and textural characteristics of batters and breads. However, microscopic techniques are valuable for a deeper understanding of the microstructure of cereal products. Recently, Durrenberger et al. (2001) showed the potential of confocal laser scanning microscopy (CLSM) for the structural characterisation of complex food systems. The advantage of this technique is its ability to produce optical sections through a three-dimensional specimen and, with application of staining procedures, to select and differentiate particular structures in the food system. CLSM has been recently applied in the development of gluten-free products (Moore et al., 2004, 2006), in order to better understand the impact of different functional ingredients on the microstructure of batters and breads.

The aim of the present chapter is to review the impact of different functional ingredients on the textural characteristics of gluten-free breads and biscuits and on the microstructure of the batters and corresponding products. Due to the fact that most of the studies in gluten-free products focus on bread, as technologically more challenging, the ingredients discussed in the subsequent sections are referring to breadmaking. A separate section of this chapter is dedicated specifically to gluten-free biscuits.

8.2 GLUTEN-FREE BREADS

8.2.1 Alternatives to wheat flour

Several flours have been used in the development of gluten-free products, alone or in combination with other flours and/or starches. The following sections give an overview of most commonly used and promising flours.

8.2.1.1 *Corn*

Corn flour is composed of the endosperm, which generally contains between 75% and 87% starch and 6–8% protein (Shukla and Cheryan, 2001). A cornstarch bread was developed by Christiansson et al. (1974) using xanthan gum as networking component. The resulting bread had good specific volume but showed a coarse crumb structure and lack of flavour. Ács et al. (1996a, b) used binding agents (xanthan, guar gum, locust bean gum and tragant) as substitutes for gluten in a gluten-free bread formulation based on cornstarch. The binding agents resulted in an increase in loaf volume and loosening of the crumb structure. Gluten-free breads with improved specific volume and crumb texture were obtained with addition of cornstarch into the formulation (Sanchez et al. 2002; Schober et al. 2004).

8.2.1.2 *Pseudocereals*

There are two major subclasses of flowering plants, that is, monocots (one seed leaf) and dicots (two seed leaves). Wheat, rye and barley are monocots whereas buckwheat, amaranth and quinoa are dicots and very distantly related to grains in the monocot subclass (Kasarda, 2001). Because of their unique chemical structures, buckwheat, amaranth and quinoa are classified as pseudocereals.

Amaranth

Amaranth can be used in the production of gluten-free products. Due to low levels of amylase, amaranth starch performs poorly in bread and cake formulations (Stone and Lorenz, 1984). However, individual species of Amaranth with high levels of amylase, that is, *Amaranth pumilus* (8.2%), could be successfully used in bread production.

The potential of amaranth for the production of gluten-free products has been recently investigated (Tosi et al., 1996; Gambus et al., 2002). By replacing 10% of cornstarch with amaranth flour, Gambus et al. (2002) found an increase in protein and fibre levels by 32% and 152%, respectively, while sensory quality was unaffected. The results indicate that amaranth flour can be used to enhance the protein and fibre contents of gluten-free breads.

Buckwheat

Buckwheat belongs to the *Polygonaceae* family and is taxonomically distant from the *Gramineae* family, which contain the cereals rice, wheat and maize. However, buckwheat seed has chemical and utilisation characteristics similar to cereal grains, and thus is usually classified as a cereal. There are two cultivated species of buckwheat for human consumption, that is, common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*Fagopyrum tataricum* Gaertner) (Ikeda, 2002). *Fagopyrum esculentum* is the most economically important species, attributing to approximately 90% of the world production of buckwheat (Mazza, 1993). Buckwheat achenes contain 55% starch (Bonafaccia et al., 2002), with a ratio of 24% amylose and 76% amylopectin, similar to what found in cereal starches (Aufhammer, 2000). Protein content (11–15%) is also similar to cereal grains.

Buckwheat has been suggested by Carroll and Hamilton (1975) to treat CD, as it does not contain gluten-like proteins (Francischi et al., 1994; Kreft and Kreft, 2000) and therefore may be used in the production of gluten-free products. Moore et al. (2004) produced good quality bread by inclusion of buckwheat flour in a gluten-free formulation. The bread had lower

specific volume and higher crumb hardness compared to a wheat bread and a commercial gluten-free bread, but the staling rate was lower in comparison to the starch-based commercial gluten-free bread.

Quinoa

Quinoa is a pseudocereal of the *Chenopodiaceae* family which is also regarded as a gluten-free cereal. The protein content of quinoa (13–14%) is slightly higher than that of most other cereal grains, while the starch content, ranging from 52% (Ruales, 1998) to 69% (Guzmán-Maldonado and Paredes-López, 1998), is lower. Quinoa is used to make leavened bread as a composite with wheat flour. It has been reported by Chauhan et al. (1992) that the addition of quinoa to leavened breads can cause a reduction in the volume. This appears to be related to the high level of starch damage found in quinoa flour and meal (Chauhan et al., 1992), to the small size of the granules and to the low proportion of amylase in the starch (Fleming and Galwey, 1995). Nevertheless, the application of quinoa for the production of gluten-free bread has still to be extensively investigated.

8.2.1.3 *Rice*

Rice is one of the leading food crops in South East Asia including India, and the production of rice in this part of the world is much higher than that of wheat (Sivaramakrishnan et al., 2004). Rice flour possesses unique attributes such as bland taste, white colour, ease of digestion and hypoallergenic properties (Kadan et al., 2001). Furthermore, low levels of sodium, absence of gliadin and the presence of easily digested carbohydrates have made it one of the most suitable cereal grain flour for preparing foods for coeliac patients (Gujral et al., 2003a, b). For baking applications, starch granule properties assume a major role in dictating the suitability of rice flour, especially if it is present in a large enough quantity, usually over 10% substitution, to influence the texture of a product (Bean and Nishita, 1985). However, rice also has low amounts of proteins, which are devoided of the visco-elastic properties typical of wheat gluten (Juliano 1985). Therefore, rice proteins are unable to retain the gas produced during the fermentation process, and this limits the use of rice flour in breadmaking (Gujral and Rosell, 2004b). Rice variety has also been found to influence the breadmaking characteristics of rice flour. Nishita and Bean (1979) showed that rice varieties having low amylose contents and low gelatinisation temperatures give superior crumb properties. Kadan et al. (2001) found that addition of 10% short grain rice in a white rice bread formula improved texture and slowed retrogradation compared to the same formulation made of 100% long grain rice. Despite the differences in the breadmaking potentials of rice varieties, hydrocolloids are essentials in any formulation in order to produce rice breads with good volume and texture. HPMC has been found to be the best alternative to gluten in rice bread formulations in order to enhance gas retention and achieve proper crumb structure (Nishita et al., 1976; Collar et al., 1998). Gujral et al. (2003a) found that increasing levels of HPMC and oil was effective in improving specific volume and crumb texture. In this study, the addition of cyclodextrin glucosyl transferase (CGTase, EC 2.4.1.19) was also investigated. CGTase is an enzyme from *Bacillus* spp. that cleaves α-1,4 glycosidic linkages in starch molecules, concomitantly linking the reducing and nonreducing ends to produce cyclic molecules (Ohnishi et al., 1997). The addition of CGTase in the formulations containing HPMC and oil was particularly effective in increasing crumb firmness. A negative correlation was also found between crumb firmness and specific volume.

The authors concluded that the product from the CGTase reaction, for example cyclodextrins, could form complexes with the rice proteins, thus decreasing their hydrophobicity, and also with lipids and other compounds present in the breadmaking process. Such complexes could be involved in better entrapment of gases, thus leading to improved volume and texture. In future studies, the application of microscopic techniques such as CLSM might provide with further evidence of such complexes and give better understandings of the microstructure of breads from rice flour. Recently, a few studies investigated the possibility of improving the breadmaking properties of rice flour by addition of protein cross-linking enzymes such as transglutaminase (TGase) and glucose oxidase (GO) (Gujral and Rosell, 2004a, b). Such findings are detailed in a specific section of this chapter.

8.2.1.4 *Sorghum*

Sorghum belongs to the grass family *Graminae* and tribe *andropoggonae*. Like other cereal grains, the primary component of sorghum is starch (Rooney and Waniska, 2000). Sorghum has a similar chemical composition to maize, but is often reported to have a slightly lower protein and starch digestibility. This is especially true for cooked sorghum and has been attributed to increased cross-linking of the proteins during cooking (Hamaker and Bugusu, 2003; Schober et al., 2006). Sorghum is often recommended as a safe food for coeliac patients, because it is more closely related to maize, than to wheat, rye and barley (Kasarda, 2001). Sorghum might therefore provide a good basis for gluten-free bread. Schober et al. (2006) showed that it has great potential as a 'functional' food. However, the majority of studies dealing with leavened loaf breads containing sorghum have focused on composite breads from wheat and sorghum, in which a maximum of only 30% low-tannin sorghum are regarded as acceptable (Munck, 1995).

Only a limited number of studies have addressed wheat-free sorghum breads and most have used relatively complex recipes incorporating xanthan gum (Satin, 1988), carboxymethyl cellulose and skimmed milk powder (Cauvain, 1998), egg (Keregero and Mtebe, 1994; Cauvain, 1998) or rye pentosans (Casier et al., 1997). Recently, Schober et al. (2004) investigated the breadmaking quality of different sorghum varieties in the development of a gluten-free bread. These authors found significant differences in crumb structure in terms of pore size and number as well as hardness. Breads differed little in volume, height, bake loss and water activity. Additionally, response surface methodology (RSM) was applied using two sorghum hybrids of opposite quality to investigate the effect of added ingredients on bread quality. Quality differences between the hybrids were maintained throughout the RSM. The authors concluded that differences in kernel hardness and damaged starch were the key elements responsible for such differences and that certain sorghum hybrids have better breadmaking potentials than others.

In the future, selection of varieties with good breadmaking potentials and further investigations on supplementation of functional ingredients in gluten-free formulations based on sorghum flour could lead to development of breads of high quality.

8.2.2 Starch-based ingredients

Starch is a major component in cereal-based products and it contributes to the texture and quality of doughs and breads. In breadmaking with wheat flour, starch contributes to dilution of gluten, interweaves with gluten, and absorbs water from the gluten by gelatinisation, thus

providing a bread structure permeable to gas so that the bread does not collapse while cooling (Sandstedt, 1961). Thus, starch sets the structure of baked-product systems and its final state contributes to the texture of the final product (Miyazaki et al., 2006).

Wheat starch has been often utilised in gluten-free products. However, many coeliac patients are sensitive to the presence of the low amounts of gliadins, which might escape the isolation procedure, and therefore starch-based gluten-free foods should use products that are naturally gluten-free (Skerritt and Hill, 1992; Chartrand et al., 1997; Lohiniemi et al., 2000). Different starches from naturally gluten-free sources such as corn, cassava, tapioca, potato and rice have been utilised in gluten-free formulations (Sanchez et al., 2002; Kobylanski et al., 2004; Moore et al., 2004; Gallagher, 2002). However, little is known on their individual contribution to the microstructure and textural characteristics of breads, as they have been used in combination with several other ingredients, in the attempt to optimise formulations. Recently, a review by Miyazaki et al. (2006) focused on the effects of modified starches on the textural characteristics of breads. It was found that by using modified starches, bakers can control texture of products and develop unique breads. Similarly, Keetels et al. (1996), investigating the mechanical properties of starch breads from wheat and potato starches, concluded that such properties are largely determined by the mechanical properties of the lamellae and beams from which they are built. Gallagher et al. (2002) investigated the application of rice starches on a replacement basis for wheat starch in gluten-free bread formulations. At inclusions of 3–9% levels, crumb hardness was reduced as well as the rate of staling. Further studies are required in order to better understand the impact of different starch types on the quality characteristics of gluten-free products.

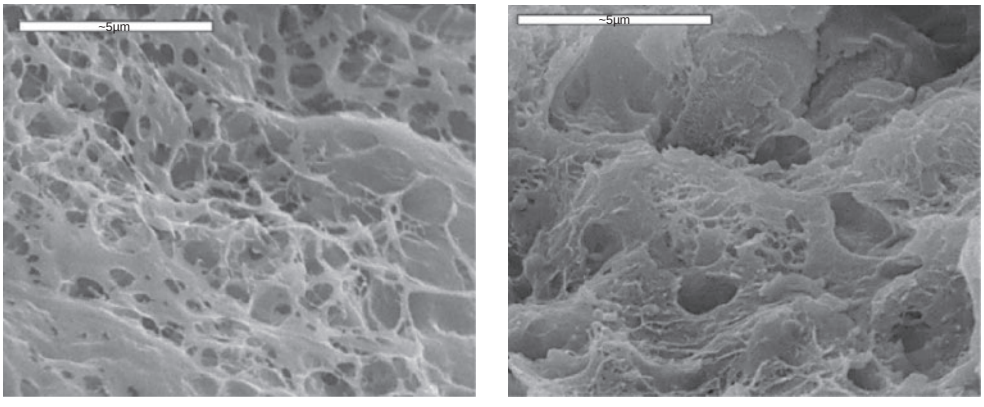
8.2.3 Hydrocolloids (gums)

The viscoelastic properties provided by the gluten network are largely responsible for gas cell formation, including stabilisation and retention of the gas cells during the proofing and baking stages (Gan et al., 1989). The lack of a gluten network determines the properties of the gluten-free dough, which is more fluid than wheat doughs and closer in viscosity to cake batters (Cauvain, 1998; Moore et al., 2004). Therefore, polymeric substances that mimic the viscoelastic properties of gluten to provide structure and retain gas are required in the development of gluten-free breads (Toufeili et al., 1994). Hydrocolloids or gums are substances consisting of hydrophilic long-chain, high molecular weight molecules, usually with colloidal properties, that in water-based systems produce gels, that is, highly viscous suspensions or solutions with low dry-substance content (Hoefler, 2004). They are derived from seeds, fruits, plant extracts, seaweeds and microorganisms, being of polysaccharide or protein nature (Norton and Foster, 2002). A wide range of hydrocolloids have been used in the development of gluten-free breads, for example HPMC, methylcellulose (MC), carboxymethylcellulose, psyllium gum, locust bean gum, guar gum, agarose, β-glucan and xanthan gum (Ahlborn et al., 2005; Gallagher et al., 2003, 2004; Gujral et al., 2003a; Haque and Morris, 1994; McCarthy et al., 2005; Schober et al., 2004; Sivaramakrishna et al., 2004; Toufeili et al., 1994; Ylimaki et al., 1991; Schwarzlaff et al., 1996). Schwarzlaff et al. (1996) used combinations of guar gum and locust bean gum to partially replace flour in bread. The introduction of guar gum resulted in crumb structure with a more even cell size distribution, while locust bean gum inclusion increased the height of the bread loaves; both gums retarded bread staling. Optimum levels for locust bean gum and guar gum were 2–4%. Xanthan gum and HPMC have been particularly used in studies on gluten-free breads,

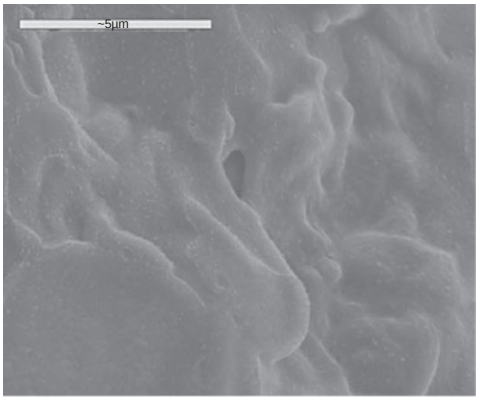
separately or in combination. Christiansson et al. (1974) reported that in order to obtain a good crumb structure in the absence of gluten in a starch-based system, xanthan gum was required. In the preparation of gluten-free bread, xanthan has been found to confer a crumb structure similar to that of wheat bread. It was suggested that xanthan may act by improving the strain hardening properties of the 'dough' (Van Vliet et al., 1992; Kokelaar, 1994). The incorporation of xanthan gum at 1% level in a gluten-free formulation based on rice flour and cornstarch did not show any effect on the loaf volume of breads, while a 2% supplementation even decreased the volume, and increased crumb firmness (Lazaridou et al., 2007). Schober et al. (2004) reported similar findings in sorghum-based gluten-free breads. In this study, loaf volume was decreasing with increasing levels of xanthan gum. Moreover, the addition of the gum had also negative effects on crumb structure as an increase in hardness was found. An adverse effect of xanthan gum on crumb hardness was also found in wheat breads (Guarda et al., 2004), which could be the consequence of the thickening effect on the crumb walls surrounding air spaces as proposed by Rosell et al. (2001). On the contrary, HPMC supplementation of wheat breads greatly improved loaf volume (Guarda et al., 2004). Positive effects of HPMC in the improvement of specific volume in a gluten-free bread system were found by Kang et al. (1997). Gan et al. (1989) and Kadan et al. (2001) also found that HPMC improves gas retention and water absorbing characteristics, which are usually conferred by gluten. A possible explanation to this is that HPMC gives some stability to the interface dough system during proofing and confers additional strength to the gas cells through the baking, increasing gas retention and thus leading to higher volume (Bell, 1990). McCarthy et al. (2005) used RSM to investigate the optimum levels of water and HPMC on a rice bread formulation. Loaf-specific volume increased as water addition increased at any of the levels of HPMC used. At levels of 0.8% HPMC and 91% water (fsb), a gluten-free bread with optimum specific volume and loaf height was obtained. However, the bread was considered of poor quality due to the presence of large gas cells, a consequence of excessive water used as confirmed by previous findings (Nishita et al., 1976; Haque and Morris, 1994). McCarthy et al. (2005) reported that increasing water level decreased crumb firmness, partly as a result of higher specific volume, but that high levels of HPMC and water had adverse effects on crumb structure, as the number of cells/cm^2 was decreased. In a second attempt to optimise recipe, most importance was given to crumb structure. Using a combination of 2.2% HPMC and 79% of water, the resulting bread was comparable to a wheat bread in terms of mean cell area, but still lower in terms of number of cells/cm^2.

Using scanning electron microscopy, Ahlborn et al. (2005) compared the microstructure of a wheat bread with a low protein starch bread containing xanthan gum and HPMC, and a gluten-free bread containing eggs and milk proteins together with xanthan gum and HPMC. A bicontinuous system of gluten with embedded starch was observed, in which the gluten created a fibrous, web-like structure (Fig. 8.2). Similarities were found in the microstructure of the gluten-free bread, while the low-protein starch formulation was lacking of matrix development (Fig. 8.2).

In conclusion, the data collected so far strongly indicate that hydrocolloids are essential in the development of gluten-free breads in order to achieve desirable characteristics such as high specific volume, crumb softness and structure. The right selection and combination of hydrocolloids, together with an optimum level of water, are necessary in order to obtain breads with a desirable balance of such characteristics. However, microstructure analysis suggests that hydrocolloids alone are not sufficient to fully replace gluten in gluten-free breads and that the supplementation with proteins is therefore necessary.



(a) (b)



(c)

Fig. 8.2 Scanning electron microscopy of bread matrix of (a) standard wheat, (b) gluten-free rice and (c) low-protein starch, lacking matrix development. All magnifications are 10 000×. (*Source*: Reprinted with permission from Ahlborn et al. (2005).)

8.2.4 Protein sources in gluten-free breads

The replacement of gluten with other protein sources such as soya and dairy proteins is one of the approaches used in the production of gluten-free products. Currently, dairy proteins, surimi, soyabean and egg proteins have been studied as supplements in gluten-free formulations.

8.2.4.1 *Dairy proteins*

Milk and dairy-based ingredients are used as components of many food products. The two major proteins in milk, that is, caseins and whey proteins, are widely used for emulsification purposes. They differ in their emulsification characteristics, which are dependent on their physicochemical properties. Dairy proteins are highly functional ingredients, and due to their versatility, they can be readily used in many food products (Gallagher et al., 2003). They may be used in bakery products for both nutritional and functional benefits including

flavour and texture enhancement as well as storage improvement (Cocup and Sanderson, 1987; Mannie and Asp, 1999; Kenny et al., 2001; Gallagher et al., 2003). Some of the useful properties of dairy proteins include the emulsifying and stabilising ability of caseinates, the gelling properties of whey protein concentrates and isolates, the water-absorption capacity of high-heat non-fat dry milk and the browning of lactose during heat processing (Chandan, 1997). Dairy proteins have functional properties similar to gluten, as they are capable of forming networks and have good swelling properties.

Gallagher et al. (2003) investigated the impact of different dairy powders with different protein content on the baking and textural characteristics of gluten-free bread formulations based on wheat starch. In general, the inclusion of any of the powders reduced loaf volume, thus confirming previous results obtained in wheat-based breads (Erdogdu-Arnoczky et al., 1996; Gelinas et al., 1995; Kadharmestan et al., 1998). Only the powders containing sweet whey, casein and whey protein isolate showed a certain recovery of loaf volume with increasing level of inclusion. The inclusion of casein and whey protein isolate also resulted in more viscous batters, due to the water holding capacity of the proteins. Batter viscosity is one of the key elements which determine the quality of gluten-free bread. In the same study, Gallagher et al. (2003) showed that increasing water addition in the batters resulted in increased loaf volumes, and these findings have been recently confirmed by Schober et al. (2004) studying gluten-free batters based on sorghum flour. The addition of dairy powders also resulted in a significant increase in crumb firmness, which did not correlate with the increased protein content of the breads (Gallagher et al., 2003). The increase in firmness varied with inclusion level and type of powder but no significant correlations were found. It was suggested by the authors that the differences in the physicochemical properties of the proteins present in the powders and particularly the water holding capacities could be responsible for the differences in the increase of crumb firmness. Kadharmestan et al. (1998) also found increase in crumb firmness with application of whey protein concentrates in wheat breads. In a different study, Gallagher et al. (2002) found that the addition of milk protein isolate together with rice starch increased loaf volume of a gluten-free bread, even though the effect was not significant. On the other hand, crumb firmness was significantly reduced with protein isolate and rice starch supplementation and a negative correlation was found between loaf volume and crumb hardness. Negative correlations between crumb hardness and specific volume were also found in other studies (Salmenkallio-Marttila et al., 2004; Schober et al., 2004). The addition of milk proteins and rice starch also affected crumb structure, as the number of cells/cm^2 was reduced and the bread had a more open structure. A positive correlation between crumb firmness and the number of cells/cm^2 was also found by Schober et al. (2004) in gluten-free sorghum breads. In this study, it was suggested that a coarse, open crumb structure might provide less resistance to the probe during TPA. In general, bread with a soft crumb and fine cell structure is regarded being of superior quality (Hoseney, 1998). In a study on gluten-free bread based on sorghum flour, the application of skim milk powder showed negative effects on the quality of the breads (Schober et al., 2004). The reduced bread height, increased bake loss and reduced crumb cohesiveness found with the addition of skim milk powder indicated a weakening in bread structure. The authors suggested that milk proteins and lactose might interfere with the starch gel, by competing for water or disrupting the uniformity of the starch gel.

Moore et al. (2004) compared the textural characteristics of a skim milk powder supplemented gluten-free bread with a non-dairy gluten-free, a commercial gluten-free (starch-based) and a wheat bread. As a result, the dairy bread had significantly firmer crumb compared to the other breads, which is regarded as a negative characteristic. Nevertheless,

the dairy bread showed a resilience and cohesiveness comparable to the wheat bread, which however, is most likely due to the presence of egg powder.

Results collected so far lead to the assumption that dairy powders do not have significant positive effects in improving the textural characteristics of gluten-free breads. Gluten-free breads supplemented with dairy powders generally show decreased loaf volume and increased crumb firmness. Crumb structure/appearance also appears negatively affected as a coarser crumb structure is detected compared to controls. Furthermore, supplementation of gluten-free breads with high lactose-content powders is not suitable for persons with CD due to their lactose-intolerance condition (Ortolani and Pastorello, 1997).

8.2.4.2 *Surimi*

Surimi is a concentrate of myofibrillar proteins obtained after mixing and water washing of fish flesh (Han-Ching and Leinot, 1993). It contains approximately 78% water, 20% protein, lipids, sugars and polyphosphates. The myofibrillar protein in the resulting products is high in actomysin, which is highly elastic and capable of forming a strong gel (Nielsen and Pigott, 1994; Trondsen, 1998; Kim et al., 1986). Hastings (1989) showed that surimi-based gels containing salt have an elastic and cohesive texture and this property can be exploited in the application of surimi as a functional ingredient. Therefore, the excellent structure forming properties of surimi (Whitehead, 1992) could be exploited for the substitution of gluten in gluten-free formulations.

Gallagher (2005) investigated the application of four different surimis on a rice flour, potato starch gluten-free formulation at 2% inclusion level (flour + starch weight). Three of the surimi types had positive impact on the baking and textural characteristics of the resulting breads. Loaf volume was improved compared to the controls, while crumb hardness was significantly decreased. Surimi from pollock also positively affected crumb structure as the number of cells/cm^2 was increased compared to the controls. A finer crumb appearance was achieved, which is regarded as desirable. Indeed, paired comparison panel tests for acceptability between the control and surimi bread samples indicated that the soft crumb breads achieved with three of the surimis were preferred.

The ultrastructure of gluten-free batters supplemented with blue whiting fish surimi showed a more even dispersion of starch and proteins, and gas cells smaller and more uniform in shape (Fig. 8.3), compared to the control gluten-free batter which revealed a disconnected network of starch, proteins and large gas cells (Fig. 8.3). Differences were not detected in the control and surimi supplemented bread samples (Fig. 8.3).

8.2.4.3 *Soyabean*

Soya flour is widely used in the production of gluten-free products. It is characteristically rich in protein, but deficient in S-containing amino acids (Belitz and Grosch, 1987). Ranhorta et al. (1975) discussed the application of soya protein to gluten-free bread. These authors formulated wheat starch-based gluten-free breads with 20, 30 and 40% soya protein isolate (88% protein content). The breads were significantly improved in terms of grain and texture as a more tender, close-grain and even texture was achieved compared to the rough, crumbly texture of the control, which is common in starch-based gluten-free formulations. Sanchez et al. (2002) studied the optimisation of a gluten-free bread formula based on cornstarch, cassava starch and rice flour. The optimal combinations of the starches and rice flour resulted in the maximum specific volume, but high specific volume was achieved at the expenses

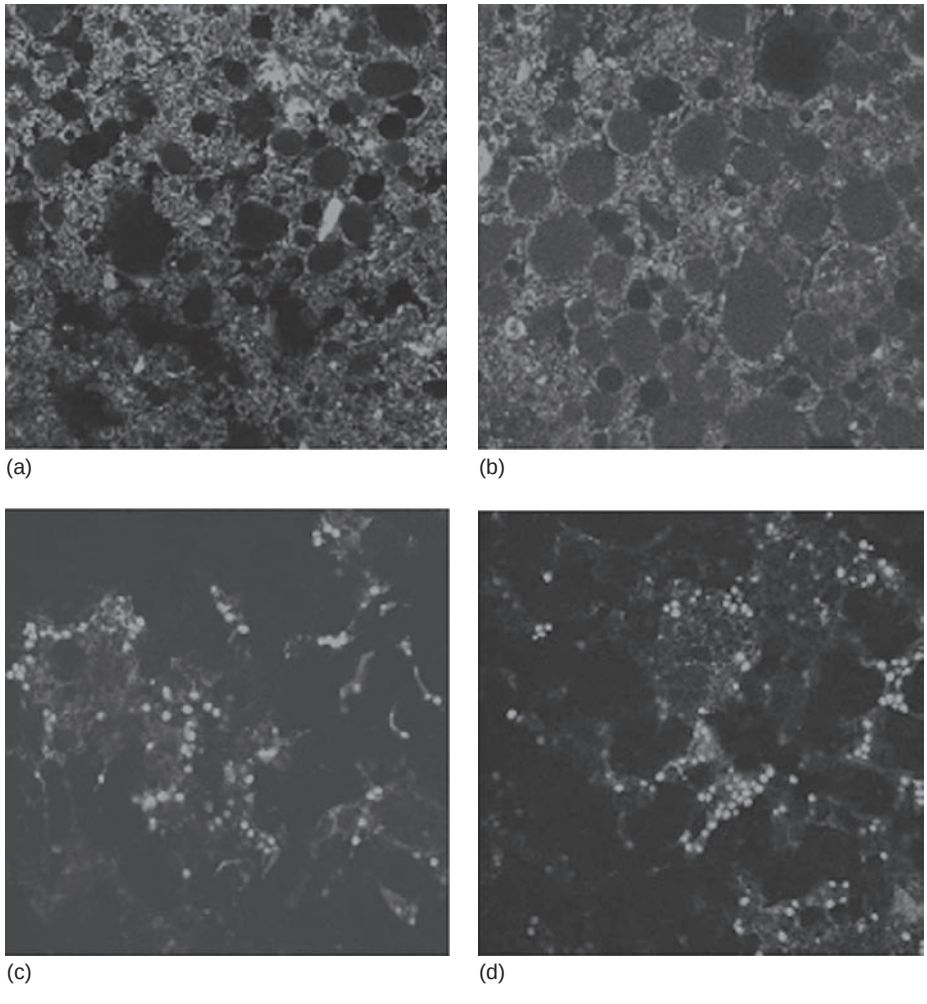


Fig. 8.3 CLSM images (40×) of control gluten-free dough and bread (a, c respectively), and gluten-free dough and bread containing fish surimi (b, d respectively). (*Source*: Adapted from Gallagher (2005).)

of crumb structure, which showed large holes in the crumb. The inclusion of 0.5% soya corrected this problem, improving crumb structure and resulting in higher crumb grain score and overall bread score. Starch-based breads are characterised by high specific volume, but at the expense of poor crumb texture. Soya proteins shows to be suitable protein source for gluten-free formulations as they can overcome problems related to crumb texture.

8.2.4.4 *Egg proteins*

Egg proteins have also been studied as supplements for gluten-free formulations. Egg proteins form strong cohesive viscoelastic films, which are essential for stable foaming (Kato et al., 1990). Egg albumen improves gas retention properties when used in gluten-free bread (Jonagh et al., 1968). Toufeili et al. (1994) used RSM to investigate the use of egg albumen in

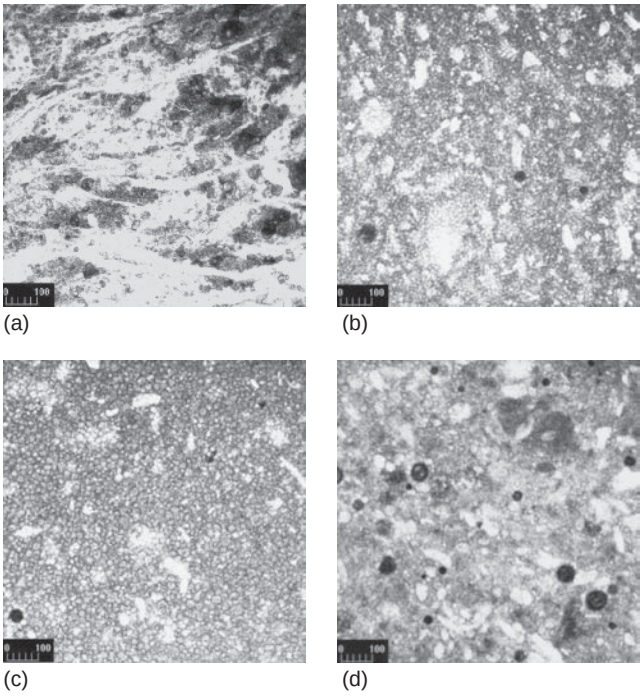


Fig. 8.4 CLSM images of wheat dough (a), commercial gluten-free batter (b), non-dairy gluten-free batter (c) and dairy/egg gluten-free batter (d) stained with Safranin O dye (magnification bar 10×, 100 μm). Proteins appear in white, starches as grey, dark granules. (*Source*: Reprinted with permission from Moore et al. (2004).)

combination with MC and gum arabic to optimise the formulation of gluten-free pocket-type flat breads. MC and egg albumen were identified as the major determinants of the product's sensory quality, which included attributes such as grain distribution, first bite hardness, adhesiveness, masticatory hardness and cohesiveness of mass. The authors concluded that with the right combination of such ingredients, gluten-free pocket-type flat breads compatible with regular wheat bread in key textural attributes can be achieved. Moore et al. (2004) also developed a gluten-free bread which, together with soya flour and skim milk powder, also included 30% of eggs. CLSM pictures of batters with such formulation showed some similarities to a wheat dough which was taken as reference (Fig. 8.4). A continuous area of proteins was visible in the gluten-free batter, in which starch granules were embedded (Fig. 8.4). In the same study, the ultrastructure of batters made from a commercial gluten-free formulation based on wheat starch and a formulation containing soya flour but not skim milk powder and eggs were also analysed. In both cases, the structure was dominated by starch granules, while no oriented network was visible (Fig. 8.4). The egg supplemented formulation showed the presence of a protein network somehow similar to the gluten one also in the bread ultrastructure, while there was no evidence of a network in the two other formulations (Fig. 8.5). The ability of egg proteins to create a protein viscoelastic network similar to gluten is even more evident in the study by Moore et al. (2006). CLSM pictures of batters and breads containing soya flour or skim milk powder or whole egg powder were compared. Batters obtained with the egg powder were characterised by a more homogeneous dispersion of proteins and starch granules compared to the soya flour batter, and with a lesser

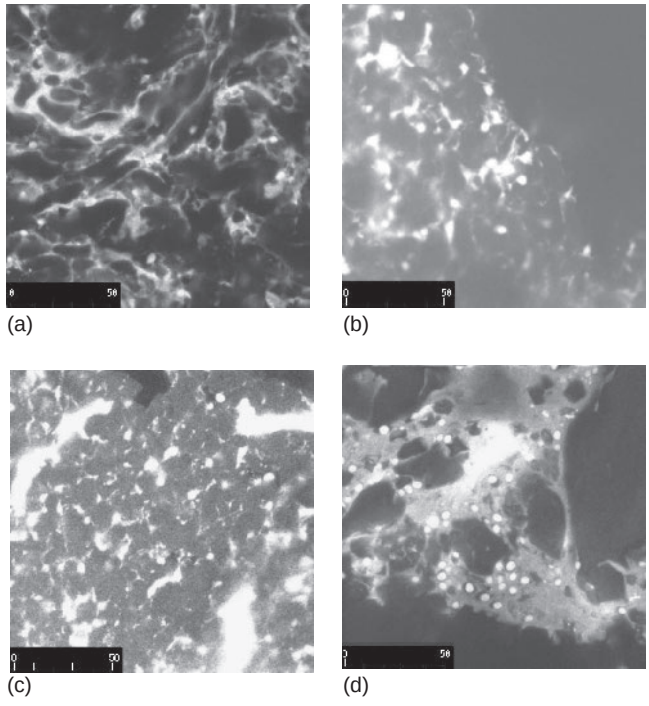


Fig. 8.5 CLSM images of wheat bread (a), commercial gluten-free bread (b), non-dairy gluten-free bread (c) and dairy/egg gluten-free bread (d) stained with Safranin O dye (magnification bar 63×, 50 μm). Proteins appear in white/grey. (*Source*: Reprinted with permission from Moore et al. (2004).)

extent to the skim milk powder batter (Fig. 8.6). Differences were even more evident in the ultrastructure of the resulting breads. The soya flour bread was characterised by the presence of large globules of denaturated proteins (Fig. 8.7a). Instead, the egg powder supplemented bread was clearly characterised by the presence of a protein network (Fig. 8.7b), while a less evident and continuous network was present in the skim milk powder bread (Fig. 8.7c). Such differences in the ultrastructures resulted in clear differences in the baking performances and bread characteristics of the three formulations. The egg powder supplemented bread showed a significant higher specific volume than the skim milk and soya flour breads, which were instead comparable. Also the number of cells/cm^2 was slightly higher for the egg powder bread, even though the differences were not significant. A high volume and a fine crumb are desirable characteristics for a gluten-free bread.

The results reported so far suggest that egg proteins are a valuable tool for replacement of gluten in gluten-free formulations, as shown by the improvements in the textural characteristics of the resulting breads. Among the protein sources discussed in the present section, egg proteins are the most likely to mimic the viscoelastic properties of gluten and promote the formation of a protein network in gluten-free breads.

8.2.5 Enzymes

Enzymes are commonly applied in the baking industry in order to improve the characteristics and quality of wheat flour based products. Such enzymes include amylases, proteases, hemicellulases, lipases and oxidases which all influence the whole baking process (Hozová

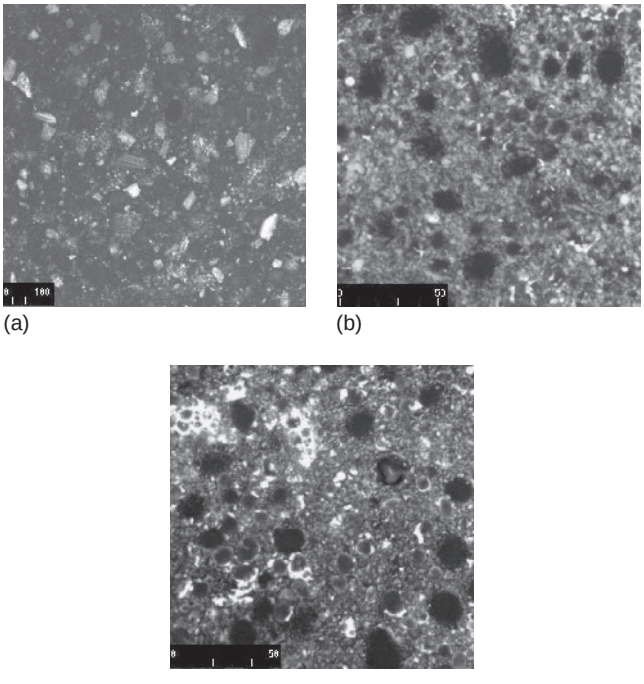


Fig. 8.6 CLSM images of batters containing soya flour (a), egg powder (b) and skim milk powder (c). Magnification 40×, bar corresponds to 100 μm. Starch granules appear as grey globules while proteins appear white. (*Source*: Reprinted with permission from Moore et al. (2006).)

et al., 2002). To date, there are few published reports on the utilisation of enzymes and their impact on gluten-free foods. Gujral et al. (2004a) investigated the addition of GO in a white rice gluten-free formulation. GO catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide, which in wheat flour either causes the formation of disulfide bonds between proteins (Haaralsita and Pullinen, 1992) or the tyrosine cross-links. Therefore, protein cross-linking might improve functionality of rice proteins, thus leading to improved quality of rice bread. Gujral et al. (2004a) found that addition of GO significantly improved bread quality as increase in specific volume as well as decrease in crumb hardness was found, thus confirming previous findings on wheat flour (Vemulapalli et al., 1998). Improvements were even more significant when 2% HPMC was added in the formulation, leading to specific volumes higher than 2.5 cm^3/g and further reduction of crumb hardness. The authors concluded that the modifications brought by GO on rice proteins, as indicated by changes in the electrophoretic pattern of rice glutelins, were responsible for such improvements. In order to promote formation of a protein network, another enzyme, that is, transglutaminase (TGase: protein-glutamine γ-glutamyltrans-ferase, EC 2.3.2.13) has been recently investigated as improver in gluten-free formulations. TGase is an enzyme capable of catalysing acyl-transfer reactions introducing covalent cross-links between proteins (Nonaka et al., 1989) as well as peptides and various primary amines. Cross-linking occurs when the ε-amino groups of lysine residues in proteins act as an acyl-receptor; ε-(γ-Glu)Lys bonds (isopeptide bonds) are formed both intra- and inter-molecularly (Ando et al., 1989). In the absence of primary

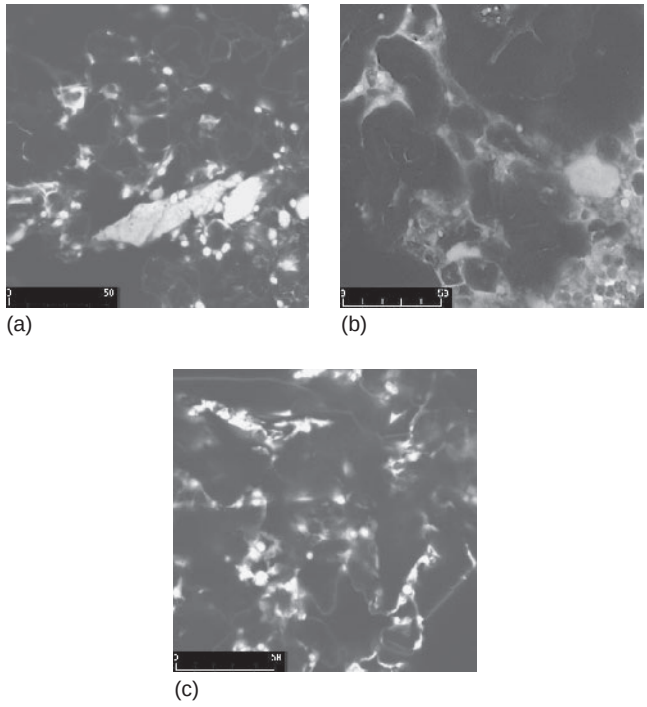


Fig. 8.7 CLSM images of bread crumbs containing soya flour (a), egg powder (b) and skim milk powder (c). Magnification 63×, bar corresponds to 50 μm. Proteins appear as white/grey strands. (*Source*: Reprinted with permission from Ahlborn et al. (2005).)

amines in the reaction system, water is used as an acyl acceptor leading to deamidation of glutamine residues (Motoki and Kumazawa, 2000). Therefore, TGase can be applied as a protein modifier (Basman et al., 2002). TGase is able to modify the functional characteristics of a wide range of proteins of different origins: casein and albumen from milk, animal protein form eggs and meat, soya protein and wheat protein. Moore et al. (2006) investigated the application of TGase on gluten-free bread formulations containing three different protein sources, that is, soya, milk and eggs. All breads contained xanthan gum as hydrocolloid. The effect of the enzyme varied depending on the protein source and addition level of TGase. In general, the application of TGase increased crumb firmness in all breads, depending on the addition level. In the case of milk and egg proteins containing breads, TGase increased the number of cells/cm^2 indicating an improvement in the crumb structure due to protein cross-linking. The dairy containing bread also showed a significant decrease in specific volume with addition of 10 units of TGase per gram of proteins present in the recipe. The increase in crumb firmness and decrease in specific volume with TGase application confirmed previous findings (Basman et al., 2002; Salmenkallio-Marttila et al., 2004). Analyses of the microstructure of batters and breads supplemented with TGase revealed that no differences could be detected on the microstructure of batters supplemented with TGase compared to their controls (Moore et al., 2006). Instead, differences in the microstructure of the breads were detected for the milk and egg proteins formulations, as TGase application enhanced continuity in the protein network.

Different results with application of TGase on white rice flour based gluten-free bread were found by Gujral and Rosell (2004a). In this study, an increase in specific volume and a decrease in crumb hardness were found with application of the enzyme at levels up to 1% (fwb) (corresponding to ≅12 U/g of proteins). Differences in both parameters were significant when 2% HPMC was added in the formulation. At TGase levels higher than 1%, an opposite trend was found, as a decrease in specific volume and increase in hardness were observed.

In conclusion, these results encourage the use of protein cross-linking enzymes to improve the textural characteristics of gluten-free bread, that is, improving crumb structure and specific volume. On the other hand, the effect of the enzymes varies depending on the protein source present, the level of addition and the type of hydrocolloid present in the formulation. Therefore, further studies are required to better understand the functionality of protein cross-linking enzymes in gluten-free formulations.

8.2.6 Sourdough

Sourdough fermentation has a well-established role in improving flavour and structure of bread (Arendt et al., 2007). Up to now, little is published on the use of sourdough in gluten-free breads. Recently, Moore (2005) investigated the potential of applying sourdough produced using a mixture of brown rice flour, cornstarch, buckwheat and soya flour to a gluten-free bread formulation. Analysis of the microstructure of the gluten-free sourdough by CLSM showed that the protein fraction was degraded over time (Fig. 8.8). However, the comparison with gluten isolated from wheat sourdough revealed that in the gluten-free system such degradation was far less obvious (Fig. 8.8). The microstructure of the gluten-free batter with 20% sourdough addition did not show any significant difference compared to the control (Fig. 8.8), which was not the case for the wheat dough containing 20% sourdough (Fig. 8.8). Consequently, the breads resulting from the gluten-free batter with sourdough addition did not show any significant difference compared to the controls, in terms of loaves volume and height, number of cells, or mean cell area. Interestingly, addition of sourdough delayed the onset of staling, thus indicating that sourdough can improve the quality of gluten-free bread. Recently, Ryan et al. (2006) found that addition of sourdough to gluten-free formulations improved the resulting breads compared to the controls by increasing specific volume and decreasing crumb firmness. The effect of sourdough was species as well as strain specific. Best results were obtained with a *Lactobacillus plantarum* FST 1.7.

Sourdough has great potential as tool for development of good quality gluten-free breads, as the above studies indicate. However, investigations on the use of sourdough in gluten-free breads are only at the early stages and extensive work is needed in order to better understand the specificity of certain species and strains rather than others, and the impact of sourdough on structure and texture of batters and breads from gluten-free formulations.

8.3 GLUTEN-FREE BISCUITS

Biscuits are based on three main ingredients: wheat flour, fat and sugar. Different combinations form the basis of a full range of products (Chevallier et al., 2000; Chevallier et al., 2002; Maache-Rezzoug et al., 1998). In contrast to bread, to achieve good biscuit quality, the gluten network has to develop only slightly, in order to give a cohesive but not too elastic dough (Contamine et al., 1995). In gluten-free biscuit manufacture, different ingredients can

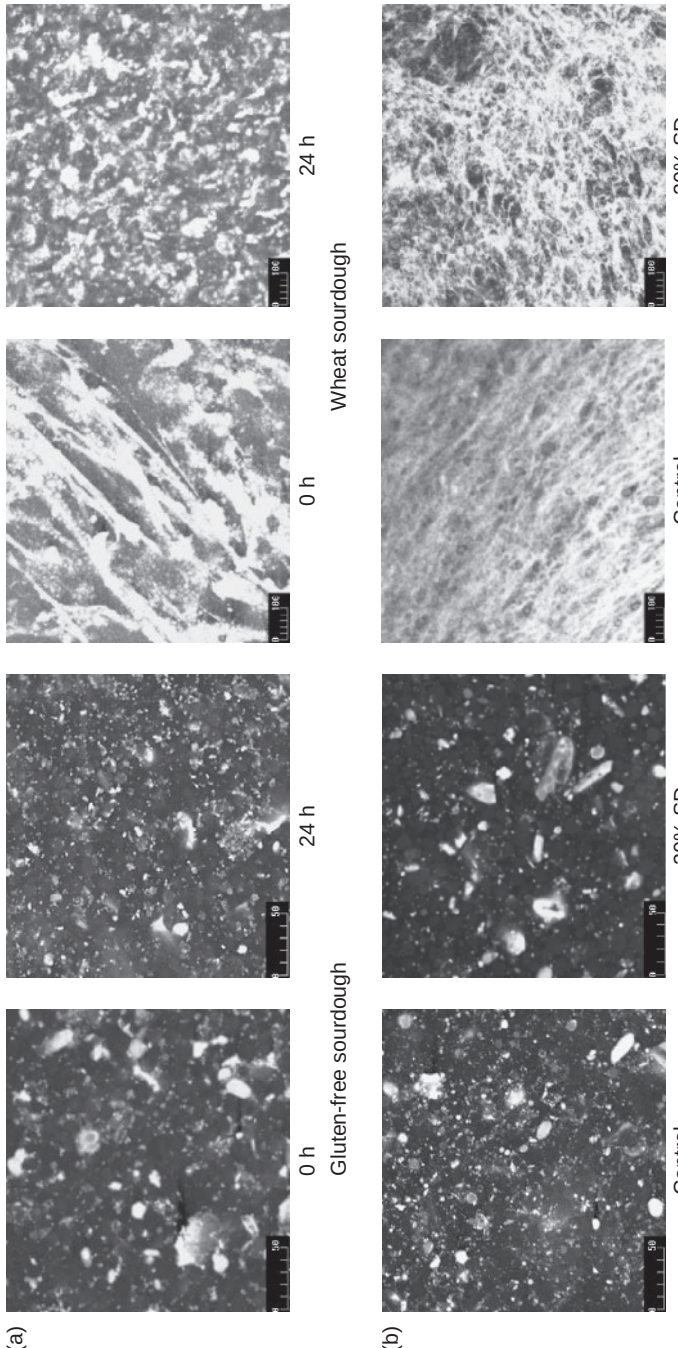


Fig. 8.8 Confocal laser scanning micrographs of (a) gluten-free sourdough and wheat sourdough at 0 h and after 24 h (magnification bar corresponds to 50 μm) and (b) gluten-free batter with 20% sourdough and wheat dough with 20% sourdough. (*Source*: Reprinted with permission from Clarke et al. (2004).)

be combined in order to obtain a similarly cohesive dough. Gluten-free biscuits are commercially available, but they are often based on pure starch, thus resulting in a dry, sandy mouthfeel (Schober et al., 2003). To our knowledge, little is published in the development of gluten-free biscuits and the microstructure has not been analysed.

Schober et al. (2003) investigated the combination of several ingredients varying in starch and protein sources, and compared them with wheat flour and a commercial gluten-free mix used in the development of gluten-free biscuits. Biscuit doughs were tested for their hardness and stickiness, while the biscuits were analysed for water activity, moisture, force in snap test and dimensions (diameter, thickness). The combination of 70% brown rice flour, 10% cornstarch, 10% potato starch and 10% soya flour gave optimum results as both dough and biscuits measured parameters were comparable to those of the wheat control. Such gluten-free dough, being firm and non-sticky as the wheat dough, resulted in firm, thin and round biscuits. The authors investigated the impact of different fat sources on the characteristics of the doughs and biscuits described in the first experiment. The inclusion of high and low fat powders resulted in products with significant differences in terms of dough hardness and stickiness, biscuit thickness and firmness. High fat powders were needed in order to obtain biscuits doughs which were cohesive and sheetable.

In conclusion, in gluten-free biscuit development, products of desired characteristics can be achieved by the right selection and combination of ingredients.

8.4 CONCLUSION

The findings reported indicate that several functional ingredients are required to develop gluten-free breads of desirable characteristics, that is, good volume and crumb structure, reflecting the technological difficulty of replacing wheat gluten. Among functional ingredients, flours and starches from naturally gluten-free sources constitute the basis for gluten-free bread formulations. However, based on the literature available, it is difficult to assess the impact of each flour and/or starch on the microstructure and textural characteristics of batters and breads as, in most cases, such ingredients are only part of a more complex formulation involving several functional ingredients, and thus interactions can play a major role. Several studies focus on optimisation of complex formulations or investigate the impact of functional ingredients on optimised complex recipes, thus limiting extrapolation of results. A valuable approach has been indicated by Gujral et al. (2003a, b) and Schober et al. (2004) using simple formulations involving only one flour and investigating the impact of several functional ingredients, that is, hydrocolloids, enzymes and starches, on such formulations. Among these functional ingredients, hydrocolloids prove to be essential in any gluten-free formulation in order to confer good gas holding properties to the batters. However, the impact of each hydrocolloid varies significantly depending on its chemical characteristics, resulting in breads with significant differences in terms of specific volume and crumb structure. Furthermore, their possible interactions with other functional ingredients must be taken into account. Microscopic techniques such as CLSM are a valuable tool for a deeper understanding of the impact of functional ingredients on the microstructure on gluten-free batters and breads. However, only few studies applied such techniques, therefore information available is still limited. In the future, extensive application of microscopic techniques and particularly CLSM can lead to a deeper understanding of the role of functional ingredients and their interactions in gluten-free bread formulas. On the other hand, gluten-free biscuits development does not constitute a major technological challenge, which explains the few investigations available

on the subject and, as already stated, gluten-free biscuits of comparable quality to their wheat counterparts can be produced by the right selection and combination of ingredients.

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9 Manufacture of Gluten-Free Specialty Breads and Confectionery Products*

Tilman J. Schober

9.1 INTRODUCTION

The present chapter aims at helping the reader in the development or improvement of gluten-free bakery products by providing an understanding of the function of ingredients and processing steps. We therefore limit the formulations presented to examples, which are relevant for an understanding of the physico-chemical principles. In order to compromise between a scientifically sound piece of literature and a concise handbook for practical use, each section explains existing knowledge in the form of a critical literature review, while at the end of the sections, we summarize the most important points in a simple way. Due to our own area of work, we put some emphasis on sorghum as an ingredient, which due to some unfavorable properties of its components – like proteins firmly encapsulated in protein bodies and high starch gelatinization temperature – is especially challenging. Section 'Example: development of a gluten-free sorghum bread' (p. 154) and Table 9.3 may provide a quick introduction into the topic for those with limited time.

9.2 FORBIDDEN, PERMITTED AND CONTROVERSIAL INGREDIENTS

9.2.1 Who needs gluten-free bread?

Potential customers of gluten-free products may be three groups of people: those with celiac disease, those with wheat allergies and possibly also people who depend on a low-protein diet. Permissible ingredients will naturally depend on the target group. Most gluten-free studies focused on *celiac disease*, which is a so-called autoimmune enteropathy (Fasano and Catassi, 2001). This means that it belongs to the larger group of autoimmune diseases, in case of which the own immune system erroneously attacks components of the body. 'Enteropathy' simply means a disease of the intestine. In case of celiac disease this encompasses damage of the small intestinal mucosa, including, as a typical sign blunting or vanishing of the absorptive villi. This results – again in typical cases – in symptoms like chronic diarrhea and malabsorption. Celiac disease is induced by prolamins from wheat, rye and barley. Recent research has shown that deamidation of glutamine to glutamate by tissue transglutaminase

*Disclaimer: Brand and company names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

within a crucial amino acid sequence plays a role, although the complete mechanism is still not known (Skovbjerg et al., 2004; Gerrard and Sutton, 2005). Celiac disease is no allergy, although sometimes erroneously called so. It is much more common than scientists assumed some decades ago, and a worldwide prevalence of 1 in 266 persons has been suggested based on modern serologic screening (Fasano and Catassi, 2001).

Typical *wheat allergies* are mediated by immunoglobulin E (IgE) and should be differentiated from other adverse reactions to wheat where IgE is not involved (Sutton et al., 1982). Sometimes adverse responses to gluten in which the immune system is not involved are termed '*gluten intolerance*' (Kadan and Schluckebier, 2007). Following this definition, gluten intolerances would exclude celiac disease and wheat allergies and be more unspecific diseases. In real *wheat allergies*, IgE antibodies may be directed against various components of wheat. Skin reactions (urticaria, i.e. hives; angioedema, i.e. swellings in the deep layers of the skin), gastrointestinal reactions (vomiting and diarrhea), asthma, hypotension (a drop in blood pressure) and in the worst case anaphylaxis (a serious, potentially life-threatening response of the whole body) may result (Sutton et al., 1982; Friedman et al., 1994; The Cleveland Clinic, 2006, *online*; WebMD, 2009, *online*). In case of the wheat-dependent, exercise-induced anaphylaxis, wheat ingestion together with physical exercise induces anaphylaxis (Lehto et al., 2003). Cross-reactions between cereals have been described (e.g. between prolamins from wheat, rye and barley; Palosuo et al., 2001).

Phenylketonuria is an example of a disease requiring a *low-protein diet*. Individuals with phenylketonuria cannot completely metabolize the essential amino acid phenylalanine, usually because of a deficiency in the enzyme phenylalanine hydroxylase. Therefore, they have unusually high plasma levels of phenylalanine, causing mental retardation via an unknown mechanism. Phenylalanine is basically present in all proteins. High protein foods like meat or dairy products are obviously a problem, but also the protein levels in normal bread are critically high (Arnold, 2007, *online*).

Summary: *Forbidden and permitted ingredients for gluten-free products depend on the target group: people with celiac disease (usually the main target group), people with wheat allergies or people on a low-protein diet.*

9.2.2 Cereals and starches

In case of celiac disease, all types of wheat, rye (*Secale cereale*) and barley (*Hordeum vulgare*) are forbidden. All three are closely related members of the grass family (Kasarda, 2001). Types of wheat include, for example, common bread wheat (*Triticum aestivum* L. ssp. *aestivum*), durum wheat (*Triticum turgidum* L. ssp. *durum*), spelt wheat (dinkel, *Triticum aestivum* L. ssp. *spelta* (L.) Thell.) and also emmer (*Triticum turgidum* L. ssp. *dicoccum*) and einkorn (*Triticum monococcum* L. ssp. *monococcum*). Since wheat and rye are forbidden, obviously also triticale is forbidden, which is a man-made cross between these two species. Occasional reports that spelt wheat might be safe for celiacs are clearly wrong, based on scientifically sound studies (Forssell and Wieser, 1995; Kasarda and D'Ovidio, 1999). Discussions about ancient, less well-known wheats like einkorn are merely of scientific interest, and it is highly recommended that celiac patients avoid them.

Safe cereals include rice, corn (maize), sorghum, millets and teff. Kasarda (2001) pointed out that only rice and corn can be regarded as safe based on scientific testing, while for example millets, sorghum and teff are just likely to be safe because they are more closely related to rice or corn than to wheat. Similarly, the pseudocereals buckwheat, amaranth and quinoa are not members of the grass family at all, and therefore only so distantly related to

grasses that it is highly unlikely that they are toxic to celiacs. For practical purposes, all these grains can be regarded as safe because they have been consumed by celiacs for a long time.

Oats are probably the most controversial of all the grains. While more recent studies suggest that they are inherently safe (e.g. Picarelli et al., 2001), there remains some controversy, and often, only moderate doses, if at all, are recommended (e.g. Murray, 1999). Another concern is the contamination of oats or oat products with wheat (Thompson, 2001a). Størsrud et al. (2003) found that levels of gluten contamination in oats were in most cases low, although there was a tendency for higher levels of contamination with increased processing. In the same study, considerable gluten contamination was also detected in some samples of maize and buckwheat. This leads to the general problem of contamination. While naturally gluten-free products like rice and corn are generally regarded as safe, there is still the risk that they might be contaminated with wheat, rye or barley. Contamination might occur at all stages (growth, harvest, transport, milling and further processing) (Størsrud et al., 2003). A quality management system that includes all stages from growth to processing is therefore required. This must include obtaining gluten-free cereals or flours from reliable sources that are aware of the contamination problem. A minimum requirement is the proper separation of gluten-containing and gluten-free cereals and proper cleaning of equipment that is also used for gluten-containing cereals. Ideally, growth, transport, milling and processing should be done by specialized producers, who solely work with gluten-free materials. Regular verification of the absence of gluten, for example by ELISA (enzyme-linked immunosorbent assay) techniques, is essential.

Besides safe cereals, a variety of isolated starches can be used in gluten-free products. Within all common starches (excluding barley or rye starch), only wheat starch remains controversial. A recent study (Peräaho et al., 2003) found that the response of patients with newly detected celiac disease to a wheat-starch-based gluten-free diet was as good as to a natural gluten-free diet. However, there remains the concern of long-term damage due to small remaining amounts of gluten in wheat starch (Ciclitira et al., 1985; Thompson, 2001b) and a study by Biagi et al. (2004) with the very descriptive title 'A milligram of gluten a day keeps the mucosal recovery away: a case report' should warn us to take also small remainders of gluten seriously. Due to the fact that alternatives to wheat starch are available, there is little reason for its use, although it should also be clearly stressed that the problem of a potential gluten contamination is not limited to wheat starch.

In the case of wheat allergies, as contrasted to celiac disease, it is not possible to make a valid statement for all patients on what to avoid, due to the magnitude of components targeted by the IgE antibodies and due to possible cross-reactions. For the producer of wheat-free bread, the strict prevention of contamination, as in case of celiac disease, and clear labeling of ingredients are of central importance. We would also strongly recommend avoiding all types of wheat listed above. It has for example been shown, that spelt (*Triticum aestivum* ssp. *spelta*) triggered strong allergic reactions in a person with wheat allergy (Friedman et al., 1994).

For a low-protein diet, isolated starches have to be used, because only they guarantee very low protein levels. In contrast to celiac disease, in this case wheat starch is not a problem.

Summary: *For people with celiac disease and those with wheat allergies, all types of wheat are forbidden, and especially tricky are: for example, spelt wheat (spelt, dinkel), emmer and einkorn, which are not automatically recognized as wheat sub-species by non-scientists, but are forbidden. Anecdotal reports that, for example, spelt is tolerated by celiacs are dangerously misleading. Also forbidden for celiacs are rye, barley and triticale. Safe cereals include rice, corn (maize), sorghum, millets, teff and the pseudocereals buckwheat, amaranth*

and quinoa. Oats and wheat starch are controversial with regard to celiac disease, and we suggest a conservative approach.

9.2.3 Other critical ingredients

9.2.3.1 *Milk*

Celiac patients often show a so-called secondary lactose intolerance. Secondary lactose intolerance is contrasted to primary lactose intolerance. The latter means that people are born without the ability to synthesize lactase and thus cannot digest lactose. In case of celiac patients, the lactase can typically be synthesized, but because it is located at the villi, it vanishes as the villi are destroyed in the course of the disease and lactose can no longer be digested. Therefore, lactose should be avoided, at least until the damage to the gut has healed due to a gluten-free diet, that is, the villi are recovered. There are, of course, some celiacs, who have at the same time primary lactose intolerance (Murray, 1996 *online*, 1999). As a consequence, milk need not be avoided generally in gluten-free bread for celiacs, but some lactose-free gluten-free products need to be available.

Milk is also a very common food allergen, ranking among the top three food allergens in various countries (Dalal et al., 2002). This might suggest avoiding it in a bread where the aim is a generally low allergenic potential.

Finally, milk is a high protein food and thus has no place in a low-protein bread.

9.2.3.2 *Egg, soy and other ingredients with allergenic potential*

Similar to milk, soy and especially egg rank high among common food allergens in various countries (Dalal et al., 2002), and both are also high protein foods, thus inappropriate for a low-protein bread. Among children, the most common food allergens are cow's milk, egg, peanut, tree nuts, soy, wheat and fish. Allergies against peanuts, tree nuts and fish/seafood usually persist lifelong (Dalal et al., 2002).

9.2.3.3 *Transglutaminase*

Transglutaminase (TGase) is an enzyme that can catalyze formation of crosslinks in proteins between lysine residues and glutamine residues. Other reactions catalyzed are the introduction of free amine groups into proteins and the hydrolyzation of glutamine residues to glutamate residues (deamidation) (Gerrard and Sutton, 2005; Fig. 9.1). Researchers have used TGase in trying to create gluten-like networks in doughs from gluten-free cereals (Gujral and Rosell, 2004; Moore et al., 2006; Renzetti et al., 2008).

As described above, deamidation of glutamine to glutamate by tissue TGase within a crucial amino acid sequence is involved in triggering celiac disease. Intuitively, this should cause concern when using microbial TGase in gluten-free products for celiacs, and similar concerns have been raised in at least two articles. Gerrard and Sutton (2005) considered the issue of TGase addition to gluten-containing cereals and expressed concern that deamidation might create the toxic epitope already during processing, thus increasing toxicity and endangering genetically predisposed individuals, whose response to gluten is dose dependent (in short: TGase might make the gluten more toxic to celiacs, making those sick who are at the edge of having overt symptoms of celiac disease). At the same time, however, Gerrard and Sutton (2005) pointed out that TGase is normally present in the human intestine. It might therefore be that there is no difference in toxicity, whether deamidation occurs during

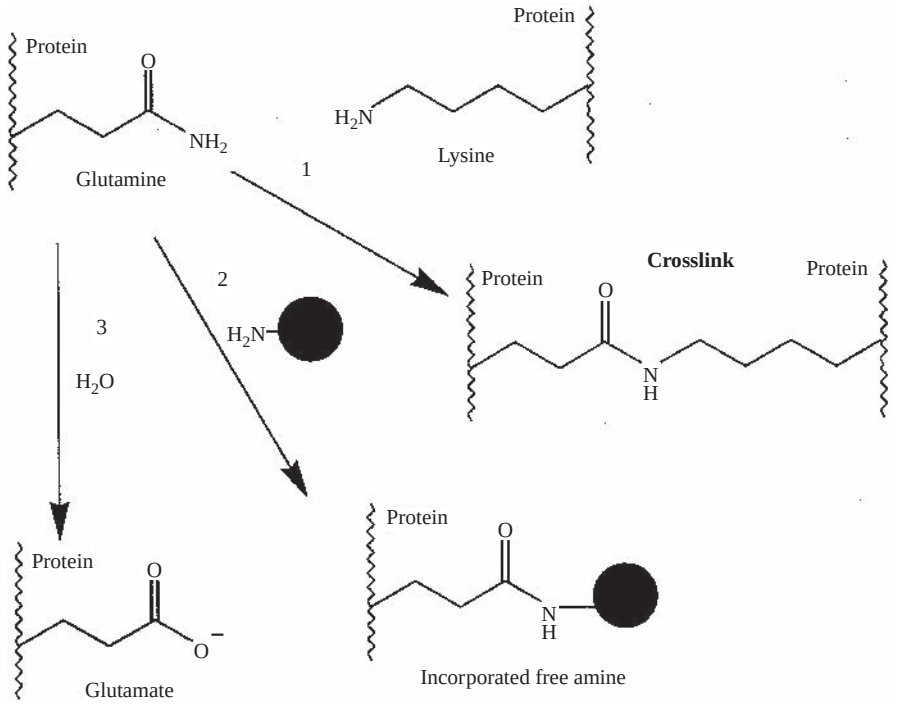


Fig. 9.1 Reactions catalyzed by transglutaminase; 1: protein crosslinking, 3: deamidation. (*Source*: Reprinted from Gerrard and Sutton (2005); copyright 2005, with permission from Elsevier.)

processing due to microbial TGase or in the intestine. These authors also mentioned that the crucial deamidation step might only be carried out by the tissue TGase in the intestine, but not by microbial TGase used in food.

Some clarification was provided by Dekking et al. (2008) and consequently, these authors adopted an even more critical position than Gerrard and Sutton (2005). Dekking et al. (2008) found that microbial TGase used in food can indeed deamidate gluten proteins, generating peptides that stimulate gluten-specific T cells from celiac patients. Microbial TGase can thus enhance immunogenicity of gluten. These authors pointed out that gluten-free foods in fact frequently and inevitably contain small amounts of gluten, so that there is no totally gluten-free diet. These small amounts of contaminants would become more toxic to celiacs due to TGase action.

The application of TGase in gluten-free bread is still in the experimental stage. Scientific studies using TGase in gluten-free bread might in fact improve the theoretical understanding of gluten-free systems. However, the above studies strongly suggest that TGase should not be used in the large-scale production of gluten-free breads destined for celiacs.

Summary: *Milk is somewhat critical for celiacs because many of them are lactose intolerant, before the damage to the gut has healed in the course of a gluten-free diet. Afterwards, it may be tolerated again. Milk, egg and soy have a high allergenic potential and are high protein ingredients. The most common food allergens for children are (cow's) milk, egg, peanut, tree nuts, soy, wheat and fish. There is increasing evidence that transglutaminase is dangerous for celiacs.*

9.3 GLUTEN-FREE BREADS

In this section, examples of bread formulations are presented, followed by a discussion of the structural principles. We start with starch breads, which are the least complex, followed by sorghum breads, rice breads and breads from other cereals/non-cereals and mixtures. We continue with some special ingredients for various types of gluten-free breads, and some more general considerations for an in-depth understanding (e.g. in how far gluten-free breads resemble rye breads). We finish the bread section with considerations on staling, which is especially important in the case of gluten-free breads, which generally stale very fast.

9.3.1 Starch-based breads

9.3.1.1 *Formulations for starch-based breads*

Table 9.1 shows different successful formulations for starch breads from the literature and two of our own experiments. Besides basic ingredients (starch, water, salt, sugar and yeast) all studies used special ingredients, that is, emulsifier (glycerol monostearate) (Jongh, 1961), soy protein isolate, xanthan gum and shortening (Ranhotra et al., 1975), xanthan gum alone (Ács et al., 1996a, b; Keetels et al., 1996; Schober and Bean, unpublished) and hydroxypropyl methylcellulose (HPMC) alone (Schober and Bean, unpublished). The breadmaking procedures were similar amongst all studies. Ingredients were mixed, followed by a rest time, and then remixed. Final proof in bread pans and baking concluded the process.

All papers reported important findings besides the mere formulations. Jongh (1961) described that without emulsifier, the bread crumb was irregular, very coarse and hard immediately after cooling. Addition of emulsifier caused, according to the author, 'regular and reasonably fine crumb'. Further effects of the emulsifier observed were: flocculation of diluted starch suspensions (large aggregates were formed resulting in a voluminous sediment), loss of dilatancy of concentrated starch suspensions (i.e. the suspensions no longer showed an increase in viscosity with shear stress and strain), instead they became plastic and their overall consistency increased. It is worth mentioning that the emulsifier used in the study of Jongh (1961), although called glycerol monostearate, contained only 50% of this component, while 40% were distearates. We could easily bake a bread similar to the one shown by Jongh (1961) with commercial wheat starch when using active dry yeast and slightly increasing the water to 67%, even without adding emulsifier. We failed, however, when using compressed yeast and a commercial glycerol monostearate preparation, containing a minimum of 90% monoester. Ultimately, we gave up further studies on the system of Jongh (1961), because hydrocolloid-containing formulations were considerably more promising in terms of volume and crumb structure (see below and Fig. 9.2).

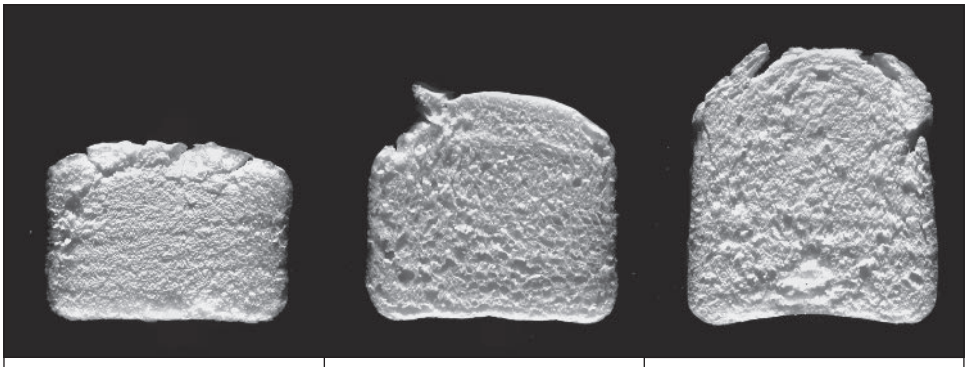
Ranhotra et al. (1975) tested the addition of different levels (0–40%) of soy protein isolate (SPI). Leavened bread with an acceptable volume (3.9 cm^3/g) could be reached without SPI, however, 20% SPI improved volume, crumb grain and texture. Without SPI, crumb was rough, crumbly and open, with SPI it was more tender, and cells were finer and more even. SPI levels higher than 20% decreased quality somewhat.

Ács et al. (1996a) compared different gums and found that xanthan gum had better effects than guar gum, locust bean gum and tragant (gum tragacanth) at any level between 1% and 5%. These authors also reported improvement of color, taste and smell by addition of 1% glucono-δ-lactone and 0.5% $NaHCO_3$.

Table 9.1 Examples of starch-based breads[a].

	Jongh (1961)	**Ranhotra et al. (1975)**	**Ács et al. (1996a, b)[b]**	**Keetels et al. (1996)**	**Schober and Bean (unpublished) xanthan gum**	**Schober and Bean (unpublished) HPMC**
Wheat starch	100	100	–	(100)[c]	100	100
Maize starch	–	–	100	–	–	–
Potato starch	–	–	–	(100)[c]	–	–
Water	60	155	100–140[d,e]	115	115	80
Soy protein isolate (SPI)	–	20	–	–	–	–
Glycerol monostearate	0.1	–	–	–	–	–
Xanthan gum	–	2	2	1.9	2	–
Hydroxypropyl methylcellulose (HPMC)	–	–	–	–	–	2
Salt (sodium chloride)	2	2	1.5	–	2	2
Sugar (saccharose)	4	14	5–10	1.9	4	4
Shortening/oil	–	10	–	–	–	–
Yeast[f]	6 (co[g])	7.5 (co)	6 (co)	3.8 (co)	2 (dr)	2 (dr)
Specific loaf volume (cm^3/g)	n.a.[h]	4.6	2.35[e]	2.6 (wheat) 3.3 (potato)	3.5	5.3
Critical ingredients	Wheat starch	Wheat starch, soy	None	None/Wheat starch[c]	Wheat starch	Wheat starch

[a] Best/optimized formulations, all standardized to 100 parts starch.
[b] Sensory improvements by addition of 1% glucono-δ-lactone and 0.5% $NaHCO_3$.
[c] Potato or wheat starch may be used alternatively.
[d] Water adjusted for comparable consistency (100%/140% water, when no additives/5% xanthan gum were used, respectively).
[e] Ács, E. (personal communication).
[f] Compressed (co) or active dry (dr).
[g] Yeast type not reported, compressed yeast assumed.
[h] Volume not reported; a slightly modified procedure in our own laboratory resulted in 3.9 cm^3/g (Fig. 9.2).



Following Jongh (1961), slightly modified (1650 ml, 3.9 cm^3/g)	Schober and Bean (unpublished), xanthan gum (2000 ml, 3.5 cm^3/g)	Schober and Bean (unpublished), HPMC (2490 ml, 5.3 cm^3/g)

Fig. 9.2 Three wheat starch breads (Table 9.1). The modifications relative to the original procedure of Jongh (1961) were: water increased to 68%; 6% compressed yeast replaced by 2% active dry yeast; omission of emulsifier due to the use of dry yeast.

Keetels et al. (1996) reported a lower density (i.e. higher specific volume) for potato starch bread than wheat starch bread, but a more even crumb structure for the wheat starch bread.

We added two experiments to directly compare the effect of xanthan gum and HPMC. This was done because the latter gum has been described to have much more beneficial effects in rice bread than xanthan gum (Nishita et al., 1976) and we wanted to find out, whether the same is true for starch bread. Wheat starch was used to allow comparison with the majority of studies in Table 9.1. We also repeated the experiment of Jongh (1961) in a modified way to get an idea how it compares to the more modern formulations: water was increased to 68% to achieve a smooth batter with the given wheat starch. The 6% compressed yeast was replaced by 2% active dry yeast. As described above, use of dry yeast superseded the addition of emulsifier.

The modified procedure of Jongh (1961) and both of our own experiments were carried out in a bread machine. All used an actual amount of 300 g starch per batch (Table 9.1, factor 3 for all ingredients). Duration of rest time and final proof was 15 min and 45 min, respectively, as in the study of Jongh; the bake time was prolonged to 45 min to account for the larger amount of batter. Preliminary tests indicated that too much water in combination with HPMC resulted in large holes, while insufficient water in combination with xanthan gum produced coarse bread. The formulations in Table 9.1 are based upon these findings. Figure 9.2 shows the results. The absolute volumes of the breads increased in the order Jongh (1961), Schober and Bean (xanthan gum), Schober and Bean (HPMC). In contrast, the xanthan gum bread had the lowest specific volume, lower than the bread of Jongh (1961). This is, because the xanthan gum bread contained considerably more water and therefore its weight was higher.

While the fresh bread of Jongh had a hard crumb that was leathery inside, and brittle toward the surface (Fig. 9.2, cracks), the xanthan gum bread had an elastic, soft, moist crumb when fresh, most similar to white pan bread from wheat flour. Also its pore structure resembled white wheat pan bread. The HPMC bread was very soft and fluffy, resembling cotton wool, and its volume was very high (>5 cm^3/g). In order to make the latter bread more suitable for celiacs, we tested its production from maize starch. A slight increase of the water addition to 88% on a starch basis was required, otherwise the batter was too stiff and lacked

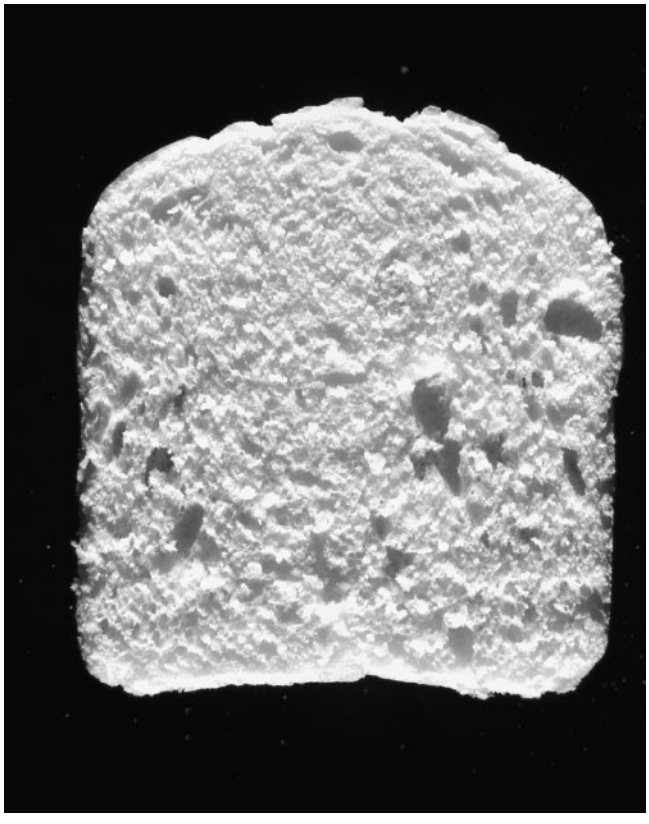


Fig. 9.3 Bread from maize starch (100%), water (88%), salt (2%), sugar (4%), active dry yeast (2%) and HPMC (2%). Specific volume was 5.3 cm^3/g.

smoothness. (Careful adjustment of the water level to reach a smooth batter, while avoiding an excess of water, appears to be a key element for bread quality.) The remaining ingredients were as described for the wheat starch-based HPMC bread. The same specific volume of 5.3 cm^3/g was reached, while the crumb was slightly coarser as for the wheat starch bread, but still good (Fig. 9.3).

In conclusion, while with pure wheat starch the greatest similarity to white pan bread could be achieved by using xanthan gum and high water addition, HPMC has the larger potential for the production of very light, highly aerated bread. The next section will address the physico-chemical background of these differences.

9.3.1.2 *Understanding the starch breads*

As always in breadmaking, there are two principally different stages: before starch gelatinization, including mixing, resting, fermenting, early stages of baking, and after starch gelatinization.

During the first stage, a simple starch batter is a suspension of starch granules and yeast cells in water, with small amounts of dissolved salt and sugar. When gas is incorporated during mixing, bubbles are suspended, which may be enlarged during fermentation. The aim is to

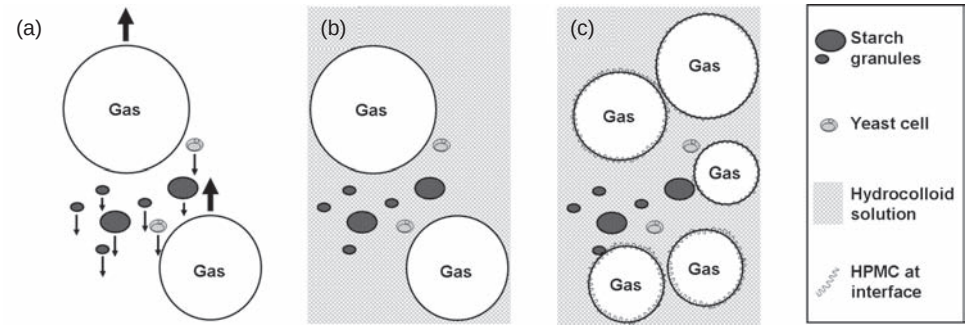


Fig. 9.4 Models for starch breads. (a) Settling of starch granules and yeast, and rising of gas bubbles, when no hydrocolloid is added. (b) Viscosity increase due to hydrocolloid addition (e.g. xanthan gum) keeps starch, yeast and gas bubbles suspended. (c) Surface-active hydrocolloids like HPMC additionally stabilize the bubbles at the gas liquid interface and prevent coalescence. A larger number of smaller bubbles results.

keep these bubbles from rising, to prevent their coalescence, and to keep the starch and yeast from settling (Fig. 9.4). A simple way to achieve this is to increase the viscosity of the liquid phase at room temperature by addition of a hydrocolloid, for example xanthan gum. Thus, rising of the bubbles and settling of the starch can be sufficiently slowed down, that the system stays homogenous during proofing, and baking until starch gelatinization. Afterwards, the starch gel provides so much viscosity that settling of remaining ungelatinized starch granules and rising of gas bubbles is no longer a problem, that is, the crumb is set. Xanthan gum differs from guar gum, locust bean gum and gum tragacanth in that its solution maintains a constant viscosity upon heating, while solutions of the latter three gums decrease in viscosity upon heating (Hoefler, 2004). This might explain that it performed best in the study of Ács et al. (1996a). The other gums would not provide sufficient viscosity during the early stages of baking when temperature increases and starch is not yet gelatinized. Additionally, locust bean gum is not completely soluble below 60°C but just swells (Hoefler, 2004).

Although it is also a hydrocolloid, HPMC differs from xanthan gum in that it is surface active (Dickinson, 2003). This originates from the hydrophobic side groups (methyl ether groups and hydroxy propyl groups) on the hydrophilic cellulose chain. A different overall substitution of cellulose with methyl ether groups and hydroxy propyl groups, and different ratios of methyl to hydroxy propyl groups can create HPMC types with different properties (Dow Chemical Company, 2005, *e-document*; Dow Chemical Company, 2007a, *online*). Surface-active substances tend to stabilize foams. They help to disperse air and thus favor formation of smaller bubbles, and they help to prevent coalescence of bubbles. Thus, in addition to increasing viscosity, HPMC also stabilizes the batter by specifically stabilizing the gas bubbles at the gas liquid interface (Fig. 9.4), and a visible consequence is the larger volume and very fluffy crumb structure of the HPMC bread (Fig. 9.2). An additional explanation is provided by one manufacturer of HPMC (Dow Chemical Company, 2007b, *online*): HPMC accumulates around the bubble surface (i.e. gas liquid interface), forming an elastic microgel there.

In order to visualize the different effects of HPMC and xanthan gum, we added 2% of the respective hydrocolloid to water and then mixed it in a blender at high speed, so that lumps were dispersed and air could be incorporated. While HPMC formed relatively stable foam almost resembling whipped egg white, xanthan gum formed a thick solution, in which only

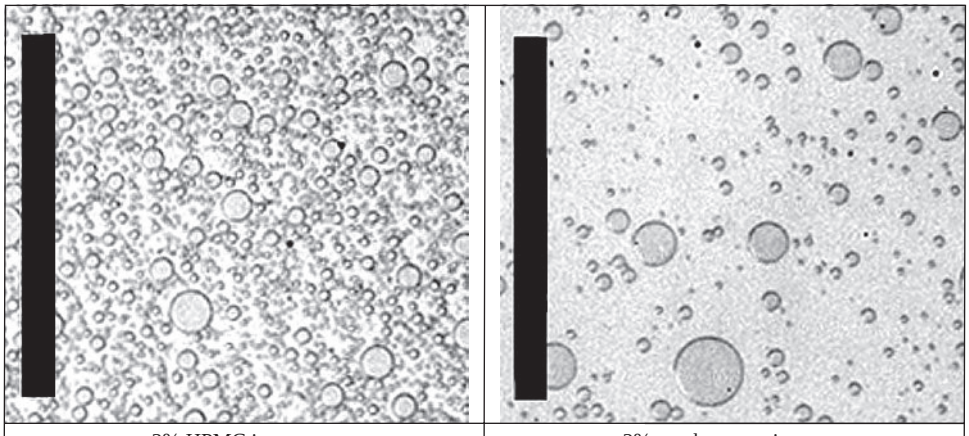


Fig. 9.5 Foams from HPMC (Methocel K4M, DOW Chemical Company, Midland, MI, USA) and xanthan gum (TICAXAN Xanthan 200, TIC Gums, Belcamp, MD, USA). Each hydrocolloid (2%) was mixed with water in a Waring-type high speed blender under standardized conditions, including 5 min rest time for swelling. For photographs, the foams were compressed between two microscope slides. Scale bars represent 5 mm. (*Source*: Reprinted from Schober et al. (2008); copyright 2008, with permission from Elsevier.)

isolated bubbles were trapped. Figure 9.5 shows the closely linked, predominantly small bubbles in HPMC foam and the individual, often large bubbles in xanthan gum, separated by wide spaces of non-aerated gum solution. Varying the xanthan gum concentration between 0.1% and 3% did not fundamentally change this behavior. At 0.1% xanthan gum, viscosity increase was too low to trap air bubbles, from 0.5% upwards, the solution/gel differed only in its viscosity/stiffness, but the coarse, distinctly separate bubbles remained. The lower density of HPMC foam (Fig. 9.5) reflected its superior aeration.

Similar to the surface activity of HPMC, other added surface-active substances may help to stabilize gas bubbles. In the case of the bread of Ranhotra et al. (1975), soy protein isolate (SPI) was added. At least two components of legume protein, 7S globulin and 11S globulin, have emulsifying properties. The 11S globulin from soybeans is called glycinin (van Vliet et al., 2002; Belitz et al., 2004). van Vliet et al. (2002) also described that glycinin forms aggregates when adsorbed at an air/water surface, which might explain that it forms a stiff protein gel layer at this interface. Ranhotra et al. (1975) could produce acceptable bread without SPI, which appears plausible because they added xanthan gum. However, the improving effects of SPI might be attributed to a stabilization of the gas bubbles at the interface, in addition to the effect of xanthan gum, which would merely keep them in suspension due to the high viscosity of the aqueous phase.

Most difficult to explain remain the results of Jongh (1961). The author claims the formation of a ‘coherent network through the whole system’, in which the starch granules are linked together at junction points, which are formed where emulsifier is adsorbed to the surface of the starch granules. The results of this starch network formation would be the previously (Section 9.3.1.1) described flocculation of starch granules, loss of dilatancy, gaining of plastic properties and improved gas holding and bread texture. However, not addressed were issues like starch-surface proteins (Seguchi and Yoshino, 1999) and their

interactions with emulsifier, or the possibility that the emulsifier might just stabilize bubbles at the gas liquid interface. Bread from starch and emulsifier instead of hydrocolloid does not seem to be very important any more, in view of much better results with hydrocolloids. Nevertheless, the possibility of linking starch granules together, forming a coherent network remains an interesting hypothesis.

9.3.1.3 *Advantages and disadvantages of starch breads*

The data in Table 9.1 and the text above show that acceptable breads with good volumes can be made from pure starches. A variety of recipes resulted in acceptable bread, and the procedures were easy, avoiding lengthy procedures (like e.g. sourdough fermentation, see Section 9.3.3.2, and Table 9.2) and even avoiding somewhat difficult process steps like rounding and shaping which are common in breadmaking procedures for wheat. Principally, pure starch breads can be made in a way that they are suitable for all classes of patients described above (celiacs, people with wheat allergy, and people on a low-protein diet), when taking into account that individual ingredients (wheat starch, soy protein) might be critical.

Disadvantages include quick staling of these breads and nutritional aspects. Quick staling is a common problem of gluten-free breads, but appears to be especially unfavorable in pure starch breads (Ahlborn et al., 2005). Keetels et al. (1996) assumed that starch breads are made up from lamellae and beams which resemble concentrated starch gels in structure. Limited water during gelatinization would prevent complete swelling and disintegration of the starch granules. A small amount of amylose would leach out of the starch granules and form a thin layer between them. It appears that amylose retrogradation occurs quickly upon cooling and stabilizes the crumb (Belitz et al., 2004). According to Keetels et al. (1996), amylopectin in the swollen granules recrystallizes during storage, rendering the granules and thus the bread stiffer. Two studies (Moore et al., 2004; Ahlborn et al., 2005) suggested that continuous protein networks (either gluten in wheat bread or added egg in gluten-free bread) can mask some of the changes due to starch retrogradation. These studies will be addressed in more detail later (Section 9.3.6.1).

Obviously, starch breads lack dietary fiber, micronutrients and protein (if protein is desired). Concerning micronutrients, enrichment is possible. Dietary fiber may be added, for example in the form of inulin, which acts also as a prebiotic (Korus et al., 2006). These aspects have been discussed by Gallagher et al. (2004). A possibly less expensive and more natural way of achieving more nutritionally balanced bread is nevertheless the use of raw materials, which are less refined than starch, for example, naturally gluten-free cereals in the form of whole meal. These ingredients also contain aroma precursors (amino acids, sugars), while starch breads have a very bland flavor.

A nutritional aspect not as easily corrected as deficiencies in nutrients and fiber is the (undesirably) quick and easy availability of glucose from starch breads. Limited available literature suggests that gluten-free breads in general tend to have a higher glycemic index (GI) than regular wheat breads, that is, the blood glucose shows a larger peak during the first 2 h after consumption for gluten-free bread than for regular bread (Foster-Powell et al., 2002; Berti et al., 2004). Theoretical considerations suggest that isolation of starch granules out of a gluten or protein matrix allows easier access of amylases to the starch (Jenkins et al., 1987; Berti et al., 2004), so that in the intestine the starch can be quicker degraded to glucose, resulting in the undesirable peak in blood glucose.

Eating much food with a high GI is undesirable and involves numerous risks, including diabetes (University of Sydney, 2007, *online*). Celiac disease and type I diabetes are

Table 9.2 Examples of sorghum-based breads[a].

	Olatunji et al. (1992a)	**Schober et al. (2005)**	**Olatunji et al. (1992b)**	**Hugo et al. (1997)**	**Hart et al. (1970)**	**Schober et al. (2007)**
Sorghum flour	70	70	70	70	80	70
Raw cassava starch	30	–	10	10	20[b]	–
Gelatinized cassava starch	–	–	20	20	–	–
Maize starch	–	30	–	–	Alternatively[b]	–
Potato starch	–	–	–	–	Alternatively[b]	30
Water	80–100	105	100–110	90	120	105
Hydroxypropyl methylcellulose (HPMC)	–	–	–	–	2	2
Salt (sodium chloride)	1.5	1.75	1.5	2	2	1.75
Sugar (saccharose)	10	1	10	8	2	1
Shortening	1	–	1	(1)[c]		–
Emulsifier	–	–	0.6[d]	(1)[c]		–
Yeast[e]	2 (co?)	2 (dr)	1 (dr)	2 (dr)	2 (co)	2 (dr)
Extra procedures/ingredients	Fungal amylase	–	–	–	–	Maltogenic α-amylase Sourdough fermentation[f]
Specific loaf volume (cm^3/g)	2.2	1.8	2.4	3.3	n.a.	2.7

[a] Best/optimized formulations, all standardized to 100 parts sorghum flour plus starch(es).
[b] Starches from sorghum, maize, cassava, arrowroot and potato had comparable effects.
[c] Emulsifier (succinylated monoglycerides) + shortening: softer, finer crumb, increased volume, off-flavor.
[d] Monoglycerol palmitate.
[e] Compressed (co) or (instant) active dry (dr).
[f] Water, sorghum flour, 1 part skim milk powder, maltogenic α-amylase, starter (*L. plantarum*) fermented for 24 h at 30°C, then 2.4 parts of calcium carbonate added for neutralization.

associated (Fasano and Catassi, 2001; Berti et al., 2004), therefore the avoidance of food with high GI is especially important for celiacs. Thus, development of gluten-free bread from naturally gluten-free cereals instead of isolated starches is important and its production will be discussed in the following sections.

Summary: *Starch breads are the simplest gluten-free breads. The only special additive required is a hydrocolloid to prevent settling of starch granules and rising of gas bubbles during fermentation. The surface-active hydrocolloid hydroxypropyl methylcellulose (HPMC) results in larger volumes and finer, fluffier crumb than the non-surface-active xanthan gum. Nevertheless, at least in one experiment presented here, starch bread with xanthan gum resembled regular white pan bread more closely. Quick staling, high glycemic index (GI), bland flavor and lack in micronutrients and fiber are problems associated with starch breads.*

9.3.2 Breads from gluten-free cereal flours versus starch breads

9.3.2.1 *A short summary of generally recognized facts from cereal science*

The most obvious difference of any flour to an isolated starch is the variety of components in the flour, including starch, proteins, soluble and insoluble fiber (e.g. pentosans, β-glucans, cellulose), lipids, minerals and polyphenols. (In fact, many of these components are also present in commercial starch, for example lipids and proteins, but only in very little amounts.) All these components can be further sub-classified, for example, soluble versus insoluble proteins or fiber, and polar versus non-polar lipids. It is known, for example, that only polar lipids can stabilize gas bubbles. The classification and functionality of proteins, beyond their solubility, is too complex for discussing it in this short paragraph.

In isolated starch, the particle size is essentially that of the starch granules. In a flour, particle size depends on the milling technique, and on the properties of the kernel, for example its hardness. We know from wheat that durum wheat, which is very hard, yields a higher amount of coarse milling product (semolina) than bread wheat. During breadmaking, particle size may influence the speed of swelling and the speed with which soluble components are extracted from the particles into the surrounding liquid phase. Particles may also directly determine structure. For example, bran particles may disrupt uniformity of gas cells. The milling technique and kernel properties also affect the amount of mechanically damaged starch, which in turn has an effect on water-binding capacity and susceptibility to enzymes. Mechanical damage enables access of water and enzymes into the inside of the starch granules, thus increasing water binding and degradation by amylases. Milling and pretreatment before milling (like polishing or decorticating) also determines the ratio of outer layers (pericarp, seed coat, aleurone layer) and germ to endosperm and consequently the composition of the flour. As a general rule, a higher percentage of outer layers results in a higher percentage of fiber components, lipids, minerals, vitamins, polyphenols and to a certain extent proteins (aleurone proteins, not storage proteins), but in a lower percentage of storage proteins and starch. Finally, the composition of flours is influenced by genetics (variety) and environment (growth conditions) of the grain. The same variety of a grain species generally has a higher protein content, when nitrogen fertilization increases. On the other hand, there are varieties, which have higher protein contents in the same environment than others.

For details and further information, see (Belitz et al., 2004, pp. 673–746 (on general cereal chemistry); Evers and Stevens, 1985 (on starch damage); Gan et al., 1995 (on gas cell stabilization and interface effects)).

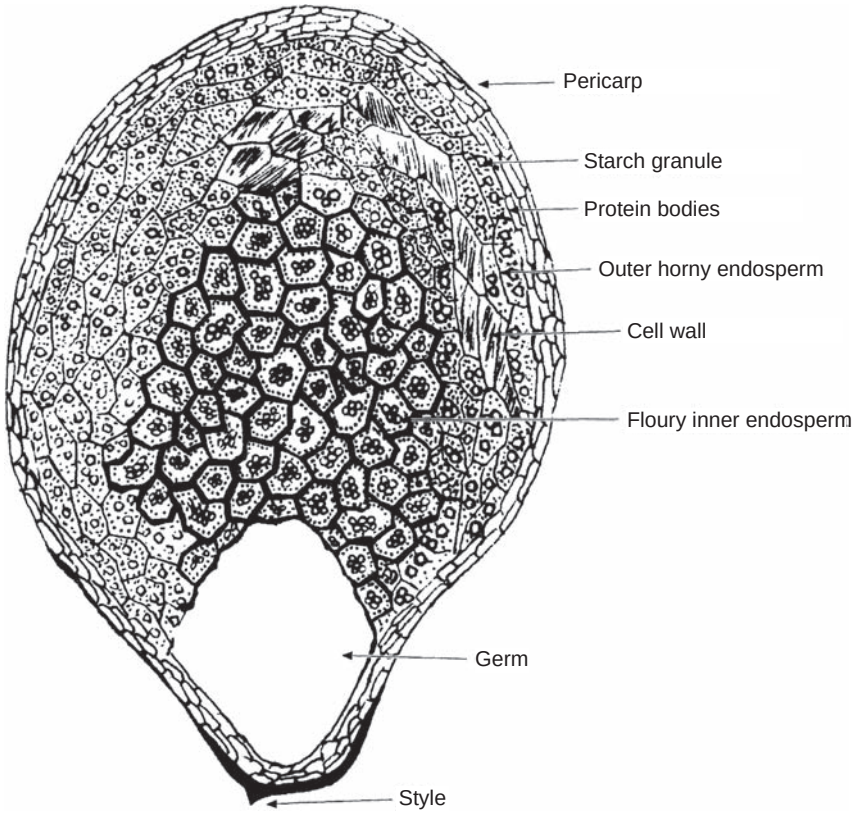


Fig. 9.6 The sorghum kernel. Mind the outer horny and inner floury endosperm. (*Source*: Reprinted from Chandrashekar and Mazhar (1999); copyright 1999, with permission from Elsevier.)

9.3.3 Sorghum-based breads

9.3.3.1 *Properties of the sorghum grain*

The sorghum kernel resembles the maize kernel in so far as it has an outer horny (corneous), vitreous and inner floury, opaque endosperm (Fig. 9.6). For practical purposes, one can assume that the outer part is at the same time vitreous and hard and the inner part opaque and soft, although Hoseney (1998) warns of a generalization in the sense that hardness and vitreousness are always the same. The horny and floury endosperm have considerable practical consequences: during initial milling, the floury part pulverizes more easily, while the horny part tends to form coarse grits (Hallgren et al., 1992). Flour from the floury part has low starch damage and tends to give less of a 'sandy' mouth feeling otherwise typical for sorghum flour (Hallgren et al., 1992). If the coarse grits from the horny endosperm are further ground into fine flour, a high amount of damaged starch results (Hallgren et al., 1992).

Protein composition is also comparable between sorghum and maize. The prolamins, called kafirins in sorghum and zeins in maize, can be further classified into α-, β- and γ-kafirins/zeins (an additional δ class is used in zeins) (Shull et al., 1991). In sorghum and maize, the prolamins (kafirins/zeins) are located in protein bodies (α-prolamins in the interior, β-, and γ-prolamins on the surface of the protein bodies) (Chandrashekar and Mazhar, 1999).

For sorghum, it has been described that the γ- and to a lesser degree β-kafirins form a disulfide-linked polymeric network that encapsulates the α-kafirins (Hamaker and Bugusu, 2003, online). Protein bodies are found in horny and floury endosperm of sorghum (Fig. 9.6) and have a diameter of roughly 1 μm. In the vitreous (horny) endosperm they are embedded in matrix protein (glutelin) (Duodu et al., 2002). Protein bodies may be relevant to technological properties in so far as they have to be disrupted in order to make the prolamins accessible. Upon cooking, sorghum proteins form disulfide-bonded oligomers, which are considerably enzyme resistant (Duodu et al., 2002). In agreement with this finding, Hamaker and Bugusu (2003, *online*) observed by laser scanning confocal microscopy that cooking causes sorghum proteins to form extended, web- or sheet-like structures with starch embedded within. Both, formation of oligomers and formation of web-like protein structures occurred to a lesser extent in maize (Duodu et al., 2002; Hamaker and Bugusu, 2003, *online*). Formation of protein aggregates upon heating may very likely affect various technological properties of sorghum.

Sorghum starch is characterized by a high gelatinization temperature, for example, relative to wheat and potato starch (Lineback, 1984), but as in all cereals, there are considerable differences between cultivars (Akingbala and Rooney, 1987).

Condensed tannins are associated by many people with sorghum. However, these polymeric polyphenols occur only in some sorghum cultivars, and it should be emphasized that virtually all sorghum cultivars grown in the US are tannin-free, for example the so-called 'white food grade sorghums', which at the same time have a white pericarp and a tan plant. The role of tannins is much more controversial than previously assumed. Tannins protect the grain against insects, weathering and birds ('bird-resistant sorghums'). Historically, focus was on nutritional disadvantages of sorghum tannins, while more recently, beneficial nutritional effects are emphasized, including a high antioxidant activity (for an overview on these aspects and further literature see Serna-Saldivar and Rooney, 1995 and Dykes and Rooney, 2006).

9.3.3.2 *Formulations for sorghum-based breads*

Table 9.2 shows formulations for sorghum-based breads from the literature. All formulations have in common that some isolated starch is used in addition to sorghum flour. Three different classes of formulations can be distinguished: Olatunji et al. (1992a) and Schober et al. (2005) added raw starch, Olatunji et al. (1992b) and Hugo et al. (1997) added mixtures of pregelatinized and raw starch, while Hart et al. (1970) and Schober et al. (2007) added HPMC in addition to raw starch. Further special ingredients were shortening and fungal amylase (Olatunji et al., 1992a), shortening and emulsifier (Olatunji et al., 1992b; Hugo et al., 1997) and maltogenic α-amylase in combination with sourdough fermentation (Schober et al., 2007).

The typical breadmaking procedure for sorghum bread was simply mixing, followed by a final proof in bread pans and baking. Olatunji et al. (1992a, b) added an additional bulk fermentation step followed by remixing before the final proof. Olatunji et al. (1992b) and Hugo et al. (1997) had to pregelatinize the starch by heating/boiling it in water. Schober et al. (2007) fermented the total sorghum flour and water together with little skim milk powder, maltogenic α-amylase and starter for 24 h at 30°C, before mixing this sourdough with the remaining ingredients, including calcium carbonate for partial neutralization.

The mentioned studies reported findings beyond the mere optimized formulations. All agreed that high water levels resulting in batters rather than doughs were required for good results, and that addition of pure starches improved results. Hugo et al. (1997) and Hart

et al. (1970) furthermore reported that, while high water levels were required, excessively high levels reduced bread quality. The use of pregelatinized starch, the need for added hydrocolloids, and other improvers was controversial. With regard to pregelatinized starch, Olatunji et al. (1992b) and Hugo et al. (1997) agreed that there is an optimum ratio between raw and gelatinized cassava starch and that the gelatinzed cassava starch has the role of providing cohesiveness, viscosity and trapping air bubbles. Olatunji et al. (1992b) added that the raw cassava starch might increase strength of the system upon baking when this starch gelatinizes. In complete contrast, Hart et al. (1970) reported that no combination of pregelatinized starch, sorghum and water produced any beneficial results.

Very controversial are the studies when it comes to the use of hydrocolloids. Hart et al. (1970) tested a large range of hydrocolloids including among others gum arabic, a guar derivative, gum tragacanth, as well as different types of methylcellulose, sodium carboxy methylcellulose and HPMC (xanthan gum was not included). They identified one type of HPMC (4000 cps Methocel) that produced clearly superior results. Without hydrocolloids, the loaves collapsed upon baking, and only HPMC types provided at the same time gas retention and prevented loaves from collapsing during baking. In the studies of Olatunji et al. (1992b) and Hugo et al. (1997), pregelatinized starch could be regarded as quasi-hydrocolloid that takes up some functions of a real hydrocolloid like providing viscosity and cohesiveness. However, the findings of Olatunji et al. (1992a) and Schober et al. (2005) are in clear contrast to Hart et al. (1970) in that they show that sorghum bread can also be made on the basis of only sorghum flour and raw starch without further special additives (Fig. 9.7a). Schober et al. (2005) also tested addition of xanthan gum and found that it lowered the volume, which is in contrast to an article by Satin (1988) that reported beneficial effects of xanthan gum on sorghum bread, however without giving detailed recipes and procedures. Finally, our own preliminary tests suggested that HPMC addition delayed staling. While our first bread (Schober et al., 2005) became unacceptably stale already after 1 day, HPMC addition produced bread that was acceptable for about a week. (For antistaling effects of HPMC see also Section 9.3.8.)

With regard to fat and emulsifier, there was agreement that there are some beneficial effects of both. Hugo et al. (1997) and Hart et al. (1970) found that shortening softened the crumb, although the former authors reported that high dosage (4–5%) caused crumbliness, while the

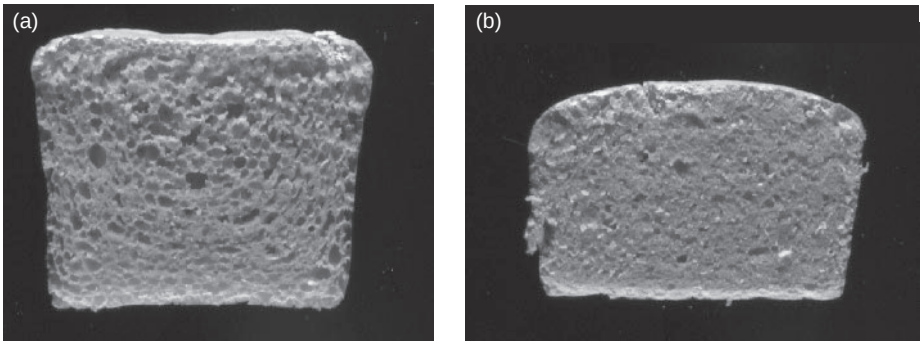


Fig. 9.7 Breads from sorghum flour (70%), maize starch (30%), water (a: 105%, b: 80%), salt (1.75%), sugar (1%) and active dry yeast (2%). Both breads were prepared from 250 g batter and proofed to height. Bread (a) reached the target height in 45 min, bread (b) was baked after 90 min (target height could not be reached, no further volume increase observed).

latter mentioned that shortening had to be combined with HPMC to prevent collapsing of the loaves. There was also agreement that some emulsifiers could soften crumb and improve crumb structure, but also weaken it or make it fragile, especially at high dosage (Hart et al., 1970; Olatunji et al., 1992b; Hugo et al., 1997). Hugo et al. (1997) furthermore reported that shortening or a combination of shortening and emulsifier reduced staling, that is, crumb firming over storage was slowed down.

Some more aspects discussed in the articles, which are relevant for an understanding of sorghum bread quality, are described as follows: Schober et al. (2005) reported overall high levels of mechanical starch damage in their sorghum flour samples. Correlations suggested that within that sample set, starch damage was higher for samples with a higher kernel hardness. On the other hand, breads from samples with higher starch damage tended to have a larger average pore size (mean cell area) but no larger volume. Schober et al. (2005) attributed the larger pore size to the fact that damaged starch is more easily degraded by amylases, resulting in more sugars for fermentation and a weaker starch gel, both resulting in a stronger expansion (and possibly coalescence) of gas bubbles. Beyond a certain point, starch damage and increase in pore size should be regarded as undesirable, especially since volume does not increase simultaneously. Two further findings by other researchers demonstrated the importance of the starch gel. Hugo et al. (1997) compared a normal, a heterowaxy (low amylose) and a waxy (virtually amylose-free) sorghum and found that the normal sorghum produced best bread, followed by the heterowaxy, while the waxy sorghum produced unacceptable bread ('with a large hole and a pudding like crumb'). The authors suggested that amylose, and possibly its retrogradation upon cooling, is critical for bread quality. Schober et al. (2007) reported that sourdough fermentation degraded proteins in the liquid phase of the batter partly. These proteins would otherwise aggregate during baking, forming strands and lumps, and interfere with the starch gel, resulting in problems like a flat top of the bread and a large hole in the crumb. Bread in which the total amount of sorghum flour had been subject to sourdough fermentation was clearly superior (Fig. 9.8).

The final question, which formulation produces the best bread, is hard to answer. Hugo et al. (1997) reported the highest specific volume, followed by Schober et al. (2007). However, for overall quality also the crumb structure plays a decisive role, and only Schober et al. (2005), Hart et al. (1970) and Schober et al. (2007) provided crumb images that would permit a direct comparison.

Summary: *Gluten-free breads can be made from sorghum flour and 20–30% pure starch, using high water levels (flour and starch to water about 1:1). A variety of successful formulations are described in the literature, but findings are sometimes contradictory between studies. Pregelatinizing part of the added starch, use of moderate amounts of certain emulsifiers, and especially use of HPMC can be beneficial. Waxy sorghum cultivars produce unacceptable bread. Damaged starch, resulting from fine milling of the horny (corneous) endosperm, has an important role on crumb structure due to its susceptibility to amylases.*

9.3.3.3 *Understanding the sorghum breads*

Viscosity increase

As in case of the starch breads, we should remember the two stages of breadmaking: before starch gelatinization, and after starch gelatinization in the course of baking. Before starch gelatinization, keeping particles and gas bubbles suspended is critical. When hydrocolloids

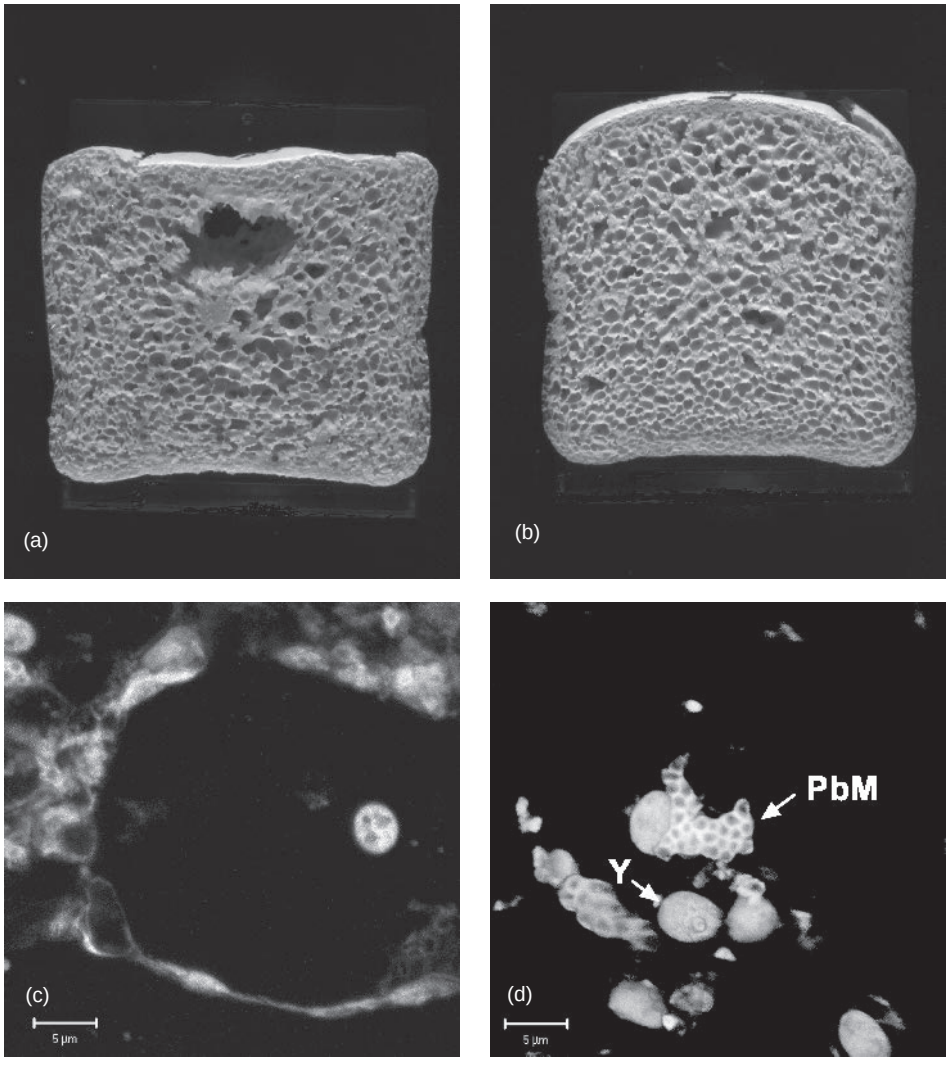


Fig. 9.8 Bread slices (a), (b) and corresponding microscopic images of the crumb (c), (d) using laser scanning confocal microscopy. (*Source*: Adapted with permission from Schober et al. (2007); copyright 2007, American Chemical Society.) Bread without sourdough (a), (c) and bread with sourdough (b), (d). In case of the sourdough bread, the total amount of sorghum flour was fermented for 24 h (formulation see Table 9.2). For the bread without sourdough, this fermentation step, as well as starter, maltogenic α-amylase and calcium carbonate were omitted. In the microscopic images, protein appears bright due to selective staining with fluorescein 5(6)-isothiocyanate. Yeast cells (Y) and protein bodies embedded in glutelin matrix (PbM) are clearly visible in the sourdough bread crumb. The crumb from bread without sourdough shows additionally strands and lumps of aggregated protein.

or pregelatinized starch are used, an increase in viscosity may be a decisive factor, as described for the starch breads (Fig. 9.4). Most interesting are however the breads without such additives (Olatunji et al., 1992a; Schober et al., 2005). In these, water levels were high and still settling of particles and rising of gas bubbles appeared to be no problem – otherwise no leavened crumb would result because the gas bubbles would rise and leave the system, and

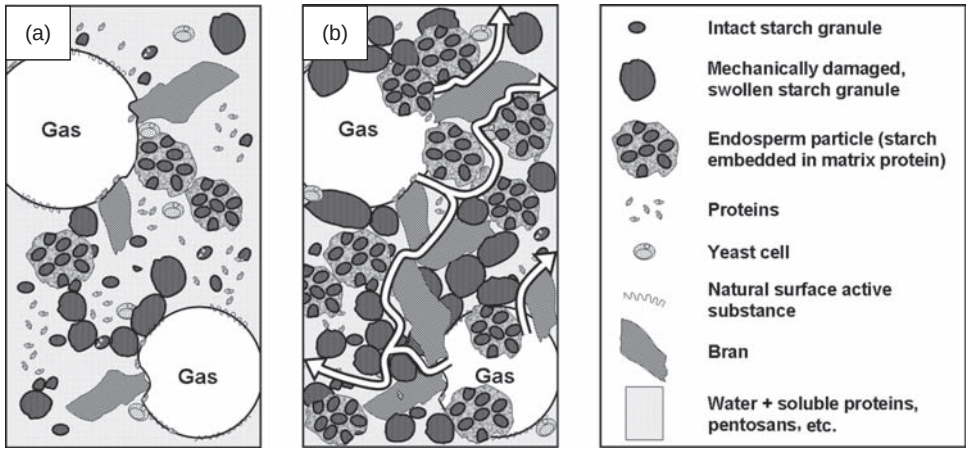


Fig. 9.9 Models for sorghum batters. (a) Thin batter with the ability to rise upon addition of extra starch and/or HPMC. (b) Thick, dough-like batter with insufficient rise.

additionally a large dense bottom layer, resulting from settled particles, could be expected in the crumb. However, although volumes were not large in these sorghum breads, and the crumb was somewhat coarse, there was still a relatively uniform leavening (Fig. 9.7a). Based on the details explained in Section 9.3.2, it is hard to draw general conclusions without having numerous analytical data on the specific flour samples used, and it is especially difficult to generalize the results from a limited sample set to a whole grain species due to differences between cultivars and growth environments. Nevertheless, data of Schober et al. (2005) in conjunction with findings of Hallgren et al. (1992) suggest that damaged starch might have a key role in at least some sorghum breads. Based on Hallgren et al. (1992), as soon as the horny (corneous) endosperm is milled into fine flour, a high amount of damaged starch will result. Likely, starch granules are so tightly packed into a rigid protein matrix in the horny endosperm (Chandrashekar and Mazhar, 1999; Duodu et al., 2002) that fine milling will break the whole matrix apart, together with the embedded starch. Accordingly, Schober et al. (2005) found very high levels of starch damage in their sample set, in which decorticated sorghum (containing horny and floury endosperm) was milled completely into fine flour on a Udy mill equipped with a small diameter screen. Interestingly, starch damage in a commercial sorghum flour was very high as well (Schober et al., 2007). Damaged starch binds more water than intact starch, and the granules swell considerably already below their gelatinization temperature (Evers and Stevens, 1985). A plausible hypothesis would therefore be that sorghum flour frequently contains numerous damaged starch granules, which at the same time bind considerable amounts of the added water and increase in size due to swelling. The result would be a sufficiently 'thick' batter, were swollen damaged starch granules and other large particles (like remaining larger endosperm particles[1] or bran) would loosely stick together, form clusters and prevent each other from settling and gas bubbles from rising by steric hindrance (Fig. 9.9a). One could compare damaged starch to pregelatinized starch in that it binds water, swells and increases viscosity at room temperature. If this hypothesis is

[1] An 'endosperm particle' would be roughly equivalent to a flour particle. We prefer using this term in order to clearly distinguish between bran particles (from the pericarp) and particles from the endosperm.

accepted, we could conclude that we either need to add pregelatinized starch or hydrocolloids, or we need to have sorghum flour with a sufficient amount of damaged starch.

Other well-known water binders like pentosans appear to be not very high in sorghum, especially if the sorghum is decorticated, because they are mainly located in the pericarp (or more simply, outer layers of the grain) (Karim and Rooney, 1972; Schober et al., 2005).

Up to here, we have discussed how to reach sufficient viscosity to keep particles and bubbles suspended. Obviously, there is also the possibility that the consistency of the sorghum batter might be too thick (all studies agreed that high water levels resulting in batters rather than doughs were required for good results, see above). We will discuss this situation later (Section 'The right balance between ingredients').

The stabilization of bubbles

Similar to added HPMC in starch breads, surface-active components in sorghum might help in the stabilization of the gas bubbles. Components to be considered in cereals in general are polar lipids, surface-active proteins (Gan et al., 1995) and water-soluble pentosans (Izydorczyk et al., 1991). Sorghum is characterized by low amounts of polar lipids due to very low amounts of glycolipids (Chung and Ohm, 2000), but still, polar lipids are present. Similarly, as stated above, sorghum is not high in pentosans, especially when decorticated. Schober et al. (2005) determined total and soluble pentosans in flours from decorticated sorghum hybrids and found very low concentrations ($<0.3\%$) of soluble pentosans. Finally, sorghum has an albumin and globulin fraction soluble in sodium chloride solution as other grains (Jambunathan et al., 1975), which might possibly help in the stabilization of gas bubbles, but there are no details available, which would allow to verify this hypothesis.

The studies of Hart et al. (1970) and Schober et al. (2007) reported decisive improvements by the surface-active hydrocolloid HPMC (see also the section on starch breads, 9.3.1.2), while the studies by Olatunji et al. (1992b), Hugo et al. (1997) and Hart et al. (1970) reported some improvements in crumb structure by moderate levels of certain emulsifiers. Both results suggest that levels of natural surface-active components in sorghum are not high enough, so that by additional amounts of added surface-active components, bubble stabilization can be improved.

Emulsifiers, starch and crumb properties

The role of added emulsifiers in the stabilization of bubbles does not really explain why these substances tend to weaken the crumb structure at the same time, especially at high levels (Hart et al., 1970; Hugo et al., 1997). Interactions of emulsifiers with starch might provide an explanation for this phenomenon. Fatty acids, including those in organic molecules like monoglycerides, and many other small molecules may form complexes with amylose molecules, so-called helical inclusion compounds or clathrates (Hoseney, 1998; Belitz et al., 2004). Emulsifiers (surfactants) that form complexes with amylose seem to cause softer bread crumb *in regular wheat bread*, presumably because they limit starch swelling (Hoseney, 1998). Similarly, Martin et al. (1991) assumed that shortening and monoglycerides decrease starch swelling during baking. For *regular wheat bread*, these authors also argued that fewer swollen starch granules and solubilized starch might form fewer entanglements with gluten. For gluten-free bread, we might argue that under the same conditions (limited starch swelling and solubilization) fewer interactions *between* starch granules and starch molecules in solution (i.e. mainly amylose) are possible. In gluten-free batter, obviously no continuous

gluten network is present that could denature during baking and thus contribute to crumb setting, so interactions within the starch phase are of central importance. The observation of Hugo et al. (1997) that waxy (i.e. practically amylose-free) sorghum produced unacceptable bread with a collapsed crumb, is in line with the important role of amylose for crumb setting (see Section 9.3.1.3). When we assume that emulsifiers and shortening limit starch swelling, and as a consequence also leaching of amylose out of the granules in the solution, there might be a trade-in between softness and crumb stability. Limited starch swelling might result in fewer interactions between starch granules and starch in solution, and therefore either in softer bread or in a weakened crumb, depending on the magnitude of the effect. We have to empirically find the type of emulsifier and shortening and the concentration level that yields the most beneficial effects. Emulsifiers and their role in staling will be discussed in Section 9.3.8.

The role of proteins and protein networks

The role of proteins in sorghum bread cannot easily be assessed in a general way. We have mentioned the *possible* contribution of soluble proteins to gas cell stabilization before. It is, however, not clear how large their role in gas cell walls really is, especially when surface-active HPMC is also used. A clearly negative effect is when proteins soluble in the liquid phase of the batter aggregate upon baking and form strands and lumps (Schober et al., 2007). It can be assumed that these aggregated proteins just interfere with the starch gel, form points of weakness, press the gel down, or reduce extensibility so that the crumb ruptures under the gas pressure and collapses, leaving a hole under the crust. We are used from wheat dough to think of protein networks as something with positive effects on bread quality. However, there is no evidence that this knowledge can be transferred to gluten-free, batter-based sorghum breads. Instead, a certain similarity to rye doughs will be discussed in Section 9.3.7.3.

The right balance between ingredients

We have discussed above how sufficient viscosity is reached in the batter, even if high water levels are added. It remains to answer the question, what the effect of low water levels, similar to those used in wheat dough, would be. Hart et al. (1970) described preliminary tests, in which sorghum dough with 35–45% moisture (dough basis, i.e. ≈54–82% water on a flour basis) did not sufficiently rise (i.e. less than double volume was reached during mixing and proofing). We made a simple experiment, in which we prepared batter from identical ingredients (70% sorghum flour, 30% maize starch, 1.75% salt, 1% sugar, 2% active dry yeast), just varying in water content. Regular water content was 105% on a flour-starch basis (formulation as in Schober et al., 2005); low water content was 80%. The regular water content produced batter of about the same consistency as pancake batter. The low water content produced a kind of dough of smooth consistency, however it lacked elasticity and extensibility when manually evaluated. The regular batter produced acceptable bread. The batter with low water hardly rose upon proofing and consequently, the resulting bread was very small and dense (Fig. 9.7).

Figure 9.9 suggests models for the breads in Fig. 9.7. Bread from thin batter (Figs. 9.7a and 9.9a) has been described above (Section ‘Viscosity increase’) – essentially water binding in damaged starch resulting in viscosity increase, swelling of damaged starch granules, and clusters of particles sticking together, resulting in steric hindrance of the settling of other particles and rising of gas bubbles. Obviously, there is an upper limit of how much water

can be bound. When exceeding this, viscosity will be too low, and dilution of particles will be too high, so that steric hindrance is insufficient. The opposite extreme, low water-containing batters resulting in breads as in Fig. 9.7b can be modeled as in Fig. 9.9b. The solids content of such dough-like batters would be so high that numerous particles would stick together. However, unlike a gluten network, these particles would only adhere loosely via their surfaces, which can be perceived upon manual testing of the thick batter in the described lack of elasticity and extensibility. We can also assume that particles will interfere with the gas cell walls, similar as suggested by Gan et al. (1995) for doughs from whole wheat. Especially large, irregularly shaped particles like bran and endosperm particles would strongly deform the bubbles. If this deformation is only at some points, as in thin batters with sufficiently high dilution, the bubble could likely extend its surface so that it surrounds the deforming object. Too many large particles, though, would deform the bubbles at too many places, so that they would penetrate the bubble walls, resulting in leaks. Once gas starts leaking out of the bubbles, it would press its way through the weakly connected particles. There would be a point reached, when all newly formed gas would just leave the dough over existing channels formed by gas pressing the particles apart. This would explain that after little initial rise the thick, dough-like batter could no longer increase in volume upon proofing.

Table 9.3 summarizes the functionality of the constituents of sorghum flour and of the major ingredients of sorghum bread as described in this whole section (9.3.3). The information provided in Table 9.3 can help with the development of new and the improvement of existing formulations. An example for a practical use of this table is given in Section 'Example: development of a gluten-free sorghum bread' below. Prior to that, however, we want to try to address contradictions between studies.

Why are there contradictions between studies?

A lot of differences between studies may be attributed to the sorghum flour. Its starch damage will depend on the milling technique and on the sorghum grain (what is the ratio of horny to floury endosperm, how hard and how brittle are these endosperm parts?) A study working with a flour with low starch damage might more likely find that it is required to add pregelatinized starch or a hydrocolloid to promote water binding in the batter than a study using flour with high starch damage.

A simple but relevant difference between studies may be the size of the bread pans used. During proofing and the initial stage of baking before starch gelatinization, the batter is very soft. Mechanical support comes from the bottom and sidewalls of the pan, but toward the center, the batter has to support itself. When increasing the size of the pan and keeping its shape and filling level similar, volume and mass of the batter increase with the third power of the length, while the surface area only increases with the length squared. This means that in larger pans, relatively more weight has to be supported by the batter itself, while in smaller pans the support by the walls is, relatively spoken, larger. This fact is likely to facilitate collapsing of the crumb center in larger batter-based breads.

Technical preparations like hydrocolloids, emulsifiers or enzymes may vary between studies. Typically they are not chemically pure substances but mixtures of components and a certain variation between batches is inevitable. (For example, How much monoglyceride do technical monoglycerides really contain, see Section 9.3.1.1 on starch breads, or how many methyl and hydroxy propyl groups does our HPMC contain, see Section 9.3.1.2?)

Some ingredients and components of cereals may interact. For gas cell stabilization in wheat dough, a competitive mechanism between polar lipids and surface-active proteins has

Table 9.3 Functionality of ingredients before and after baking (sorghum bread used as an example).

		Before baking (batter)	After baking (crumb)
Sorghum	Intact starch	Binds little water	Binds much water, amylose: quick retrogradation (setting), amylopectin: slow retrogradation (staling)
	Damaged starch	Binds much water, swells strongly, degraded to sugars by amylases	Functionality depends on degree of amylolytic degradation
	Endosperm particles	Stick to each other (?), interfere with gas cells, steric hindrance	Gritty mouth feel, limit starch swelling/gelatinization (depends on size)
	Bran particles	Interfere with gas cells, steric hindrance	Gritty mouth feel, health benefits
	Insoluble proteins (protein bodies, glutelin matrix)	Little functionality	Aggregation upon heating, this may interfere with starch gel
	Soluble proteins	Possibly gas cell stabilization	Contribute to aggregation upon heating
Pure starches (not pregelatinized)	For example from maize, potato, cassava	Bind little water, dilute sorghum flour (i.e. lower percentage of damaged starch, endosperm particles, bran, proteins)	Bind much water, amylose: quick retrogradation (setting), amylopectin: slow retrogradation (staling), easily digestible (increase glycemic index)
Additives	Non-surface-active hydrocolloids (e.g. xanthan gum)	Bind much water, increase viscosity	Bind water, retain moisture in crumb during storage
	Pregelatinized starches	Bind much water, increase viscosity, susceptible to amylases	Water binding basically unchanged (unless degraded by amylases)
	Surface-active hydrocolloids (HPMC)	Stabilize gas cells, bind much water, increase viscosity	Bind water, retain moisture in crumb during storage, antistaling
	Emulsifiers	May stabilize gas cells (depends on type/concentration of emulsifier)	May affect starch retrogradation (depends on type/concentration of emulsifier), possibly antistaling
Water		Dilutes all components, reduces interactions between particles and soluble components, reduces viscosity, facilitates bubble expansion, but also coalescence and collapsing (?)	Has to be bound in set crumb mainly by gelatinized starch

been described, that is, each component alone stabilizes the gas cells better than a mixture of both (Gan et al., 1995). Similar antagonistic (or synergistic) interactions might occur in gluten-free batters between polar lipids from the grain or added emulsifiers, proteins and even HPMC. Thus, the effect of each additive would depend on the system it is used in.

Another central question is the definition (or rather feeling) for an acceptable bread. The bread in Fig. 9.7a may be rated acceptable – or not. It is obviously not very similar to white pan bread, but is leavened. It has advantages over pure starch breads (see Section 9.3.1.3), it is inexpensive, because no additives are required and it might be promoted as 'natural' and 'healthy' for the same reason. However, if we aim for a more wheat-like quality, at present the use of HPMC is the best option.

In some cases, there are even very obvious conflicts of interest between promoting health and improving quality. For example, the more starch we add the higher the resulting volume. To put it a little sarcastic: the fluffiest sorghum bread contains only added starch and HPMC, no sorghum. However, we can reasonably expect that the more starch we add the higher the resulting glycemic index (GI). To illustrate another example: higher decortication rate reduces bran and can be expected to improve volume, but with the bran, we also lose fiber and vitamins, as is well known from wheat. It could even be speculated that, if sourdough fermentation degrades proteins, as reported by Schober et al. (2007), the GI might increase, because starch is more readily accessible to amylases in the intestine. More research is required to specifically address these health aspects.

Example: Development of a gluten-free sorghum bread

(Please look at Table 9.3 for a better understanding of this example.)

Before we even start, let us think about the bread pan: It might be a good idea to use one that is not excessively large (e.g. 1 l volume or smaller), because we can then expect less trouble with a collapsing of the crumb. (More crumb weight, relatively spoken, is supported by the sides of the pan and the crust in a smaller pan). Then, there are various starting points when developing a new formulation. Let us assume that we want to keep the amount of sorghum flour as high as possible, which is desirable for a low glycemic index, so we start with 100% sorghum flour. Let us also say that we want to avoid too much trouble with bran, but also keep our bread healthy, so we might use sorghum, which has been decorticated to lose 10% of its original weight (if we can control this variable and if we do not just have to take the flour that is available). Let us then look at the crumb. We need amylose for quick retrogradation to facilitate setting. Hence, we do *not* select flour from a waxy cultivar. We also need a certain ratio of starch to water so that the crumb can set properly and is neither moist nor dry. The starch content of our sorghum flour is a given parameter. Most recipes used about 80–110% water on a flour-starch basis, so let us start with 100% and regular amounts of salt, sugar and yeast (see Table 9.2). We will now observe the consistency of our batter, which will largely depend on the amount of damaged starch – again a given parameter. If the flour has a high starch damage, the batter will be pretty thick, so we add more water (to a total of, let's say, 120% on a flour basis). When we bake our bread, we will likely encounter problems like a coarse crumb, possibly even a large hole and/or a collapsed bottom layer – both are the effects of the excessive degradation of damaged starch granules by amylases in combination with too much water. In order to dilute our damaged starch and to promote structure formation in the crumb, we add 30% pure starch (no waxy starch). We would probably not want to add pregelatinized starch if we have high starch damage, because it would bind even more water in the batter, so we add all starch raw (e.g. 70% sorghum flour,

30% ungelatinized maize, potato or cassava starch). We will now probably need less water because undamaged added starch binds less water than sorghum flour with high damaged starch content, so we can go back to about 100%. The resulting bread might look similar to Fig. 9.7a. If we are satisfied with this quality, we can finish here, and just slightly modify fermentation and baking conditions to get the best possible result out of this formulation. It is the most inexpensive, natural option.

If we aim for a higher volume, a more regular crumb structure and a slower staling rate, we might add 2% HPMC (hydroxypropyl methylcellulose e.g. Methocel K4M, DOW Chemical), which we carefully mix with our flour and starch, so that it does not form lumps upon water addition. We now have to raise the water again (e.g. to 105% or higher) – HPMC will bind more water in the batter and help in stabilizing the crumb during baking. We may further improve our bread by sourdough fermentation of the sorghum flour, in order to degrade interfering proteins. If we add amylase into our sourdough, we might even degrade undesirably high amounts of damaged starch – but we have to add an amylase that is not very thermostable (e.g. a fungal amylase), so that it does not degrade starch after it is gelatinized. We can also try a bacterial α-amylase for its antistaling effect, which will be discussed below (Section 9.3.8). And we can try emulsifiers and shortening – Table 9.2 makes some suggestions. If the bread is still not good, locate the author of this chapter and beat him up . . . this will not improve the bread, but maybe your mood.

Let us just assume the opposite of the above situation: we obtain a sorghum flour with only little starch damage. Then we need to increase our water binding, viscosity and cohesiveness of the batter. We can either add HPMC, or pregelatinized starch. If we add a total of 30% pure starch (e.g. cassava starch), we however do not want to add all of this starch in pregelatinized form. Most likely, we would otherwise run into difficulties similar to those resulting from excessive starch damage, that is, too much water bound in the batter, too much starch degraded because pregelatized starch is readily accessible to amylases, not enough starch for gelatinization and crumb setting during baking. Let's instead look at Table 9.2: 10% raw, 20% pregelatinized starch has been successfully used before. For pregelatinization, we mix part or all of the water with the starch portion to be gelatinized, boil it for some minutes and let it cool, before mixing it with the remaining ingredients. If we are about satisfied, we can make final improvements like optimizing fermentation and baking conditions.

9.3.4 Rice-based breads

9.3.4.1 *Properties of the rice grain*

Rice is harvested as so-called *paddy rice* with the hull (husk) attached. After removal of the hull, so-called *brown rice* remains. Brown rice is equivalent to the whole grain of non-hulled cereals like wheat. Removal of bran and germ from brown rice results in *white rice* (regular milled white rice, polished rice) (Hoseney, 1998; Wilkinson and Champagne, 2004). White *rice flour* can be ground from whole or broken polished kernels. As broken kernels are a by-product of polishing that sell at a lower price, rice flour is typically made from these. Brown rice flour, ground from raw brown rice, has a limited shelf life due to lipolysis by lipases (Wilkinson and Champagne, 2004; Kadan and Schluckebier, 2007).

Rice has a relatively low protein content in comparison to other cereals. Nevertheless, rice protein seems to inhibit swelling of rice starch granules (Shih, 2004). However, Hamaker and Bugusu (2003, *online*) reported that after cooking, rice proteins appeared aggregated together forming denser structures. This was in contrast to sorghum proteins, which formed

Fig. 9.10 A compound rice starch granule. (*Source*: Reprinted from Cheng and Lai (2000), with permission of the American Society for Nutrition.)

extended, web- or sheet-like structures with starch embedded within, and might indicate that the rice proteins have less negative impact on the starch gel strength and uniformity. Storage proteins in rice are located in several different types of protein bodies (diameter about 1 μm). Rice starch granules are compound, that is, very small individual starch granules with diameters of 2–4 μm aggregate to a larger unit, the compound starch granule (Fig. 9.10) (Hoseney, 1998; Champagne et al., 2004). In general, rice starch relative to wheat starch is characterized by a high gelatinization temperature similar to sorghum, and by a relatively low amylose content (Lineback, 1984). Both, gelatinization temperature and amylose content also depend on grain type (long, medium, short grain) and variety (Moldenhauer et al., 2004; Wilkinson and Champagne, 2004). As a general rule, US long grain rice varieties have higher gelatinization temperatures and amylose contents than medium and short grain types. Additionally, there is waxy (virtually amylose-free) rice (Wilkinson and Champagne, 2004). Mechanical starch damage occurs during grinding of rice to flour, and depends on the milling process (wet, semidry, or dry milling). Wet milling generally produces lower starch damage due to the cooling and lubricating effects of water, while dry milled flours, especially those with very small particle size, have high starch damage (Yeh, 2004).

It is often mentioned as an advantage that rice has a bland taste (e.g. Kadan et al., 2001; McCarthy et al., 2005). Naturally, bland taste may also be a disadvantage and be considered boring. Bland taste may be desirable for gluten-free bread when targeting groups of people who are repelled by stronger or more unusual flavors. Flours with bland taste may also be used in mixtures with ingredients that contribute intense flavors like buckwheat.

9.3.4.2 *Formulations for rice-based breads*

The suggested recipes for rice breads (Table 9.4) are comparatively uniform. In contrast to the starch and the sorghum-based breads, all used HPMC, but no other hydrocolloids or

Table 9.4 Examples of rice-based breads[a].

	Nishita et al. (1976)	Kadan et al. (2001)[b]	Kadan and Schluckebier (2007) white[b,c]	Kadan and Schluckebier (2007) brown[b,c]	McCarthy et al. (2005)	Gujral and Rosell (2004)
White rice flour	100	90.9	100	95.1	50	100
Potato starch	–	–	–	–	50	–
Rice bran (milled, defatted)	–	9.1	–	4.9	–	–
Water	75	103	110	104	79	90
Hydroxypropyl methylcellulose (HPMC)	3[d]	2.7[d]	2[d]	1.9[d]	2.2	2[d]
Salt (sodium chloride)	2	2.3	1.1	1.1	2	2
Sugar (saccharose)	7.5	12	13	12	5	7.5
Oil (vegetable, v, or rice bran oil, rb)	6 (v)	5.4 (rb)	6 (rb)	5.7 (rb)	6 (v)	6 (v)
Yeast[e]	3 (co)	2.7 (dr)	2.2 (dr)	2.1 (dr)	5 (co)	3 (co)
Extra ingredients	–	–	–	–	10 (skim milk powder)	Transglutaminase (1%, flour basis)
Specific loaf volume (cm^3/g)	5.0–5.3	1.9	4.0	4.2	3.0	2.7

[a] Best/optimized formulations, all standardized to 100 parts rice flour (plus bran or added starch if applicable).
[b] Re-calculated from weight of ingredients in published recipes, slightly rounded (Kadan and Schluckebier, 2007: % sugar appears to be miscalculated in original publication; brown rice bread re-calculated on a flour plus bran basis for the present table).
[c] Patented (USA and Kadan, 2006).
[d] Methocel K4M (Dow Chemical Company).
[e] Compressed (co) or dry (dr).

pregelatinized starch. Only one formulation used isolated starch (McCarthy et al., 2005). Possibly, the early success of Nishita et al. (1976) may have inspired successive studies. The approaches by McCarthy et al. (2005) and Gujral and Rosell (2004) are somewhat different from the rest. The former used high levels of skim milk powder, the latter focused on the effects of TGase.

The typical breadmaking procedure for rice bread does not differ from that described for sorghum bread above (Section 9.3.3.2). All studies involved mixing, final proof in bread pans and baking. Kadan et al. (2001) and Kadan and Schluckebier (2007) used home bread machines and added additional intermediate rising and punching steps between initial mixing and final proof. Similar intermediate fermentation and remixing steps have been applied by Olatunji et al. (1992a, b) in sorghum breads and are common practice in wheat bread.

Additional results reported by Nishita et al. (1976) were that other hydrocolloids besides HPMC did not produce leavened bread. Gums included were sodium carboxy methylcellulose, xanthan gum, carrageenan, locust bean gum, guar gum, gum tragacanth, gum arabic and alginates. Most other types of HPMC tested were inferior to Methocel K4M. Nishita et al. (1976) also found that water levels were critical, with insufficient water producing a stiff dough that rose only little, and excessive water causing overexpansion and thus bread loaves with large holes. Distinct decreases in volume were described when adding solid fat and emulsifiers. The reported volume (Table 9.4) is slightly ambiguous. The highest specific volume (5.3 cm^3/g) appears to have been reached without added oil. The authors reported even higher volumes (6.5 cm^3/g) for bread with 85% water, which however contained large holes. The nature of the rice flour remains also slightly ambiguous. The authors (Nishita et al., 1976) reported an ash content of 1.38%, which would be closer to what could be expected from a brown than from a white rice flour. However, they described the bread crumb as 'very white'.

The finding that excessive water produces large holes was confirmed by McCarthy et al. (2005) applying response surface methodology, but appears to be in contrast to Kadan et al. (2001) and Kadan and Schluckebier (2007), who used distinctly higher water levels than the other studies (Table 9.4). While higher water binding in batter and bread containing bran might be explained by the high water absorption of hemicelluloses in the bran, the white rice bread of Kadan and Schluckebier (2007) remains in contrast to the other studies. Possibly, the type of rice or the size of the rice flour particles might play a role. Kadan and Schluckebier (2007) defined that the majority of the flour particles should be between 100 and 150 μm. In contrast, Nishita et al. (1976) reported that 56% of their rice flour was retained by a 100 mesh sieve (i.e. in theory, 56% were larger than 149 μm). Finally, as in case of the sorghum breads, it remains difficult to compare studies. Although both Nishita et al. (1976) and Kadan and Schluckebier (2007) provided pictures of the crumb structure, a direct comparison remains difficult due to different image sizes and quality, and different loaf sizes.

Gujral and Rosell (2004) observed synergistic effects between HPMC and transglutaminase (TGase). An optimum level of TGase was found, which produced maximal specific volume and minimal crumb hardness (in their case, 1% TGase on a flour weight basis with an activity of 100 units/g). The optimal TGase level together with 2% HPMC produced the maximal volume of ≈2.7 cm^3/g (Table 9.4). In the absence of HPMC, volumes were very low (≈1.5 cm^3/g), and only slightly improved by TGase at its optimum level. The authors put forward the hypothesis that TGase could cause the formation of a protein network that might retain carbon dioxide formed during fermentation.

All studies that included storage trials (Nishita et al., 1976; Kadan et al., 2001; Kadan and Schluckebier, 2007; and McCarthy et al., 2005) mentioned the quick staling of rice

breads. Kadan and Schluckebier (2007) suggested to freeze the bread slices for storage, and to refresh them after thawing by microwave heating or toasting.

As in case of sorghum, the question for the best rice bread cannot be answered so easily. Highest volumes were achieved by Nishita et al. (1976) and the crumb structure of these breads appears acceptable, as far as can be judged from the provided photographs. When health-promoting bran is added, there appears to be a trade-in between volume and health. Low levels of about 5% can be added without adverse effects on volume as in case of the brown rice bread of Kadan and Schluckebier (2007); however, higher levels around 10% lower the volume markedly as in case of Kadan et al. (2001) (Table 9.4).

Summary: *Rice breads are typically based on white rice flour, high water levels (75–110% on a flour basis) and added HPMC. Hydrocolloids other than HPMC apparently do not work. There is no need for the addition of isolated starches. Rice bran can be added at moderate levels (e.g. 5%) without notably negative effects on bread quality, but high levels (e.g. 10%) result in low volumes. Together with starch breads, rice breads can be regarded as the 'classics' among gluten-free breads, characterized by high volumes and bland flavor. Bland flavor can be an advantage or disadvantage, depending on the customers' preferences.*

9.3.4.3 *Understanding the rice breads*

Many of the results can be interpreted with the theoretical background explained for the sorghum breads, summarized in Table 9.3. As pointed out by Hoseney (1998), rice endosperm is in general both hard and vitreous. Similar to what we discussed above (Section 9.3.3.1) for the horny endosperm of sorghum, we can expect some coarser endosperm particles and damaged starch in rice flour (depending also on milling techniques, see Section 9.3.4.1). Bran obviously is only present in brown rice flour or when it is added, as in case of Kadan and Schluckebier (2007). Rice contains insoluble and soluble proteins, and also protein bodies, similar to sorghum (Shih, 2004). Upon heating, proteins tend to aggregate together, forming denser structures, somewhat different from sorghum, where they tend to form more extended structures (Hamaker and Bugusu, 2003, *online*, see Section 9.3.4.1). Because of its different focus, the formulation of Gujral and Rosell (2004) will be treated separately in the section on TGase (Section 9.3.6.4).

Most obvious is the negative effect of bran on volume. Small amounts as in case of the brown bread of Kadan and Schluckebier (2007) may be tolerated – as hypothesized above (Section 'The right balance between ingredients'), bran particles would then deform the bubbles, but the latter could extend their surface and surround the deforming object. Large amounts of bran as in case of Kadan et al. (2001) would, however, ultimately penetrate bubble walls and cause leaks. Large amounts of bran particles could also be expected to just compress the starch gel by their weight. With these hypotheses, the slight volume increase of the brown versus the white bread of Kadan and Schluckebier (2007) cannot be explained. However, this might be simply an effect of water binding. Due to the addition of bran on top of the rice flour, the relative amount of water in the formulation decreases and additionally, bran binds water due to its hemicellulose content. (For example, insoluble pentosans swell extensively and are located mainly in the outer layers of the grain, that is bran, Belitz et al., 2004.) As in the case of sorghum, it appears that the right balance between water, hydrocolloid, damaged and intact starch has to be reached – if the resulting batter is too thick, it would not rise, if it is too thin, too high dilution would result in large holes (see Section 'The right balance between ingredients'). It has been suggested that damaged starch is undesirable for

rice bread production (Yeh, 2004) and also for the patented rice bread, use of starch with low damage is recommended (USA and Kadan, 2006). However, at the same time there is a connection between milling technique, particle size and starch damage (see Section 9.3.4.1). In line with this latter hypothesis, Nishita et al. (1976) used coarser flour with presumably lower starch damage and therefore also lower water levels, while Kadan and Schluckebier (2007) used finer flour and higher water levels. It remains unclear if there is another effect of small particle size besides starch damage, like faster swelling due to better accessibility of the inside of the endosperm particles.

Similar to the balance between water and water binders, there appears to be also an optimum balance between amylose and amylopectin. In the patent (USA and Kadan, 2006), use of rice flour with about 20–26% amylose is recommended. Kadan and Schluckebier (2007) also suggested the addition of small amounts (10–20%) of waxy rice flour when certain long grain varieties are used. The patent additionally claims that waxy rice flour at the same time decreases volume but may cause softer texture. As mentioned in Section 9.3.4.1, long grain rice generally is higher in amylose. While amylose is required for crumb setting due to its quick retrogradation (Section 'Emulsifiers, starch and crumb properties' and Table 9.3), amylopectin might counteract excessive crystallinity in the crumb directly after cooling and thus cause the fresh bread to be softer.

Nishita et al. (1976) already stated that the lack of success with xanthan gum in rice breads is not understood, in view of the fact that this gum works well in wheat starch breads. We can only suggest hypotheses for an explanation. During baking, the high gelatinization temperature of rice starch would result in a longer time period, in which the bubbles expand due to heat, before they are stabilized by starch gelatinization. A surface-active hydrocolloid might help to keep the bubbles stable in this critical phase. Additionally, all breads from gluten-free flours contain coarser particles, while starch breads contain only (small) starch granules. Coarser particles would more strongly interfere with the bubbles, so that again, a surface-active hydrocolloid would be beneficial. These ideas are in line with the finding of Hart et al. (1970), who reported that basically only HPMC could be successfully used in sorghum bread, and Schober et al. (2005), who reported lack of success with xanthan gum in sorghum bread (see Section 9.3.3.2). It is important to remember the similarities between sorghum and rice in terms of high gelatinization temperature and endosperm particles.

It is interesting to note that clearly higher volumes can be reached with rice than with sorghum (Tables 9.2 and 9.4). This fact agrees favorably with the hypothesis that protein aggregation upon baking has negative effects on bread quality (see Section 'The role of proteins and protein networks'). In rice, the low flour protein content and the tendency of the proteins to just aggregate to denser structures, but not to form extended structures would then be beneficial (see Section 9.3.4.1).

9.3.5 Other cereals, pseudocereals and their mixtures

9.3.5.1 *Maize breads*

The similarity between sorghum and maize has already been pointed out (Section 9.3.3.1). Thus, it is not astonishing that Olatunji et al. (1992a) also produced a maize bread applying the identical recipe and procedure as for sorghum (70% maize flour, 30% raw cassava starch, see Section 9.3.3.2 and Table 9.2). This bread reached a slightly lower specific volume of

2.0 cm^3/g than the sorghum bread. The theoretical basis would be the same as for sorghum (Section 9.3.3.3 and Table 9.3). Very likely, the sorghum breads in Table 9.2 could all be produced also from maize with little adaptation, applying the information from Section 9.3.3 and Table 9.3.

A somewhat different type of maize breads was described by Sanni et al. (1998) and Edema et al. (2005). These so-called sour maize breads were made from maize flour and maize starch (70:30) in case of Sanni et al. (1998) and different maize flours, soy flours and blends of maize (80–90%) and soy (10–20%) in case of Edema et al. (2005). In both studies, leavening was achieved with mixed cultures of lactic acid bacteria and yeast, and salt, fat, sugar and high water levels were added. In case of Sanni et al. (1998), in addition one egg per 100 g flour was used. Specific volumes of these breads were very low (<1 cm^3/g). The authors emphasized that these breads are specialty breads, and have advantages like improved mold-free shelf life relative to yeast leavened breads, or improved protein quality in nutritional terms when maize–soy mixtures are used.

9.3.5.2 *Mixtures*

Various mixtures of gluten-free cereals, pseudocereals and other ingredients like beans have either been described in the literature or are commercially available for bread production. The more complex the mixture, the more difficult is the understanding of the contribution of each component. We will therefore limit this section to a few examples, and the explanation of only the most obvious facts.

Sanchez et al. (2002) optimized a bread formulation from corn starch, rice flour and cassava starch, with and without soy addition, applying response surface methodology. The optimum formulation contained 74% corn starch, 17% rice flour and 9% cassava starch (100% total flour), plus 0.5% soy flour. Other ingredients comprised an undisclosed gum, salt, sugar, fat, yeast and 83–100% water on a total flour basis. As expected, soy improved crumb structure, presumably due to surface-active components like glycinin (see Section 9.3.1.2). With regard to the effect of the starches and rice flour, different gelatinization temperatures (maize and rice: high, cassava: low), particle sizes and starch damage might explain the existence of an optimum.

Moore et al. (2004) developed and studied two gluten-free bread formulations from a variety of ingredients. The non-dairy (ND) formulation had a flour-starch basis of brown rice flour (25%), corn starch (54%), buckwheat flour (8.5%) and soy flour (12.5%). Other ingredients were xanthan gum, salt, sugar, sugar syrup, yeast and 105% water on a flour-starch basis. We can see that only a limited amount of buckwheat flour was used, reflecting its intense flavor. Furthermore, soy flour was added, which likely contributed to gas cell stabilization, and the flours were diluted by >50% pure starch (compare Table 9.3). Xanthan gum contributed viscosity. This bread staled faster than a wheat bread control, as detected especially by a much more dramatic drop in cohesiveness over 5 days of storage. The second formulation (dairy bread, D) in the same study showed a better keeping quality, especially distinctly less loss of cohesiveness than the ND formulation. This indicates that the dairy bread became less brittle over storage. Its flour-starch basis was brown rice flour (50%), potato starch (25%), corn starch (12.5%) and soy flour (12.5%). A very high amount of 37.5% skim milk powder on a flour-starch basis was included. Other ingredients were xanthan gum, konjac gum, salt, sugar, yeast, baking powder, a considerable amount of egg (30% fresh

whole egg on a flour-starch basis) and water (105%). For this bread, a lower amount of pure starches was added relative to the ND bread, while stabilizing factors included the two gums, soy and egg. The effects of skim milk powder are not clear. Both, egg and skim milk powder require a separate, in depth discussion (Sections 9.3.6.1 and 9.3.6.2).

A formulation somewhat comparable to the dairy bread by Moore et al. (2004) was developed by Ahlborn et al. (2005). The flour-starch basis of their gluten-free rice bread was white rice flour (70%), tapioca (cassava) flour (13%) and potato starch (17%). Stabilizing ingredients were fresh whole egg (17% on a flour-starch basis), xanthan gum and HPMC. Other ingredients were salt, sugar, yeast, oil, skim milk powder (4%) and water. This gluten-free rice bread showed less staling than wheat and low-protein starch breads, measured as resistance to mechanical collapse in two uniaxial compression cycles.

Many commercial gluten-free bread mixes or mixes described in cookbooks for celiacs contain bean flours, namely garbanzo bean (chickpea, *Cicer arietinum*) and fava bean (broad bean, *Vicia faba*) flours (Fenster, 2004; Bob's Red Mill, 2008, *online*). A bean flour mix suggested by Fenster (2004) contained over 50% of bean flour, and from the position of garbanzo bean flour in the list of ingredients one can conclude that also many commercial mixes contain substantial amounts (Bob's Red Mill, 2008, *online*). The mentioned beans are members of the *Fabaceae* family (legumes), as are soybeans (Belitz et al., 2004). We have mentioned the surface activity of legume proteins before (7S and 11S globulins, see Section 9.3.1.2). However, the findings of Sanchez et al. (2002) reported in the present section suggest that low amounts (<1%) of soy flour are sufficient for an improvement of crumb structure. Other reasons for addition of large amounts of legumes would be their high content in protein and dietary fiber (Belitz et al., 2004). It is also important that proteins from legumes and cereals supplement each other in their biological value, as lysine is the limiting amino acid in cereals, while methionine is the limiting amino acid in beans (Hegarty, 1995; Belitz et al., 2004). Simplified, the combination of cereal protein and bean protein is 'healthier' or 'more useful' for the body than each individual protein, as the combination contains a more favorable mixture of essential amino acids.

Similar to legumes, the pseudocereals amaranth, quinoa and buckwheat may be added to gluten-free products in order to improve the nutritional value. The favorable amino acid composition of pseudocereals has been pointed out by Kuhn (1999) and Kuhn et al. (2000). The USDA National Nutrient Database for Standard Reference (USDA, 2008, *online*) shows lysine contents for all three pseudocereals of 0.6–0.8 g/100 g versus 0.3–0.4 g/100 g for whole wheat.

Summary: *Formulations for gluten-free breads from mixtures of a variety of ingredients have been described in the literature. Similar to commercial gluten-free bread mixes, the contribution of each individual ingredient is not always easily understood. Examples for ingredients used in mixtures are corn starch, potato starch, cassava starch, white and brown rice flour, soy flour, buckwheat flour, skim milk powder, egg, bean flours (chickpea and broad bean), xanthan gum, konjac gum and HPMC. Reasons for the addition of an individual ingredient may be technological (e.g. in case of gums or egg the improvement of the crumb structure), sensory (e.g. buckwheat, in case of which small amounts may add a more intense flavor, but large amounts may taste too strong) or nutritional (e.g. bean flours add protein and fiber, and the amino acid composition supplements with cereals resulting in an increased biological value of the protein). Pseudocereals (amaranth, quinoa, buckwheat) may also be added for their nutritional value.*

9.3.6 Special ingredients and additives for gluten-free bread

9.3.6.1 *Egg products*

Important functions of egg are surface activity and emulsifying properties of egg white and yolk, and heat coagulation of egg white (Forsythe, 1970; Satin, 1988; Cauvain, 1998). From these properties we can expect that egg addition to gluten-free bread helps in the stabilization of the gas cells due to its surface activity as well as in the setting of the crumb due to heat coagulation of the egg white. These effects are well known from cake making, and indeed such breads have some similarity with cakes (*personal observation*).

The studies of Moore et al. (2004) and Ahlborn et al. (2005) agreed that formulations with egg produced breads with delayed staling (see previous Section 9.3.5.2). Both found web- or film-like structures resembling gluten in crumb from gluten-free breads containing egg, but not in egg-free gluten-free formulations. In line with the theory, these structures were likely denatured egg white. Both studies hypothesized that these protein matrices were the factor counteracting staling, for example, by simply masking some of the changes originating from starch retrogradation. A trade-in for the delayed staling upon egg incorporation is that we add an ingredient with allergenic potential (see Section 9.2.3.2). Egg-containing breads might therefore be better regarded as specialty than as a mainstream gluten-free product.

9.3.6.2 *Milk products*

For wheat breads, it is generally assumed that milk products like skim milk, casein, whey and buttermilk have beneficial effects like increasing the water-binding capacity of the dough and the moistness of the crumb due to the increased protein content (Belitz et al., 2004). It should, however, not automatically be assumed that the same is true for gluten-free breads. In the absence of a gluten-network, the gas cell stability and strength of the starch gel become more relevant. The right balance between water binding in the batter and water binding after baking appears to be the relevant factor rather than increasing water absorption, and it has already been described for sourdough fermentation of sorghum that protein degradation was beneficial (see Section 9.3.3.3 and Table 9.3).

Gallagher et al. (2003) examined the effects of different dairy powders added to a starch-based commercial gluten-free flour. Included were types of whey, skim milk powder and other milk solids, sodium caseinate, and milk protein isolate, ranging between 6.5% and 90% protein, in levels of 3%, 6% and 9% on a flour weight basis. Overall, these powders reduced loaf volume, although there were differences between type of powder and addition levels. Dairy powders improved crust browning and in some cases also softness of the crust. Both of these effects may be regarded as desirable. However, all dairy powders reduced crumb softness. Increasing the water content could increase volume and crumb softness of dairy-containing breads. Sensory results pointed toward a higher acceptability of the dairy breads. Nutritionally, dairy powders increase protein content, while those with high lactose content can be problematic for celiac patients with a secondary lactose intolerance. Milk has also an allergenic potential (see Section 9.2.3.1).

The results of a response surface study on sorghum bread by Schober et al. (2005) were more critical with regard to the technological effects of skim milk powder. Skim milk powder decreased loaf height by causing a collapsed top of the breads and reduced crumb cohesiveness. Only improved crust browning was found to be a positive effect.

Latest approaches involve the use of a casein, at specific pH and ionic strength, in order to form gluten-like masses (Gallagher, 2006, *online*).

9.3.6.3 *Other animal products*

Other animal proteins that have been suggested for use in gluten-free breads are fish surimi (Gallagher et al., 2004) and gelatin (Kieffer, R., German Research Center for Food Chemistry, Garching, Germany, *personal communication*). Surimi is muscle protein from fish that has been water washed. Together with water, it forms a solid cohesive gel (Belitz et al., 2004). Gelatin is extracted from animal bones or skin under acid or alkaline conditions. Like surimi, it is a gelling agent (Belitz et al., 2004; Hoefler, 2004). In contrast to what might possibly be expected, Gallagher et al. (2004) reported that taste panels could generally not detect a difference between control and surimi breads. At the same time, loaf volume and crust and crumb softness were improved by addition of most types of surimi. It remains, however, questionable whether consumers would accept a bread made with fish or gelatin, even if they could not taste any off-flavor.

Summary: *Egg has doubtlessly technological benefits in gluten-free bread, while the role of milk products is more controversial. Both have allergenic potential, and lactose from milk is critical for celiacs when starting the gluten-free diet as long as the damage to the intestine has not healed (see Section 9.2.3.1). Both should therefore be used with care and have to be clearly labeled.*

9.3.6.4 *Antistaling α-amylases*

Some α-amylases are very promising for delaying the staling of gluten-free bread. Details about these enzymes will be discussed in the section on staling (Section 9.3.8).

9.3.6.5 *Transglutaminase*

The basic idea for the use of TGase in gluten-free bread is very straightforward. The main reaction catalyzed by this enzyme is the formation of new covalent crosslinks in proteins via lysine and glutamine residues (see Section 9.2.3.3 and Fig. 9.1). The proteins in all gluten-free cereals do not aggregate to continuous networks in the dough at room temperature, unlike wheat gluten. Therefore, it appears logical to artificially crosslink them and thus create a network. However, up to now, it appears that no attempt has lead to a gluten-like product.

Moore et al. (2006) added protein sources (12.5% of soy flour, skim milk powder, or whole egg powder) to a gluten-free base mix from white rice flour (35%), potato starch (30%) and corn starch (22.5%). Other ingredients were salt, yeast, sugar, xanthan gum and water. The study aimed at how these protein sources, rather than flour protein, would be crosslinked by different levels of TGase. Bread volume was affected by the type of protein source. Egg addition resulted in higher volumes than the other protein sources, in line with the theoretical background (see Section 9.3.6.1). TGase had little effect on volume, except that at its highest dosage in combination with skim milk powder, bread volume was lowered. These results suggest that TGase has no beneficial effects on gas holding capacity in combination with any of these protein sources. The authors observed network formation in the protein phase

due to the added TGase in the systems with skim milk powder and egg powder. It appears however that these networks did not improve bread quality.

The study of Gujral and Rosell (2004) has already been described above (Section 9.3.4.2) and in Table 9.4. In contrast to Moore et al. (2006), no protein source was added, but the effect of TGase directly on rice proteins studied. Several variables indicated that rice proteins were indeed crosslinked: with increasing levels of TGase, free amino groups decreased. Rheological measurements (dynamic oscillatory frequency sweeps) showed an increase in elastic and viscous modulus with increasing levels of TGase, indicating more resistance to deformation at small shear deformations. Increasing levels of TGase also increased farinograph consistency. However, bread with acceptable volume could only be produced, if HPMC was added in addition to TGase. Therefore, the protein crosslinking achieved with TGase most likely did not produce a network with properties similar to gluten. More research would be required to understand, how exactly the bread volume was improved by the combination of TGase and HPMC.

Finally, Renzetti et al. (2008) studied the effect of TGase in formulations from different cereal and pseudocereal flours (buckwheat, brown rice, oat, sorghum, teff and corn flour), with only water, yeast, salt and sugar added. Due to the absence of added hydrocolloids, this is an especially challenging system. As in the case of Gujral and Rosell (2004), cereal proteins rather than added protein sources were the target for TGase action. The authors found that the effect of TGase differed between the studied cereals. In buckwheat and brown rice, increasing levels of TGase caused a decrease in specific volume. In sorghum and corn, low levels of TGase increased the specific volume. However, all specific volumes were low ($<$2.2 ml/g) and in sorghum and corn, the specific volume was improved by TGase to only 1.6–1.7 ml/g. The most remarkable positive effect of TGase was an improvement of the crumb structure of buckwheat, brown rice and corn bread from unacceptable (coarse, central hole or collapsed) without TGase to quite uniform with TGase. The authors identified protein crosslinking and possibly also deamidation as responsible for these effects of TGase. Nevertheless, the overall low volumes suggest that no protein structures with a gas holding capacity comparable to wheat gluten were formed.

In order to understand the limited success with TGase in gluten-free breads, we first have to address wheat bread. The decisive critical question to ask would be, whether wheat gluten is really crosslinked. Although there is still a lot of discussion among cereal scientists about the nature of gluten, there is also at least some consensus. Gluten is not a material dominated by covalent crosslinks like (vulcanized) rubber. Otherwise, dough could not be permanently deformed, as for example in case of sheeting, but would inevitably assume its original shape after the outer force is removed. Instead, it appears that the formation of high molecular, linear aggregates from glutenin subunits via disulfide bridges is decisive in wheat gluten. This can be concluded, because rheological dough properties (resistance to extension) were highly correlated to x-type HMW glutenin subunits, which tend to form linear polymers, but not to y-type subunits, which tend to form covalent crosslinks (Belitz et al., 2004). It is also important to remember, that gluten contains gliadin, which remains monomeric and acts as lubricant for the aggregated glutenins. Analogies from polymer science suggest that the linear glutenin polymers are linked to each other only via transient, non-covalent crosslinks, so-called ‘entanglements’ (Singh and MacRitchie, 2001).

Thus, the one important question for the use of TGase would be whether, at sufficiently low dosage, this enzyme would be able to form large, predominantly linear protein aggregates out of non-gluten proteins (equivalent to glutenin polymers). At the same time, a considerable portion of the proteins should remain unchanged or only aggregated to a small

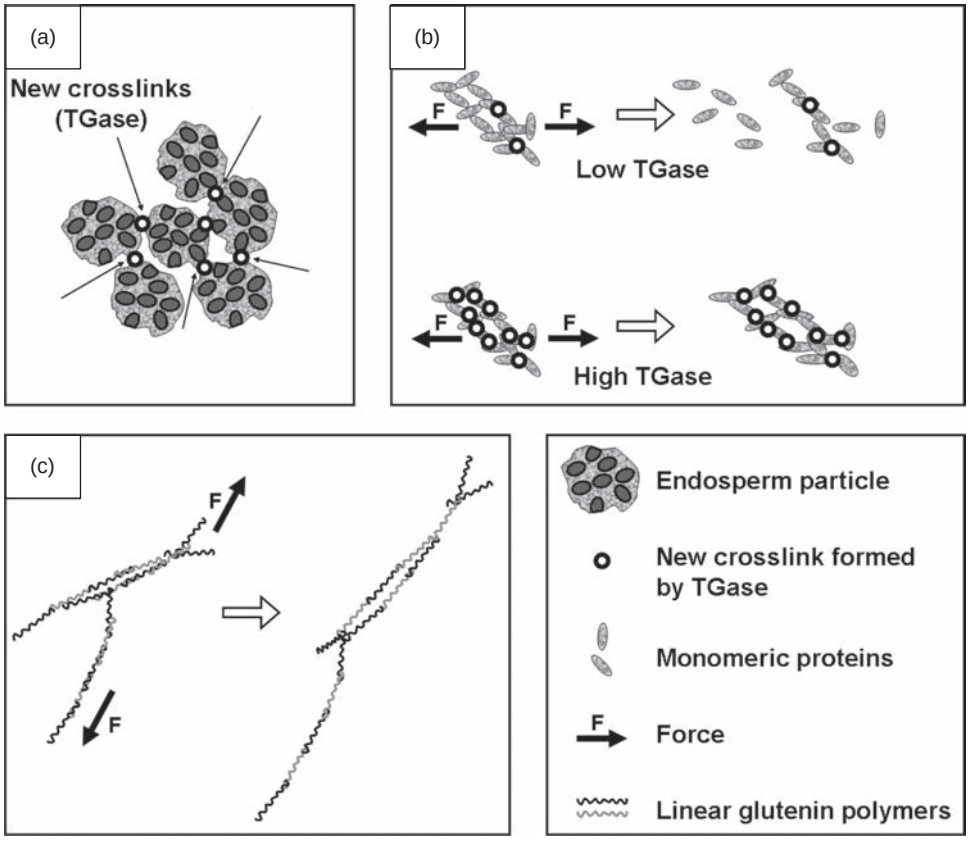


Fig. 9.11 Models for the effects of transglutaminase (TGase) on proteins in gluten-free cereals (a, b). (a) Flour particles (endosperm particles) glued together at their surfaces. (b) Soluble monomeric proteins with low and high dosage of TGase. (c) Wheat gluten for comparison (linear glutenin polymers forming entanglements).

degree (so that we have something that functions like gliadin). This is a considerably more complex question than whether this enzyme can create 'crosslinks' or 'networks'. Another prerequisite for the formation of a gluten-like polymer would be that the storage proteins are accessible. If they are encapsulated in protein bodies, the formation of an extended network through the whole dough (i.e. before baking!) appears impossible. At best, we can then stick protein bodies or endosperm particles together via their surfaces. Models for the effects of TGase (not leading to a gluten-like network) are suggested in Fig. 9.11, and a model for wheat gluten is given for comparison. It must be emphasized that these models solely explain why TGase can most likely not create artificial viscoelastic gluten. Whether it may possibly have other beneficial effects, like improving the ability of proteins to stabilize the gas–liquid interface in bubbles or increasing the cohesiveness of otherwise crumbly gluten-free bread, is not addressed in this figure. These issues require further research.

It has been pointed out before that a certain risk for celiacs is associated with the use of TGase (for details, see Section 9.2.3.3).

Summary: *Up to now, the use of transglutaminase (TGase) has not resulted in the development of greatly improved gluten-free bread. It appears that no protein network with viscoelastic properties similar to gluten could be produced. According to our opinion, the assumption that any protein network will improve gluten-free bread is not sufficiently supported by theoretical considerations and experimental data. More attention has to be paid to the nature of the protein network, and the details of the structure of wheat gluten have to be taken into account.*

9.3.7 Wheat bread, rye bread and gluten-free bread

9.3.7.1 *Wheat-like viscoelasticity of zein dough*

We have discussed some aspects of the functionality of wheat gluten in the previous section. In short, linear glutenin polymers form entanglements and thus contribute elasticity. Gliadins remain monomeric and act as a lubricant for the glutenin polymers. The use of TGase was one attempt to create viscoelastic protein networks from storage proteins of gluten-free cereals. Another was described by Lawton (1992). Isolated zein, maize starch and water could indeed form a viscoelastic dough, provided that they were mixed at elevated temperatures (e.g. 35°C), above the glass transition temperature of zein (around 28°C, Lawton, 1992). Such dough had a protein fiber network, which could be visualized by scanning electron microscopy (Lawton, 1992). Studies in our lab (Schober et al., 2008) showed that addition of HPMC to such zein-starch dough yielded well-leavened bread. The resulting dough was less elastic than wheat dough, but could be handled similar to the latter. For example, it could be rolled into strands and these could be slung into the shape of pretzels. Zein dough might therefore have its niche for the production of gluten-free products other than pan breads (hearth-type breads, braided breads, soft pretzels and various types of rolls). A technological challenge is that zein dough must not be cooled below zein's glass transition temperature. We could overcome this problem by preparing it at 40°C, that is, close to the maximum temperature that regular baker's yeast can tolerate. Then, it would not easily cool below glass transition while being worked at room temperature (e.g. into pretzels). Obviously, all proofing steps must also be carried out at elevated temperatures.

Summary: *Viscoelastic dough can be made from zein (maize prolamin), water and added starch by mixing at elevated temperatures (e.g. 35–40°C). Such zein dough might have its niche for specialties like hearth-type breads, braided breads, soft pretzels and rolls.*

9.3.7.2 *Pitfalls when determining viscoelasticity*

Frequently, dynamic oscillatory tests are used to determine the viscoelastic behavior of dough or batter. Such tests are easily misinterpreted. An example would be as follows:

We have shown the effects of different water contents on sorghum bread above (Section 'The right balance between ingredients', p. 151) and in Fig. 9.7. While 105% water on a flour-starch basis resulted in acceptable bread, 80% produced very dense, hardly leavened bread. We studied both batters, omitting the yeast, by dynamic oscillatory frequency sweeps in the linear viscoelastic region (Fig. 9.12). Unexpectedly, the phase angles were very low (10–15°) for both batters. (For any material, the phase angle is between 0° and 90°, where

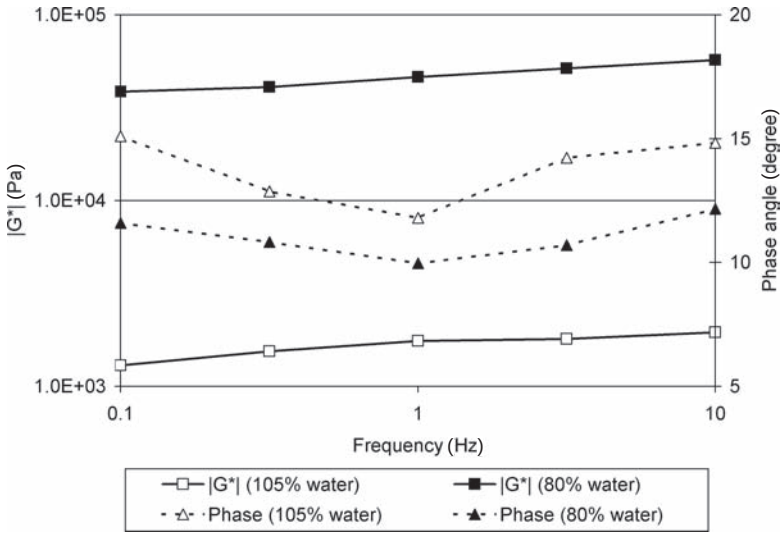


Fig. 9.12 Fundamental rheological properties of the sorghum–maize starch batters from Fig. 9.7 (yeast omitted). The absolute value of the complex dynamic modulus ($|G^*|$) was significantly different between the two samples for all measured frequencies ($P < 0.05$), the phase angle only at 0.1 Hz ($P < 0.05$). Measurements were done in triplicate. (Parallel, serrated plates, 25 mm diameter, target strain 5×10^{-4}, gap autoadjusted around 3 mm to reach a normal force target of 0.01 N, temperature 30°C.)

0° corresponds to ideal elastic behavior, and 90° to ideal viscous behavior). For comparison, wheat doughs measured under similar conditions in the linear viscoelastic region showed phase angles between 20° and 24° in the same frequency range (Clarke et al., 2002, 2004). Can we thus conclude that our sorghum batters are more elastic or have stronger 'networks' with more covalent crosslinks than wheat dough? Obviously not, because when just manually evaluating the sorghum batters, the batter with 105% water showed liquid-like behavior, that is it flowed without regaining its original shape, while the low water content produced a dough-like product, that however was brittle and broke upon deformation in the centimeter range, rather than regaining its original shape after being deformed. (Ideal elastic means by definition that a body regains its original shape immediately after the deforming force is removed.) So, what is the cause of this apparent contradiction? Dynamic oscillatory tests in the linear viscoelastic region are done at very small deformations. Let us assume that we have a cube that is sheared with a strain of 5×10^{-4}. Shear strain (γ) is defined as displacement of the top of the cube (x) divided by its height (h):

$$\gamma = \frac{x}{h}$$

Thus, a strain of 5×10^{-4} at a height of 3 mm as in measurement in Fig. 9.12 would correspond to a displacement of 1.5 μm. This is smaller than all common starch granules (Belitz et al., 2004) and obviously much smaller than most endosperm particles, which comprise starch granules embedded in matrix protein. Therefore, we measure various interactions, like interactions between starch granules or between endosperm particles or between starch and proteins. However, if there should be a gluten-like protein network, its properties would be masked by the abundant starch. Therefore, these measurements cannot clarify, whether

there is a continuous gluten-like network or not. These interpretations have been put forward already more than a decade ago by Amemiya and Menjivar (1992) for wheat dough. Comparable to the present study, Parkkonen et al. (1994) found that rye dough had substantially higher elastic moduli (G') than wheat dough, which they attributed to larger size and rigidity of the rye particles. These interpretations fit well to the model for sorghum batter (Section 'Viscosity increase', p. 148 and Fig. 9.9). Damaged starch, swollen to a considerable size, endosperm and bran particles would stick together and result in an elastic response (therefore the low phase angles in Fig. 9.12). This is true, as long as the deformations are small enough, so that the particles are not torn apart, but can reset to their original configuration after removal of the force, similar to a weak network. When we extend our batter or gluten-free dough in the centimeter or possibly only millimeter range, their weak interactions are broken, and the response is no longer elastic (this is what we feel, when we manually assess these systems). A more appropriate test method in order to find out, whether a viscoelastic protein network is present, would be large deformation extension tests (e.g. extensigraph or Kieffer extensibility rig). If we are unable to form the dough strands required for these instruments, than we most likely have no continuous viscoelastic protein network through our dough or batter. If there is one, then we should get extension curves comparable to those for wheat dough.

An important rheological aspect of gluten-free batters is their viscosity (or, more accurate, apparent viscosity, a term that applies to non-Newtonian fluids). We saw in the section on rice, that an optimum water content exists (insufficient water levels resulted in stiff dough that rose only little, excessive water resulted in overexpansion and thus large holes in the breads, see Section 9.3.4.2). This would suggest that an optimum viscosity exists, which is determined by the water level and the properties of the flour and added hydrocolloid. Possibly, dynamic oscillatory tests could be used to determine this optimum (e.g. by defining an optimum absolute value of the complex modulus ($|G^*|$) at a certain frequency). Figure 9.12 indeed indicated that higher water (105%) resulted in a significantly lower $|G^*|$ over the whole frequency range than lower water (80%). (Lower $|G^*|$ means less resistance to deformation, or 'softer'). However, due to the sensitivity of this type of measurement to particle size and particle rigidity, and to interactions between various components like damaged starch, endosperm and bran particles, we cannot expect that a comparison of $|G^*|$ between different types of flours would work. We would therefore recommend large deformation measurements for the determination of an optimum (apparent) viscosity, so that particle size and short-range interactions become less important. Examples would be determination of the force required for extrusion (Schober et al., 2005), or standard methods like Newport Rapid Visco Analyser (RVA) or Brabender Amylograph (ICC Standards No. 162 and 126/1; ICC, 2000). The latter methods also measure viscosity increase upon heating and thus starch gel strength, and are widely used for the evaluation of rye. This leads to the next section.

Summary: *We cannot conclude from low phase angles in dynamic oscillatory measurements that gluten-like protein networks are present in a gluten-free dough. Low phase angles might also originate from interacting particles like starch, endosperm particles or bran. More useful are large deformation measurements (e.g. extension tests). For batters, the optimum viscosity, measured at large deformations, appears much more important than dynamic properties, and we recommend extrusion tests, Amylograph or Rapid Visco Analyser.*

We should be very careful when claiming that in gluten-free breads, 'elasticity' or 'networks' are required. We need a clear definition (elasticity: in which deformation range; what kind of networks).

9.3.7.3 *The analogy between rye breads and gluten-free breads*

At the beginning of this section, we have to emphasize that rye is not suitable for celiacs (see Section 9.2.2). However, we want to include it in the discussion, because it helps to understand gluten-free bread. It is also important to emphasize that we relate to a type of rye bread, which is made from at least 90% rye flour. Such bread is popular in Central, Eastern and Northern Europe. Its production has been described by Meuser et al. (1994) and Seibel et al. (1978). Such rye bread is distinctly different from rye bread sold for example in the USA, which contains mainly wheat and only a comparatively small portion of rye in order to modify flavor and appearance (Hoseney, 1998).

When comparing gluten-free batters, rye doughs and wheat doughs, gluten-free batters resemble rye dough much more than wheat dough. Gluten-free batters and rye dough are highly viscous, but have little elasticity and extensibility upon deformation in the centimeter range. Rye dough and those gluten-free batters made with hydrocolloid addition both leave a 'slimy' feeling at one's hands. On a more scientific level, both are characterized by a bubble structure not supported by a continuous viscoelastic gluten network. Water binding and dough cohesion are achieved by natural hydrocolloids in rye (pentosans and to a lesser degree β-glucans, Parkkonen et al., 1994), while in gluten-free batters, added hydrocolloids like xanthan gum and HPMC play an important role. These substances also cause the 'slimy' feeling just mentioned. HPMC and soluble pentosans have in common that they are surface active and may therefore stabilize gas cell walls (see Sections 9.3.1.2 and 'The stabilization of bubbles', p. 150).

During baking, in wheat bread, besides starch gelatinization, the denaturation of gluten contributes to crumb setting. In rye and gluten-free breads, it is largely the starch that causes crumb setting, first by gelatinization, later by amylose retrogradation (see Section 'Emulsifiers, starch and crumb properties', p. 150). Therefore, excessive degradation of starch has to be avoided. This is done by acidification in rye bread (sourdough or added acids), so that the dough pH is well below the pH optimum of the α-amylase. This pH optimum is about 5.5–5.7 (Belitz et al., 2004). In gluten-free bread, we have to take care that we do not overdose added amylolytic enzymes. This point is relevant for the next section.

The role of proteins in rye bread and (egg-free) gluten-free breads is generally most difficultly to understand. The idea that only starch and pentosans are responsible for rye dough and crumb formation is an over-simplification. Parkkonen et al. (1994) reported that proteins play an important role in the rye dough structure directly after mixing, when degradation of cell walls is still limited and therefore not enough pentosans have been released into the dough. Fluorescence microscopy, in which cell walls and proteins had been specifically stained, revealed that rye dough directly after mixing contained unbroken bran, aleurone, endosperm particles and starch granules dispersed in a protein matrix. The protein content of the flour appeared to be a decisive factor for the continuity of the protein matrix. In apparent contrast, Tuukkanen et al. (2005) concluded that proteolytic breakdown of rye proteins (mainly secalins) during sourdough fermentation may have a key role in rye breadmaking. These authors mentioned various aspects of proteolysis, like the possibility that soluble rye protein structures might stabilize foams, or that small peptides and free amino acids might act as flavor precursors and nutrients for the microorganisms in sourdough. The situation in gluten-free breads is similarly controversial. We have mentioned a possible positive role of soluble proteins on gas cell stabilization, and a negative role of protein aggregation during baking in the section on sorghum bread (Section 'The role of proteins and protein networks', p. 151). Some degradation of sorghum proteins during sourdough fermentation was therefore beneficial, as it prevented this aggregation upon baking (see Section 9.3.3.2 and Fig. 9.8). In

yet unpublished experiments, we also tried to degrade the sorghum proteins completely, using a high dosage of an endo-protease. However, the resulting bread was of very low volume and had a sticky crumb, suggesting that a partial, but not a complete protein degradation is required. Thus, it appears that proteins have some role in the crumb formation of gluten-free batter breads. This is also in agreement with results of studies like Gujral and Rosell (2004), where TGase showed some beneficial effects in combination with rice flour and HPMC. As TGase acts upon proteins, its effect on bread quality shows that proteins must play a certain role. Clearly, more research is required to identify the exact role of proteins in these types of gluten-free breads.

Summary: *Gluten-free breads and rye breads (from >90% rye flour) resemble each other technologically, as they are both made from soft, batter-like doughs and contain natural or added hydrocolloids rather than a continuous, viscoelastic gluten-network (pentosans and β-glucans in rye vs. added xanthan gum and HPMC). It appears that this analogy has been largely ignored in the literature. There might be a chance for a better understanding of both systems, if results from both areas were compared, and the analogies might be exploited for the improvement of bread quality.*

(Rye bread serves as a technological model only, it is not safe for celiacs!)

9.3.8 Staling

Quick staling – or increase in crumb firmness – is one of the most unpleasant properties of gluten-free bread. The mechanisms of staling are still being debated. Historically, it has been associated with starch retrogradation. However, more recently, for regular wheat bread the involvement of gluten in the staling process has been suggested, in a way that starch might interact with gluten fibrils and crosslink them (Martin et al., 1991; Hoseney, 1998). Meanwhile, this model has been questioned again. At least, it has been reported, based on a comparison of regular wheat bread and gluten-free starch bread, that interactions between starch and gluten are not essential for the crumb firmness increase. Starch retrogradation alone is sufficient to cause bread firming (Morgan et al., 1997). In gluten-free bread, we should focus on the starch alone. Within the starch phase, amylose retrogradation occurs very fast upon cooling and helps to stabilize the crumb. In contrast, amylopectin retrogradation is slower and seems to be the decisive factor for aspects of staling like crumb firming and loss of elasticity (Belitz et al., 2004). For pure starch breads from potato or wheat starch, Keetels et al. (1996) suggested a detailed model for crumb structure and staling (see Section 9.3.1.3).

It is known that crumb firmness increases over storage time also when no drying occurs (Hoseney, 1998). Nevertheless, drying doubtlessly speeds up the perceived firmness of bread. Hydrocolloids, including xanthan gum and HPMC, have been shown to reduce moisture loss in wheat bread that was stored unpacked (Guarda et al., 2004). HPMC, but not xanthan gum, lowered the increase in crumb firmness over 24 h of storage in the same study, and the authors assumed that HPMC might inhibit amylopectin retrogradation by binding to the starch.

Shortening and emulsifiers (monoglycerides) are widely used in wheat bread to delay staling (Hoseney, 1998; Belitz et al., 2004). Several mechanisms for their antistaling effect have been suggested. Both substances might limit starch swelling (Martin et al., 1991; Hoseney, 1998), and therefore subsequently starch interactions that would lead to staling. This is essentially the same argument used above to explain, why these substances soften or weaken the crumb (Section ‘Emulsifiers, starch and crumb properties’, p. 150). As before, we have to carefully balance between desirable prolonged softness of the crumb and undesirable

crumb weakening. It has also been reported that during baking, emulsifiers (in this case, monoglycerides) form complexes with amylose and amylopectin, retarding retrogradation (Belitz et al., 2004). Again, it is obvious that we should not prevent amylose retrogradation too much, because we would otherwise prevent the proper setting of the crumb.

Use of bacterial α-amylases is a well-established method to delay staling in wheat bread (Martin and Hoseney, 1991). Thermostability of these amylases is a critical factor. There is a so-called 'window' for the amylase activity. It starts upon starch gelatinization, because ungelatinized starch (unless mechanically damaged) is not notably attacked by amylases, and ends upon thermal inactivation of the amylase (Martin and Hoseney, 1991). While bacterial α-amylases are generally relatively heat stable (Akers and Hoseney, 1994), it might be desirable to select such enzymes with only intermediate temperature stability to avoid excessive starch degradation and dextrin production (Gerrard et al., 1997). This appears especially important in gluten-free bread due to the important role of starch.

There is considerable debate in the literature about how these α-amylases delay staling. While there is agreement that α-amylases produce specific mixtures of dextrins from starch, there is no consensus whether these dextrins are the cause of the delayed staling, or whether they just reflect the degradation of starch. In the latter case, the modification of the starch itself would be the cause of the delayed staling and the dextrins just a symptom. Martin and Hoseney (1991) and Akers and Hoseney (1994) suggested that dextrins of a certain size are the cause of delayed staling, for example, by interfering with crosslinks between gluten and starch in staling wheat bread (Martin and Hoseney, 1991).[2] Gerrard et al. (1997) and Morgan et al. (1997) disputed this view and put forward the hypothesis that the modification of the starch itself is the cause and dextrins just indicate this modification.

One bacterial α-amylase of intermediate temperature stability (Novamyl® by Novozymes, Switzerland) that has considerable potential in delaying firming of the crumb has been used by Gerrard et al. (1997) in regular wheat bread and by Morgan et al. (1997) in gluten-free starch bread.

Summary: *Staling is a large problem in gluten-free bread. We can slow it down by adding hydrocolloids (especially HPMC), shortening and emulsifiers and/or bacterial α-amylases. In the case of shortening and emulsifier, overdosage may easily weaken the crumb, and we also have to pick the right emulsifier (which may involve trial and error). In a similar way, bacterial α-amylase may destroy the crumb structure by excessive starch degradation, therefore we should take care that we do not select an excessively thermostable enzyme and that we do not overdose.*

9.4 CONFECTIONERY PRODUCTS

9.4.1 General

In most confectionery products, excluding puff pastry and sweet yeast leavened breads, full gluten development is undesirable and instead egg, fat and/or sugar play an important role for the physical structure. Therefore, the problems in producing these products from gluten-free flours are small. The technological steps for a successful production can mostly

[2] This interpretation would obviously require modification when applied to gluten-free bread, in a way that dextrins would interfere with starch-starch interactions.

be derived from wheat-based formulations. Other aspects, like desirable color or flavor, become more central questions. These, however, depend largely on the consumers' taste in various countries and are beyond the scope of this book chapter. We therefore want to limit the following sections to a small number of examples, where technological problems were encountered.

9.4.2 Gluten-free cakes

As with all bakery products, cakes vary between countries. Hoseney (1998), with a US background, differentiates layer cakes (high ratio, i.e. more sugar than flour), angel food cakes (based on foam from egg white and sugar, only little flour) and pound cakes (heavy, rich cakes). For the present section, we focus on layer cakes, as described in AACC Standard 10–90 (AACC International, 2000). The formulation is (on a flour basis) 100% flour, 140% sugar (saccharose), 50% shortening, 12% non-fat dry milk, 9% dried egg whites, 3% salt (NaCl), optimum (typically 125–145%) water and baking powder. The procedure comprises sifting of dry ingredients, addition of shortening and part of the water, mixing in several steps with addition of the remaining water, scaling into pans and baking.

Although the study of Glover et al. (1986) addressed wheat–sorghum composite flours (obviously not appropriate for celiacs and people with wheat allergies), several important principles for gluten-free cake production can be derived. The basic problem was that cake volume decreased as the percentage of sorghum increased in the sorghum–wheat flour mix. At high levels (30–50% of sorghum), crumb became very brittle.

Problems associated with the sorghum flour were related to large particle size, lack of polar lipids, especially glycolipids, and high starch gelatinization temperature. The high gelatinization temperature resulted in a high percentage of ungelatinized starch. High gelatinization temperature and lack of glycolipids are in agreement with the literature (Chung and Ohm, 2000; Lineback, 1984). Glover et al. (1986) could improve the cakes by technological means. Finer milling, using a pin mill, resulted in smaller particle size and higher starch damage. The water binding and resulting increase in batter viscosity due to starch damage appear to be desirable specifically in high ratio cakes (Evers and Stevens, 1985) and pin milling improved cake quality to a limited degree. Use of glucose instead of saccharose in the recipe improved cake volume, crumb grain and crumb texture considerably. A higher degree of starch gelatinization was found as a consequence of the use of glucose. Spies and Hoseney (1982) reported that sugars delay starch gelatinization and that saccharose has a stronger effect than glucose. They attributed these findings to two effects of the sugars: The first effect would be lowering the water activity, that is, less water is available for starch gelatinization because it is bound by the sugar. The second effect would be interactions between sugars and starch. Sugars would bind to the starch chains and promote their interactions, that is, the sugars would act as bridges between starch chains. Longer sugar molecules could be expected to be more efficient in forming such interactions and bridges. The longer saccharose molecule (disaccharide) would therefore increase starch gelatinization to a larger degree than glucose (monosaccharide). Use of glucose instead of saccharose in the cake would cause the starch to gelatinize earlier. Similar to sorghum, rice is generally characterized by a high starch gelatinization temperature (see Section 9.3.4.1). We could therefore expect similar problems with rice flour as with sorghum flour. In (wheat-based) layer cake baking tests with reconstituted flours, where in a commercial cake flour wheat starch had been replaced by a variety of other starches (rye, barley, maize, rice and potato), rice starch did indeed perform worst (Sollars and Rubenthaler, 1971).

If in a grain like sorghum, the content of natural emulsifiers (i.e. polar lipids) is insufficient, emulsifiers should be added to the batter. In layer cakes, propylene glycol monostearate is widely used to facilitate air incorporation (Hoseney, 1998).

Summary: *It appears that the following traits of a gluten-free flour are undesirable for layer cake production: large particle size, insufficient content in polar lipids and high starch gelatinization temperature. Beneficial are fine milling of the flour, and addition of emulsifiers to compensate for a lack in polar lipids. For a given flour with a given starch gelatinization temperature, exchange of sugars is possible, and use of glucose instead of saccharose may lower the starch gelatinization temperature in the batter. However, we might better try to select gluten-free flours with lower starch gelatinization temperatures.*

9.4.3 Gluten-free biscuits

Biscuits (British English, equivalent to 'cookies' in American English) are based on formulations high in sugar and shortening, but relatively low in water (Hoseney, 1998). They can be produced using either rotary molds, or by sheeting and cutting, or by extrusion through an orifice and cutting. Important are a tender bite of the biscuits and their size. Size (width and height) is important in industrial production because the biscuits have to fit into their boxes, and is governed by the amount they spread during baking (Hoseney, 1998). It has been furthermore pointed out that damaged starch is undesirable in biscuits, because it binds water. Since the baked biscuits are very low-moisture products, this extra water has to be evaporated during baking, increasing the required energy (Evers and Stevens, 1985). A different argument would be that the degree of starch damage affects how much the biscuits spread. The higher the starch damage the more water from other ingredients is bound and consequently the spread is lower (Thomas and Atwell, 1999). At least for formulations with high sugar and low water (e.g. 60% sugar and about 23% water on a flour basis), little or no starch gelatinizes during biscuit baking due to limited water availability (Abboud and Hoseney, 1984). We could therefore conclude that, in contrast to cake, starch gelatinization temperature is relatively unimportant in biscuits. However, upon baking shortening melts and sugar dissolves, which increases fluidity and thus allows the biscuits to spread (Abboud and Hoseney, 1984; Hoseney, 1998). The latter author furthermore assumed that proteins might play a role in controlling viscosity during baking and thus biscuit spread as starch is not gelatinized. This might require a more careful selection of the flour mixture in gluten-free cookies. A successful formulation for a sheeting and cutting procedure used brown rice flour (70%), corn and potato starch (10% each) and soy flour (10%) in combination with egg (Schober et al., 2003).

A study on biscuits from sorghum and pearl millet covered many of the aspects just mentioned and is therefore a good example for problems encountered in gluten-free biscuit development and finding of solutions (Badi and Hoseney, 1976). These biscuits were produced following the micro method of Finney et al. (1950). The formulation (on a flour basis) was flour (100%), sugar (saccharose, 60%), shortening (30%), $NaHCO_3$ (1%), NH_4HCO_3 (0.75%), non-fat milk solids (3%), salt (NaCl, 1%) and water (to optimum). The procedure involved creaming of sugar, shortening, non-fat milk solids, salt, leavening agents and water. Finally, flour was added with very short mixing. Sheeting, cutting and baking followed. According to Finney et al. (1950), sufficient spread and a well-broken top with numerous small cracks are desirable for these biscuits. In contrast, sorghum and millet biscuits produced by Badi and Hoseney (1976) with the exact procedure of Finney et al. (1950) lacked spread and top cracks and were, according to the authors, 'tough, hard, gritty and mealy'.

Badi and Hoseney (1976) could identify several problems associated with the sorghum and millet flours, including the lipid composition, and a high degree of starch damage. Improvement of the biscuits was possible by several steps: The first step was adding emulsifiers (unrefined soy lecithin or refined lecithin plus monoglycerides), which improved top grain and spread. This is in line with the lack of polar lipids in sorghum described above and in the literature (Chung and Ohm, 2000). Next, incubation of the sorghum or millet flours with malt syrup or just water for several hours and air drying was done to remove damaged starch. This improved spread and top grain even more, and also reduced grittiness. The authors suggested that only malt treatment removed damaged starch, however, it would appear plausible that incubation with water could also reduce the amount of damaged starch due to the action of grain amylases. (It should be kept in mind that barley malt is not gluten-free, thus it should be replaced by microbial amylases in a gluten-free formulation.) Grittiness could be further reduced by increasing the pH of the biscuit dough (use of Na_2CO_3 instead of $NaHCO_3$). Our own data suggest that sorghum proteins can be solubilized under alkaline conditions (Fig. 9.13). Therefore, we might assume that the protein matrix of endosperm particles is dissolved as the biscuit dough gets more alkaline, thus eliminating these particles and reducing grittiness. Finally, Badi and Hoseney (1976) addressed the remaining problem of fragility of the biscuits by blending the gluten-free grains with wheat. As this step is impossible in gluten-free biscuits, we would suggest to try egg addition if gluten-free biscuits are too fragile.

Summary: *Gluten-free flours for biscuit (cookie) production should have low starch damage, while starch gelatinization temperature appears to be less important because starch tends to not gelatinize in biscuits anyhow due to high sugar and low water concentrations. Controlling the biscuit spread is important in the industrial production, so that the biscuits fit in their boxes. Starch damage, water level and possibly proteins from flour or added egg affect the spread.*

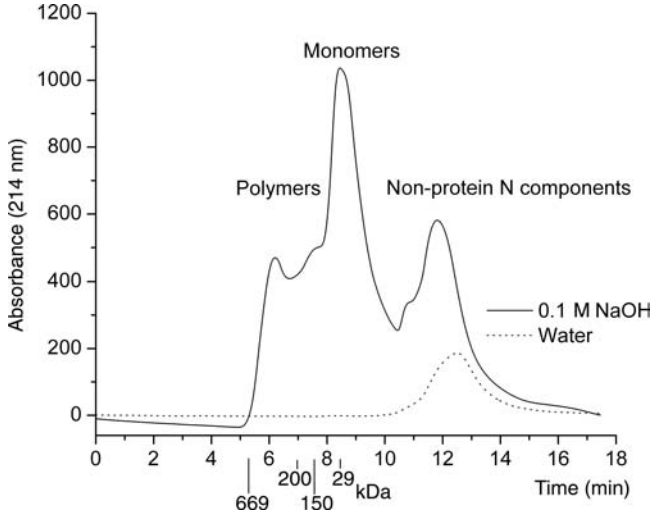


Fig. 9.13 Size-exclusion HPLC of sorghum flour extracted for 15 h with water or 0.1 M sodium hydroxide solution (100 mg flour plus 400 µl liquid). Separation on a Phenomenex® BioSep-SEC-S 3000 column (300 × 7.8 mm), mobile phase 50% acetonitrile in water plus 0.1% TFA, flow rate 1.0 ml/min, column temperature 40°C, 15 µl injection, detection at 214 nm.

9.5 CONCLUDING REMARKS

We know that this chapter contains a lot of information. We are also sure, that some of it might be too simplified, too complicated, too detailed or not detailed enough, depending on you, our reader and your specific background and needs. We hope that you could benefit from some of the information provided and that the literature cited will provide additional help. If you are new in the area of gluten-free bread, do not be too easily discouraged. People who are used to wheat bread will sometimes be quite critical about gluten-free bread – but we have to advance step by step, improving quality, healthiness and maintaining safety of the products.

ACKNOWLEDGEMENTS

Special thanks to Dr. Scott R. Bean for his scientific input and his personal support as a friend. I would also like to thank Dr. Sajid Alavi and Ms. Jhoe Stonestreet for critical review and constructive critics. Thanks also to Dr. John R. N. Taylor for many important inspirations.

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10 Production of Gluten-Free Beer

Gernot Zweytick and Emmerich Berghofer

10.1 BEER – THE CLASSICAL POINT OF VIEW

10.1.1 History of brewing

Beer is one of the oldest fermented beverages. The history of beer is closely connected to the history of human civilisation. It is not exactly known when, where and how the process of brewing was invented. Brewing and beer drinking are shown in ancient drawings of the Mesopotamian culture (dated ~ 2800 BC), as well as in drawings of the old Egyptian culture (dated ~1700 BC). At that time, soaking bread in water and allowing spontaneous alcoholic fermentation to take place is the most likely way that beer could have been produced. Therefore, taste and quality of this beverage must certainly have been different from today's beer types (Fischer, 1999).

In Europe, beer was a favourite beverage of the German tribes and of the Scythians and the Celts. As baking and brewing were women's work in all primitive cultures, beer was brewed by women for daily household nourishment. In the fourteenth century AD, the type of beer we know today was created by addition of hops as the only flavouring ingredient. At this time, beer was usually brewed in monasteries and nunneries, thus brewing became men's work. The German 'Purity Law', proclaimed in 1516, made a very important contribution to the improvement of beer quality. It defines beer as a beverage made of water, malt and hops. The yeast which plays an important role in beer production was not known as starter of alcoholic fermentation in the sixteenth century (Kunze, 2004).

The invention of the refrigeration machine by Carl von Linde (1876) and the development of the railway network led to building and expansion of large breweries in all developed countries. An additional milestone in beer brewing was set, when the Austrian Anton Dreher (1841) developed the bottom-fermented beer, the so called 'lager' or 'Viennese type' beer applying mechanical refrigeration. For the production of these light coloured beers, which gradually displaced the dark Bavarian beers, lower temperatures and longer fermentation times were necessary (Fischer, 1999).

For many centuries, the Bavarian 'Purity Law' was the prevailing law in many countries, but, due to economic advantages, American brewers began to use maize and rice grits as additives in the middle of the nineteenth century. This new type of beer and the raw grain processing in brewing has gained global importance (Kunze, 2004).

10.1.2 Common beer types

Depending on the yeast used and the parameters of the fermentation process, beer types can be classified into bottom- and top-fermented beers. The characteristics of these beers are quite different.

10.1.2.1 *Bottom-fermented beers*

All bottom-fermented beers, which cannot be categorised as special beer types, are called 'lager' beers or entire beers. Their common characteristic is a main fermentation at low temperatures and a long secondary fermentation step. The primary fermentation is carried out at approximately 5–7°C for about 10 days; during the second fermentation step, the beer is ripened at temperatures close to the freezing point for 2–6 months (Fischer, 1999).

The main raw material for the production of these beers is barley malt; maize and rice are often added as un-malted grain. Well known beers of this category are 'Munich-type', 'Viennese-type', 'Pilsner-type', 'Dortmund-type', Bock and Double Bock (Kunze, 2004).

10.1.2.2 *Top-fermented beers*

Top-fermented beer is fermented at about 20°C; a long second fermentation step is not required. Typical top-fermented beers are South German wheat beer, 'Berliner Weisse', Trappist-beer, Lambic beer, Kölsch, Alt, Ale, Porter and Stout (Kunze, 2004).

10.1.3 Traditional malting process

Malting can be defined as a controlled natural process, in which barley and other cereal grains are allowed to grow under precisely defined conditions in order to bring about specific, desirable changes in the properties of the kernel. The aim of the malting process is the formation of hydrolysing enzymes in the kernels for further decomposition of the starch during the brewing process. Approximately 23% of the contained starch is decomposed due to this operation and about 4.5% of the resulting fragments are completely metabolised. Traditionally, classical beer is produced from barley malt – only for special beer types, wheat malt or un-malted maize and rice are used. There are many advantages when using barley in malting:

- Barley is a modest plant.
- The germination can be easily controlled.
- The amount of enzymes produced during germination is high.
- The spelts of barley work as additional filtration aid.
- The taste of beer made from barley is widely accepted.

10.1.3.1 *Steeping*

The water content of the cleaned and graded cereal is increased by soaking in water (12–16°C) to about 42–44% for pale malts and 44–47% for dark malts. The water uptake is highest during the first few hours. With increasing water content, the cereal begins to respire. As the produced carbon dioxide inhibits the growth of the germ, it has to be removed continuously and oxygen has to be supplied to prevent the conversion of respiration to fermentation. Every

malting-house has its own typical scheme of alternating cycles of soaking and aeration to obtain the desired water content.

10.1.3.2 *Germination*

In the traditional malting process, the wet grain is brought to threshing floors up to a height of 15–50 cm. The piles of grain have to be turned over several times to prevent the germs from intertwining and to equalise humidity and temperature. The duration of germination is 7–8 days for light malts and 8–11 days for dark malts (Kunze, 2004).

Nowadays, the pneumatic malting is applied more often. The layer of the grain is higher and has to be cooled continuously with a moisture saturated air stream. The duration of this process is comparable to the traditional one but the expenditure of work is lower (Kunze, 2004).

10.1.3.3 *Kilning*

The green malt has to be dried to obtain a longer shelf life and to stop all biochemical reactions that occurred during germination. Another aim of kiln drying is the formation of the typical malt flavour. There are two steps in the drying process: initial drying and kilning. The water content is first reduced from 40–50% to about 10% at temperatures of 45–65°C. After 10–12 h, the end of this first phase is marked by an increasing outlet air temperature. In the second phase, the drying air has to be heated up to 80–85°C to decrease the moisture content of the malt to 3.5–4.0%. While some germinating reactions take place in the first step, only chemical and biochemical reactions occur in the second step. Enzymes are not affected by this treatment (Kunze, 2004).

10.1.4 Traditional brewing process

There are four main steps in the brewing process: mashing, wort boiling, fermenting and ripening. A flow chart of the brewing process is shown in Fig. 10.1.

10.1.4.1 *Mashing*

The cleaned and ground malt is mashed with brewing water and heated up in steps to the optimum temperatures of the enzymes to be activated. The first rest at 45–50°C provides the optimum temperature for proteinases and glucanases, the next rest at 62–65°C is the maltose production rest and the third rest at 70–75°C is the saccharification rest. Depending on how these temperatures are obtained, two different types of mashing can be applied: the infusion method and the decoction method. When applying the infusion method, the temperature of the entire mash is increased up to the resting breaks continuously. In the decoction process, the temperature is increased by adding a previously removed and then heated part of the mash. When returning this hot mash into the tun, the resulting temperature of the next resting break is obtained after mixing. During the decoction process, one, two or three of these heating steps can be applied. Particularly during the maltose production rest and the saccharification break at about 60–62°C, the enzymes produced in the malting process convert the starch to maltose and glucose. At the end of this process, the undissolved substances – the spent grains – are separated from the wort usually using a lauter tun. The spent grains are washed with hot water. The second wort is then added to the first (Kunze, 2004).

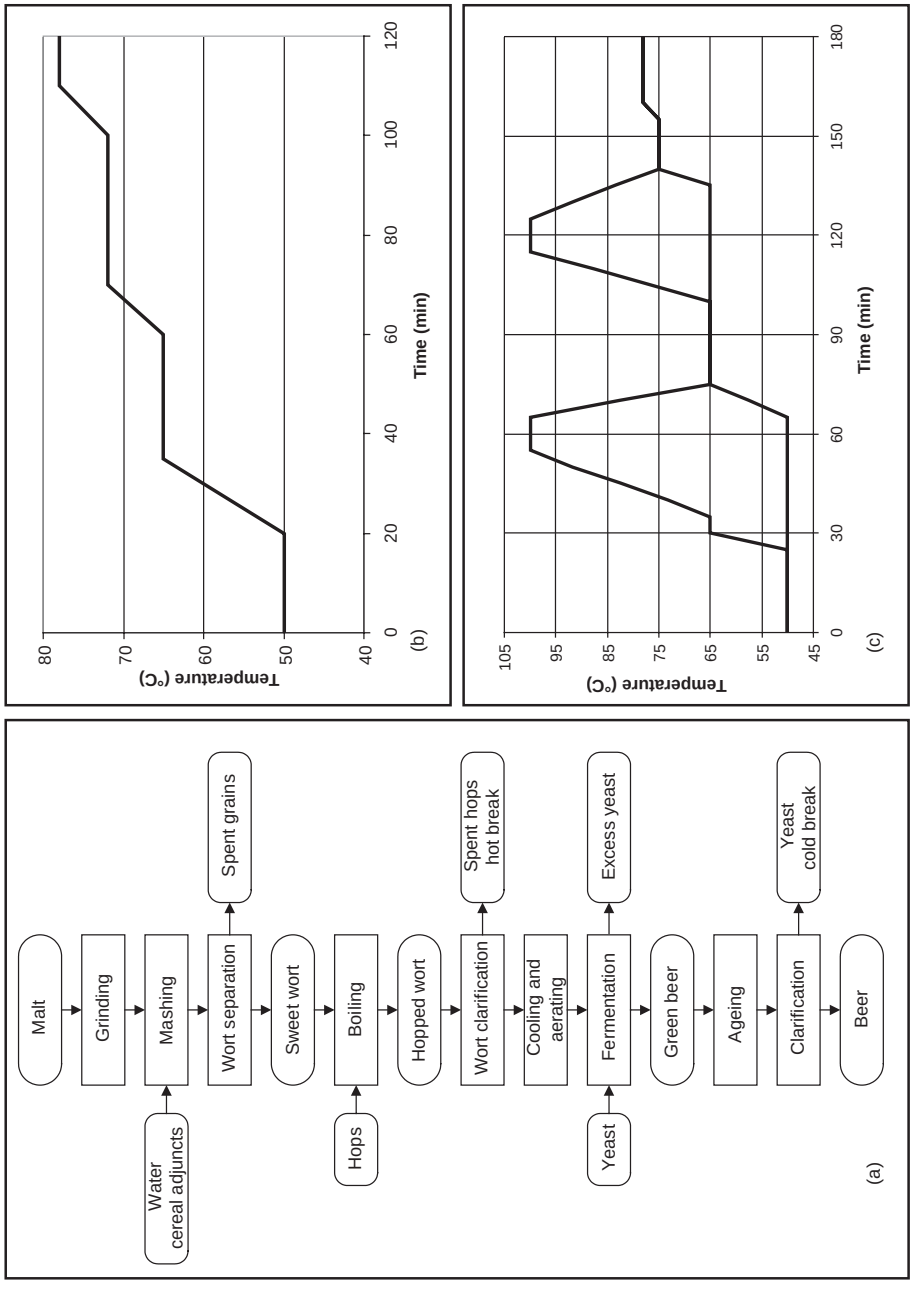

Fig. 10.1 General flow sheet of the brewing process (a) and temperature profiles of infusion method (b) and decoction (c) method.

10.1.4.2 *Wort boiling*

Ground or pelletised hops are added to the obtained wort, which is then boiled for 50–60 min in a special wort kettle. During this process, a number of important physical and chemical processes occur:

- extraction and transformation of hop components;
- evaporation of water and standardisation of the original gravity;
- inactivation of the enzymes and sterilisation of the wort;
- formation and precipitation of protein–polyphenol compounds;
- removal of undesirable flavours which would give a bitter taste to the beer;
- lowering of the pH of the wort.

As some of the hop components are volatile oils, the hops are not added at once but in two or three steps. When using aromatic hops, these are added at the end of the boiling process. The amount of hops used depends on the type of beer to be produced (Kunze, 2004).

10.1.4.3 *Wort clarification – cooling – aerating*

The wort has to be clarified in a whirlpool by separation of the hot break (caused by the coagulation of the proteins during cooking) and the cold break (formed at temperatures below 60°C during wort cooling). Aerating the wort is necessary for the initial growth of the yeast.

10.1.4.4 *Fermentation – ageing*

During fermentation, the carbohydrates of low molecular weight are metabolised to alcohol and carbon dioxide. The two types of yeast used in beer production are *Saccharomyces cerevisiae*, which is used for brewing top-fermented beer, and *Saccharomyces carlsbergensis*, which is used in the production of bottom-fermented beer (Kunze, 2004). The differences between top- and bottom-fermented beer are shown in Table 10.1.

When producing bottom-fermented beer, the wort has to be cooled during fermentation, due to the formation of fermentation heat. After the primary fermentation, the yeasts agglomerate and precipitate. Currently, in the production of bottom-fermented beer, the precipitated yeast can be removed at the bottom zone of cylindrical–conical tanks; for example,

Table 10.1 Main differences between top- and bottom-fermented beer.

Type of yeast	**Top-fermented beer** ***Saccharomyces cerevisiae***	**Bottom-fermented beer** ***Saccharomyces carlsbergensis***
Formation of colonies	After cell division, cells stay together for a long time	Mother and daughter cell separate soon
Location of the yeast during fermentation	Yeast agglomerates move to the surface during the intensive fermentation	Yeast cells remain in the fermenting wort and precipitate at the end of fermentation
Primary fermentation Secondary fermentation	2–3 days at 15–20°C The final specific gravity is obtained after 3–6 days	6–14 days at 4–12°C 1–6 months at 0–2°C

Table 10.2 Original gravity, final degrees of attenuation and fermentation times of different types of beers.

Beer type	Original gravity	Final degree of attenuation	Fermentation time
Draught beer	10–12°	75–77%	3–7 weeks
Lager beer (entire beer)	12–14°	75–77%	2–4 months
Dark beer	13–14°	70–74%	
Special beer	13–16°	78–82%	3–5 months
Strong beer, bock beer	>16°	80–83%	4–7 months

in light entire beer production, the final degree of attenuation is 75–82%, of which 64–72% is obtained during the primary fermentation. Consequently, the necessary time for secondary fermentation is very short. On the contrary, strong beers have low degrees of fermentation but a long phase of secondary fermentation. The freshly fermented beer (green beer) still contains fermentable carbohydrates and some components which can only be removed by a further fermentation and ripening step. The aims of this secondary fermentation are:

- getting the final degree of fermentation;
- saturation of the beer with carbon dioxide;
- metabolism or removal of off-flavour substances;
- clarification and ageing (Kunze, 2004).

When producing top-fermented beer, the wort does not have to be cooled during fermentation. The final degree of attenuation is reached during the first fermentation step as far as possible. No long secondary fermentation is needed, though this is sometimes applied to improve quality. Characteristic production parameters of different beer types are shown in Table 10.2. At the end of the ageing process, the beer still contains a large amount of yeast cells and other turbidity causing substances as tannin–protein complexes and hop resins. This 'cold break' has to be removed to obtain a long shelf life and provide a clear and stable appearance of the beverage. This is achieved by filtration (Kunze, 2004).

10.2 DEFINITIONS AND LEGAL ASPECTS

10.2.1 Beer

The International Codex Alimentarius Commission (2004) and the German beer law define beer as an alcohol and carbon dioxide containing beverage, produced by mashing and cooking of cereals such as barley, wheat, rice and maize or their derivatives with hops and water. In the 'Dictionary of Beer and Brewing' compiled by Rabin and Forget (1998), beer is defined as 'a generic name for alcoholic beverages produced by fermentation of a cereal or a mixture of cereals and/or alcoholic beverages made by fermentation of malt with or without other cereals and flavoured with hops'. Hardwick et al. (1995) distinguish between classical beer, made of barley malt and hops with or without raw grain, and beer-like beverages that are not produced by conventional beer brewing, such as sake, chicha or pulque.

10.2.2 Gluten-free products

The International Codex Alimentarius Commission (2006) defines 'gluten' as a protein fraction from wheat, rye, barley, oats or their crossbred varieties and derivates thereof, to which some persons are intolerant and that is insoluble in water and 0.5 M NaCl. The prolamins are a fraction from gluten, which can be extracted by 40–70% of ethanol.

According to the International Codex Alimentarius Commission (2006), gluten-free products are defined as food:

(a) 'consisting of or made only from ingredients which do not contain any prolamins from wheat, durum wheat, rye, barley, oats or any *Triticum* species such as spelt (*Triticum spelta* L.), kamut (*Triticum polonicum* L.) or their crossbred varieties with a gluten level not exceeding [20 mg/kg] in total based on the food ready for consumption;
(b) 'consisting of ingredients made from wheat, rye, barley, oats or any *Triticum* species such as spelt (*Triticum spelta* L.), kamut (*Triticum polonicum* L.) or their crossbred varieties, which have been rendered 'gluten-free'; with a gluten level not exceeding [100 mg/kg] in total based on the foods ready for consumption;
(c) 'any mixture of the two ingredients as in (a) and (b) with a gluten level not exceeding [100 mg/kg] in total based on the food ready for consumption.'

Oats can be tolerated by some people with coeliac disease, therefore its use in gluten-free foods for dietary management may be determined at national level (International Codex Alimentarius Commission, 2006).

10.3 TRADITIONAL GLUTEN-FREE BEERS AND BEER-LIKE BEVERAGES

Some alcoholic beverages traditionally brewed from gluten-free grains or malts such as sorghum, maize and rice can be considered as beers, as these raw materials contain starch or other high molecular carbohydrates as metabolites for further alcoholic fermentation. Common to all of these beverages is the necessary presence of amylolytic enzymes for the hydrolysation of the starch to fermentable sugars. Most of these beers are not brewed with hops, are not filtered, and they cannot be classified as top-fermented or bottom-fermented beers (Hardwick et al., 1995).

While beers of Western Europe, which are mainly made from barley malt, are well known and have spread to America, Australia, New Zealand, and so on, beers made from other raw materials are less popular. Nevertheless, some special types of different beers and beer-like beverages can be found in countries all over the world. Brewing of such beers has a long tradition in these countries and due to their raw materials, they can be considered gluten-free. Some well known examples of such beverages are the African sorghum beer or kaffir beer, sake, which is a Japanese beer-like beverage made from rice, and chicha, a beer-like beverage from Latin America, which is mainly made from maize (Hardwick et al., 1995).

10.3.1 Sorghum-beer

10.3.1.1 *Raw materials*

Sorghum and millet are the major food sources of energy and protein in many African countries. The ground grain is used for the production of porridges, kisra, which is a type of sorghum bread, and fermented beverages (Makki, 1998).

Sorghum beer is brewed in many African countries from north of the equator down to Ciskei in the Cape. In former times it was called kaffir beer; more recently it is named African, opaque, or sorghum beer. As it is brewed throughout African continent, there are numerous African names for this beverage. In comparison to sorghum, the millets *Pennisetum typhoides* and *Eleusine coracana* are less important for the brewing industry. Maize, which is not an original African cereal as it came from the New World, is well integrated in the brewing process of sorghum beer. Its properties as malted grain are not very good, but it is mainly used as adjunct. The fermenting cultures used for sorghum beer brewing are *Saccharomyces cerevisiae*, and *Lactobacillus* cultures (Hardwick et al., 1995).

10.3.1.2 *Characteristic properties of sorghum beer*

Compared with European beer, sorghum beer is an opaque and rather viscous beverage. Its colour can be yellowish when sorghum malt and millet are used for brewing, or pinkish, when sorghum malt and maize are used. The depth of the colour is dependant on the pH of the beer. Sorghum beer may have estery or fruity flavours, which are quite different from European beers. The taste is slightly sweetish, and due to the formation of lactic acid, it can be a little sour. The touch of bitterness or astringency on the tongue is mild compared with the bitterness of hops. Depending on the formulation, the number and the particle size, the mouthfeel of sorghum beer can vary from thin to creamy. The presence of yeast cells, lactic acid bacteria and fermentable sugars underlines the unfinished state of this kind of beer (Hardwick et al., 1995).

10.3.1.3 *Sorghum beer production*

The origin of sorghum beer is a beverage, which was traditionally produced like chicha, a south American maize beer. The enzymes of the human saliva, not the amylases of sorghum malt, were used for the saccharification of the sorghum starch (Maurizio, 1933). In industry, the usual way of processing is souring, cooking, mashing, straining and fermenting. The souring process is carried out at temperatures of approximately 48°C with a water suspension of ground malt inoculated with *Lactobacillus leichmanii* as the souring culture, usually from a previous souring. At this temperature and the low pH value, the occurrence of contaminations is unlikely. When the desired degree of acidification is reached, the suspension is diluted with water and adjuncts are added. The sour is then cooked and the starch of the un-malted grains is gelatinised. During the mashing process, the boiled sour is cooled to 60°C and sorghum malt is added. Due to the high amount of α-amylase in sorghum malts, the viscosity of the mash decreases rapidly and the following saccharification proceeds more slowly over the next 1–2 h. As the straining step, accomplished by centrifugal decantation, takes a long time, it is sometimes started before saccharification is finished. Fermentation, started by addition of the yeast, is carried out at temperatures of 20–25°C and is finished after a few days. After this time, the sorghum beer is packaged. As a result of ongoing fermentation, problems

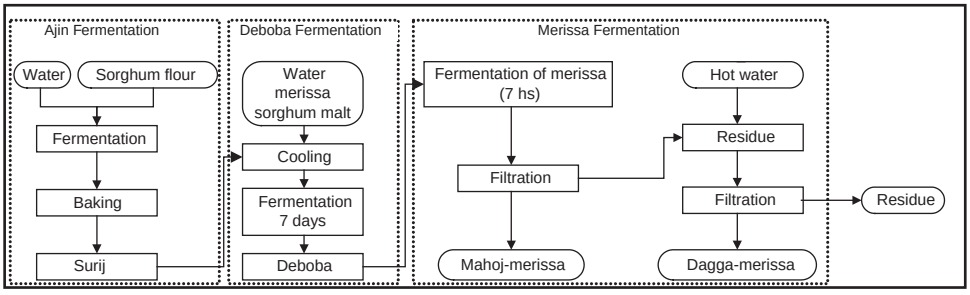

Fig. 10.2 Fermentation steps of Merissa production (Makki, 1998).

in packaging can sometimes occur. Bottled sorghum beer is usually fully fermented and pasteurised (Hardwick et al., 1995).

Merissa is a variety of sorghum beer that is brewed in some African countries. It is considered a national drink in many countries and it usually contains about 5–6%vol of alcohol. The method of merissa production as shown in Fig. 10.2 is divided into three stages of fermentation: ajin fermentation, deboba fermentation and merissa fermentation. In the first step of merissa production, sorghum flour and water are mixed forming a type of viscous suspension. The mixture is left for spontaneous lactic acid and acetic acid fermentation and then baked resulting in 'surij'. After cooling, the baked 'surij' water, sorghum malt and merissa from a former production are added and left for fermentation for approximately 7 days. This so called 'deboba' fermentation is a starter activating phase. The third step is the final 'merissa' fermentation, an alcoholic fermentation, which results in a pale and opaque beer (Makki, 1998).

10.3.2 Sake

A typical and traditional beer-like beverage made of rice is the Japanese sake. It is not produced by conventional beer brewing, but there are several similarities between beer and sake (Hardwick et al., 1995).

10.3.2.1 *Raw materials*

There are approximately 65 varieties of rice used for brewing, and each type has its own characteristic flavours and properties (Hardwick et al., 1995).

In former times, when mechanical cooling was not highly developed, sake brewing was limited to the winter months as the cool temperatures provided the best conditions for brewing and storage. Due to these circumstances, large-scale brewing was unprofitable resulting in many different types and regional brands. The degree of milling – meaning the reduction of the size of the rice grain – has an important influence on the taste of the resulting product. Premium sake is brewed with special rice, where the starch is concentrated in the centre of the grain while the fats and proteins are located outside. Depending on the degree of milling, more or less of these undesirable components are removed and the pure starch remains for the brewing process. For premium sake, about 40% of the rice grain is removed – for super premium sake, at least 50% (Gauntner, 2003).

10.3.2.2 *Properties of sake*

Sake is an almost transparent, clear, pale yellow, un-carbonated liquid with an alcohol content between 15 and 17%vol. Due to the presence of many esters such as isoamyl acetate, ethyl capronate and phenylethyl acetate, sake has a characteristic estery flavour, which is closer to that of wine than to that of beer.

Four main types of sake can be found:

- **Junmai-shu**: Junmai-shu is pure rice sake with no other additives except water, rice and koji added. It is made of polished rice and only about 30% of the outer kernels are removed. The taste is heavier and fuller in comparison to other sake types.
- **Honjozo-shu**: The production of Honjozo-shu is comparable to that of Junmai-shu but there is an additional step: at the final stages of production distilled ethanol is added. By blending it with more water in a final step, the alcohol content is adjusted to the same value as for other sake types. The taste and flavour is lighter and drier and the fragrance is more prominent.
- **Ginjo-shu**: For this sake type, the size of the rice has been reduced to 60% of the original rice grain. The removal of fats and proteins causes less off-flavour to be produced during fermentation. Ginjo-shu is fermented at lower temperatures for a longer time. The flavour is more complex and delicate and the fragrance is flowery and fruity.
- **Daiginjo-shu**: The production of Daginjo-shu is similar to that of Ginjo-shu but approximately 50% of the rice grain is milled. Sometimes, for even more delicate types, 65% of the kernels are milled (Gauntner, 2003).

10.3.2.3 *Sake-brewing process*

Fig. 10.3 gives an overview on the production of steamed rice, koji and sake.

Brown rice is milled and polished according to the desired degree. The fine particles resulting from the milling step have to be washed away; then the rice is soaked to attain a certain water content. The more the rice is polished, the shorter the time for soaking. The next step is steam cooking, which gives the rice a firmer consistency in comparison to cooked rice. After cooking, koji mold – which is mainly *Aspergillus oryzae* – in the form of a fine dark powder, is sprinkled on the steamed rice (Gauntner, 2003).

This mixture is placed in a room with high humidity and temperatures of 34–36°C for 36–45 h. The final product – koji – is then used in the brewing process (Gauntner, 2003). About 50 different enzymes can be found in koji, the most important being the amylases and proteases. Depending on the cultivation conditions, the activities of the enzymes are different. Higher temperatures favour the activation of amylases while lower temperatures favour the development of protease activity (Hardwick et al., 1995).

The first step of fermentation is the production of a seed mash. The steamed rice is mixed with water, yeast and koji and fermented at approximately 20°C for 15 days. Due to the presence of lactic acid bacteria, the mash is acidified. This naturally acidified mash is mixed with water, steamed rice and koji at 8°C. After a few days, the mash is warmed slowly to about 15°C. During this phase of about 15 days, the composition of the micro-flora changes. Nitrate-reducing bacteria such as *Pseudomonas* are replaced by lactic acid bacteria such as *Leuconostoc mesenteroides* and *Lactobacillus sake*. The production of lactic acid and the low pH favour the growth of yeast, which further increases the mash temperature up to 20–23°C. The seed mash is then cooled and stored for 5–7 days before it is used for the main mash

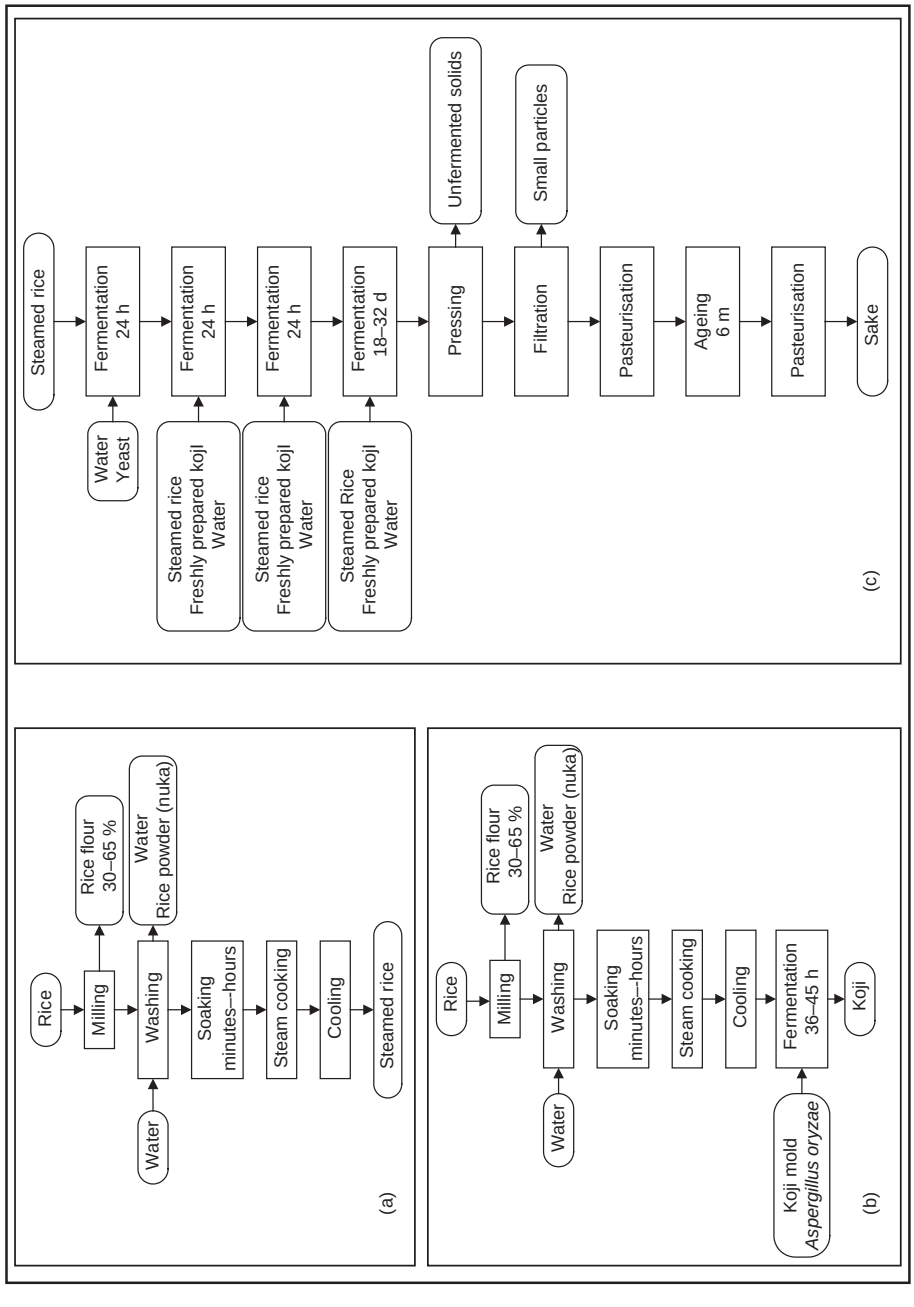

Fig. 10.3 Production processes of steamed rice (a), koji (b) and sake (c) (Gauntner, 2003).

preparation (Hardwick et al., 1995). Steamed rice, koji and water are added to the seed mash at 12°C. The quantity added is about twice that of the seed mash. After 2 days, this addition step is repeated at 10°C, and on the following day, there is a third addition at 7–8°C. For every batch, the quantity of mash is doubled (Gauntner, 2003).

After 15–25 days, when the fermentation is almost complete, ethanol of high concentration is added to increase the alcohol concentration up to 20%vol. This addition gives the sake a smooth taste and prevents the growth of microbial contaminants. Sometimes, steamed rice is also added to increase the final sugar content and to adjust the sweetness of the beverage. On the same day of alcohol addition, the sake is pressed (Hardwick et al., 1995). In former times, canvas bags were used to squeeze out the clear, fresh sake or simply to let it drip out. Nowadays, hydraulic presses are used. After a few days when more solids and particles are precipitated, the sake is usually charcoal filtrated to adjust flavour and colour. The enzymes are then inactivated and the number of colony forming units is decreased by pasteurisation at 63–65°C, which provides longer shelf life and stability of flavour and colour. The pasteurised sake is then aged for several months. After this period of maturation, the sake is diluted with water to about 15–16%vol alcohol content, bottled and pasteurised again. Non-pasteurised sake, which is called 'Namazake', keeps a certain freshness of flavour, but it has to be chilled and the shelf life is quite short (Gauntner, 2003).

10.3.3 Chicha

Chicha, which is originally made from maize in Andean regions, can also be brewed from other domestic grains such as amaranth and quinoa as well as roots such as yuca (chicha de yuca), or fruits such as chonta (chicha de chonta) or from a combination of these. For the Amazonian cultures, chicha is not only an alcoholic beverage, it is also a highly nutritious food, which is consumed in times of food shortage, and it is a typical drink which is used to welcome special guests (Sighartner, 2006).

In the traditional production of chicha, the source of enzymes, which are needed to hydrolyse the starches in the raw materials, is human saliva. Older women and children of Indian tribes masticate a coarsely ground meal, incorporating the amylases of their saliva. The masticated meal has a dough-like consistency and can be formed to small cakes, which are then dried and stored. When chicha is produced, these cakes are crumbled into water and heated. The human amylases complete their conversion of starch to fermentable sugars, and yeast and lactic acid fermentation is started by addition of a batch of a prior fermentation. Depending on the temperature, fermentation is complete after 6–10 days. Today however, this chewing process is no longer acceptable or practised, due to obvious hygiene issues. Instead of saliva, maize malt is used (Hardwick et al., 1995).

Traditionally, chicha de yuca is only produced by women, and everyone produces their own typical chicha. Usually, the root of *Yuca amarilla*, a yellow-coloured type of yuca root is used as raw material; white yuca can also be used. The peeled yuca roots are cut into smaller pieces and cooked. After 30 min, the cooking water is removed and the root pieces are mashed. Water and mashed sweet potatoes are then added. A starter culture taken from a former chicha production is used to start fermentation, which takes 1–5 days, but on average 3 days. The fibres are separated and the remaining beverage is diluted with water resulting in a low viscous beer, which is not absolutely free of any fibres (Sighartner, 2006).

Chicha de chonta is a palm which is originally grown in the Andes of Peru and Ecuador. The palm is covered with dark spines and its round fruits are yellow to red, closely resembling peaches. In contrast to chicha de yuca, chicha de chonta is a 'fruit' beer as the fruits, which are

rich in starch and fat (and not the roots), are used for brewing. The fruits are cooked, peeled, chewed and mixed with water. The mash is then left for spontaneous fermentation. The production process of chicha de yuca and chicha de chonta is shown in Fig. 10.4 (Maurizio, 1933).

10.4 GLUTEN-FREE BEERS COMPARABLE TO THE CONVENTIONAL TYPE

Innovation in food production and diversification in agriculture are common trends today. Also, allergies and food intolerances such as coeliac disease are an increasing problem. Thus, the production of beer free of any gluten-containing raw materials would widen the range of consumable food products for those affected people. Due to their chemical composition, maize, rice, sorghum, the pseudo-cereals amaranth, buckwheat and quinoa as well as roots and fruits, which are rich in starch, are suitable raw materials for gluten-free beer production.

When having a look at an online database, where various even very special food products from all over the world can be found, seven gluten-free beers are listed (http://www.productscan.com):

- 'COOP Free From – Gluten-Free Beer': is launched in a pack containing 4 × 0.33 L in Switzerland. The alcohol content is 4.5%vol. It is gluten-free but the raw materials used are not specified.
- 'Schnitzer Braeu German Premium Beer': is a new organic beer from SchnitzerBraeu GmbH & Co. KG, Germany. It contains 5%vol of alcohol. It is free of gluten and is brewed from millet.
- 'Green's Gluten-Free Discovery Beer, Explorer Stout, Pioneer Lager': These beers are produced by Green's in Leeds (UK). The Discovery Beer and the Explorer Stout contain 6%vol of alcohol and the Pioneer Lager 5%vol. They are claimed as gluten-free but the raw materials used are not specified.
- 'New Grist Beer – Traditional Ale': It is brewed by the Lakefront Brewery, Inc., Milwaukee, WI, USA. This beer is brewed from sorghum, hops, water, rice and 'gluten-free' yeast grown on molasses. It is available in the US in six packs of 0.33 L.
- 'Hambleton GFA Ale': This ale is brewed by Nick Stafford Hambleton Ales, UK. It is described as a full-flavoured beer with an alcohol content of 4.2%vol. It is available in 0.5 L bottles.

Several patents also exist, which describe possible methods for the production of gluten-free beer or beer-like beverages. In the patent of Fritsche (2006), a beer-like beverage is produced, which is said to be indistinguishable from conventionally brewed beer. It is produced by mixing ethanol, sucrose, caramel sugar syrup, saponins, isomerised hop extract, liquid malt aroma, hop essential oils and gluten-free dextrins. The pH is adjusted to 4.4 and the beverage is carbonated. This easy way of processing should help to avoid problems occurring when brewing gluten-free beers from quinoa, rice, buckwheat or millet. The brewing process of gluten-free beer made from buckwheat, sorghum and millet is described in the patents of MacCagnan et al. (1999a). This beer is obtained by a process which includes the saccharification of a mixture of gluten-free cereals, preferably buckwheat, sorghum or

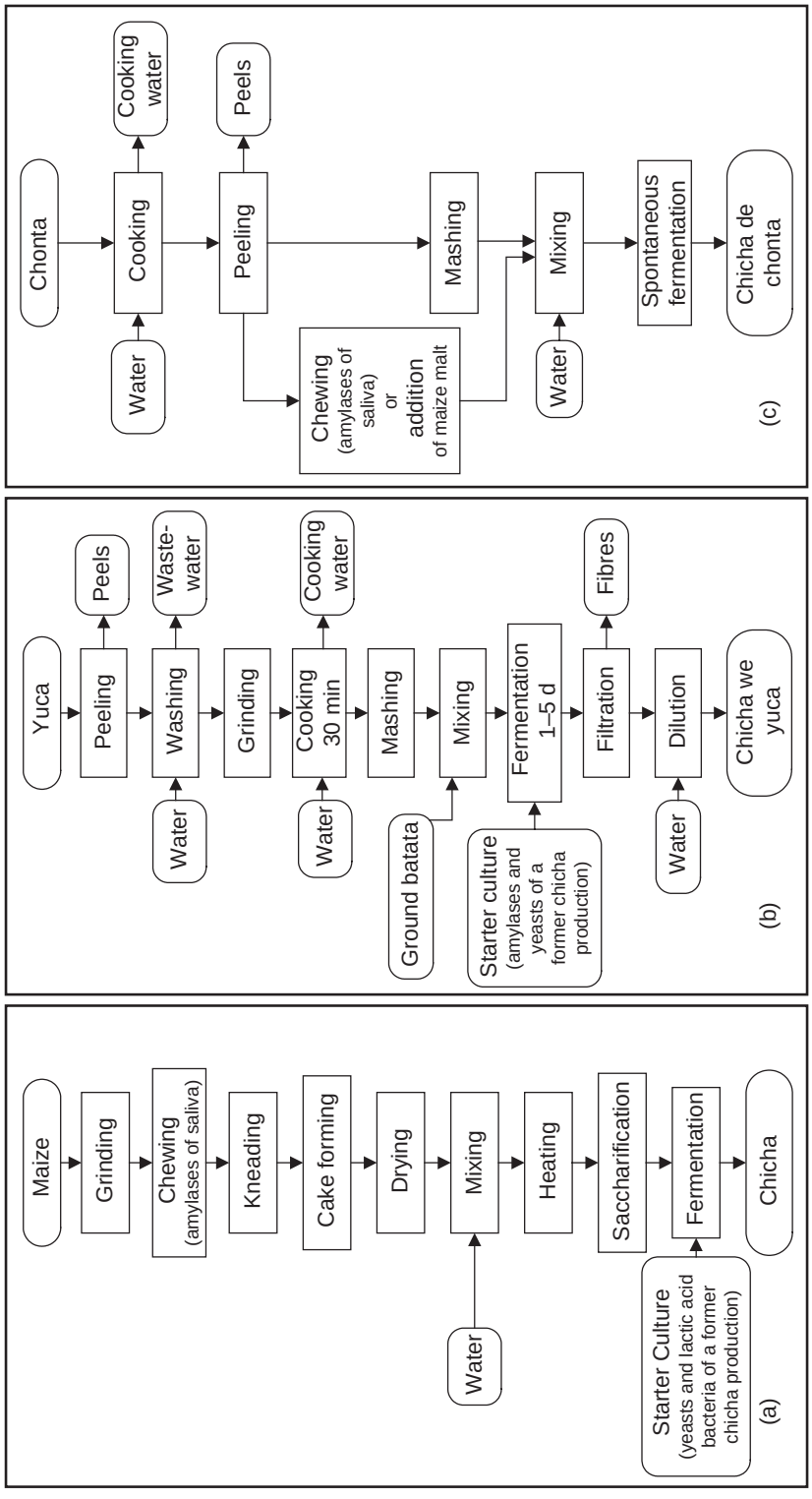

Fig. 10.4 Production of chicha (a), chicha de yuca (b) and chicha de chonta (c) (Sigharfner, 2006).

millet, and syrup obtained by the hydolysis of a gluten-free starch, preferably maize starch, as well as amylolytic enzymes. Best results are obtained when the mixture is composed of cereals and syrup in a ratio of 50:50. If the mixture of starting materials does not contain a sufficient amount of water-soluble proteins to give the beer the required organoleptic properties, the addition of proteolytic enzymes to the starting mixture is suggested. In another patent of MacCagnan et al. (1999b), rice malt is used instead of or in addition to the saccharification enzymes. The proposed ratio of buckwheat, rice malt and maize syrup is 20/60/20% (w/w) without addition of amylolytic enzymes. For another proposed ratio of 40/10/50% (w/w), the addition of saccharifying enzymes is suggested, as the amount of rice malt is relatively low. The sensory properties of this gluten-free beer are described as comparable to conventional beer.

10.4.1 Maize beer

Maize is mainly grown in areas of warm climate. It has a high lipid content (4–5%); its protein content is approximately 9–12%, and starch approximately 65–70%. Maize originates from South America, where it was used for brewing chicha. For the production of conventional type beer, only un-malted, degerminated maize, mainly maize grits or maize flakes, is used as adjunct. The gelatinisation temperature of maize starch is 60–70°C (Kunze, 2004).

In spite of the long tradition of chicha brewing from maize, only a few studies exist which relate to the production of conventional beer using maize malt. Zweytick et al. (2005) produced maize malt on a pilot scale to brew bottom-fermented beer using 100% maize. The seeds were washed with water at 10°C for 15 min. The following steeping step lasted 2 h and the water was changed every 30 min. The germination process took 7 days at 15°C and 95% relative humidity. The moisture content at the end of germination was about 40%. When the radicles of the germinated kernels had reached approximately double the length of the grain, germination was stopped by kilning at 80°C for 18 h.

Due to the gelatinisation temperature of the maize starch being higher than that of barley, the decoction method, using the two-mash process, was chosen. The ground malt was suspended in water at 50°C. The pH value of 5.5 was adjusted using lactic acid. After a mashing time of 30 min and a resting time of 20 min, the mash to be utilised was removed from the mash tun and heated to 85°C. This temperature was held for 5 min to caramelise some of the sugars, giving the beer a deeper flavour and colour, and to release more starch. The portion of wort drawn off for decoction at 50°C was calculated so that the next rest temperature of 65°C could be obtained by simply putting the boiled portion back into the mash tun. Before drawing off for decoction, the mash was allowed to settle. The more dense part of the mash was removed for decoction, as the enzymes were mostly dissolved in the liquid phase. After a resting time of 20 min, this procedure was repeated for a second time to reach 71°C. The whole mash was then heated up to 76°C and filtered after 20 min. The obtained wort was boiled for 90 min – three quarters of the hop pellets were added after 15 min, one quarter after 75 min. After cooling the wort down to 12°C, yeast was added and the beer was left for fermentation. The primary fermentation was carried out at temperatures between 12°C and 6°C in the straining vat; a second fermentation was made at 4°C in kegs, which allowed the retention of carbon dioxide and flavour. The resulting beer was clear and had a light yellow colour. The foam stability was good and the taste was reported to be comparable to that of conventional beer (Zweytick et al., 2005).

10.4.2 Sorghum beer

Sorghum is a drought resistant annual grass which is mainly grown in developing countries. In Europe and in the US, it is mostly used as fodder plant (Ternes, 1994). The protein content of sorghum is approximately 6.9–12.8%, the fat content approximately 3.0–4.1% and the starch content approximately 72.3–78.4% (Elobeid, 2003).

The two most important differences between sorghum and barley are the significantly higher gelatinisation temperature of the sorghum starch and the lower level of β-amylase activity in sorghum malt. The consequence of this difference is that in current industrial brewing, sorghum is almost exclusively used as a starchy adjunct. Sorghum grain or sorghum malt is first cooked to gelatinise the starch, which is then saccharified by the addition of commercial enzymes. The gelatinisation temperatures of sorghum starch for sorghums grown in Africa are in the range 67–73°C, and 71–81°C for sorghums grown in India. These are much higher than that of barley starch (51–60°C). Recently, a South African sorghum variety, which was selected for its good malting and opaque beer brewing properties, was found to have a very low gelatinisation temperature of 59.4°C (Taylor et al., 2006).

The activity of β-amylases in sorghum reaches only 20–25% of the enzyme activity that is found in barley malt. The optimisation of the steeping regime, especially air rests, water temperatures and steep-out moisture, could significantly enhance β-amylase activity and thus, malt quality. Steeping the sorghum grain in dilute alkali (up to 0.3% $Ca(OH)_2$, KOH or NaOH) could significantly enhance sorghum malt diastatic activity (Taylor et al., 2006).

Another important aspect regarding the malting properties of sorghum and maize is the fundamentally different composition of their cell walls in comparison to barley. In the cell walls of sorghum and maize, the water-insoluble glucuronoarabinoxylans (GAX), which are much more complex than in barley, are predominant; in barley, the β-glucans predominate. It was found that β-glucans in sorghum endosperm cell walls were only poorly degraded during malting and it was suggested that the activity of β-glucan endohydrolase is very low. The resistance of the cell walls to enzymatic attack during germination inhibits the access of amylolytic enzymes the starch inside the cells during the brewing process (Taylor et al., 2006).

Investigations on mashing conditions in relation to sorghum starch hydrolysis and production of fermentable sugars showed that a calcium ion concentration of 200 ppm resulted in highest reducing sugar production and wort yield. Malt α-amylase activity was found to be directly related to calcium ion concentration and it was concluded that the effects could be derived from preventing thermal inactivation of the α-amylase (Taylor et al., 2006).

The effects of various mashing methods have also been investigated by Taylor et al. (2006). The highest yield of extract could be obtained applying the decantation and the decoction process. For the decoction method, portions of the mash were removed and boiled to gelatinise the starch, and then added back to the mash. When applying the decantation process, an enzymatic extract was produced and only the remaining solids were cooked for gelatinising the starch. After cooling the mash, the extract is added back to hydrolyse the starch. Due to the complexity of these methods, in practice the sorghum malt amylase activity is supplemented with industrial enzymes.

Zweytick et al. (2005) applied an adapted malting and mashing trial (as described for the production of maize beer) to produce beer from 100% sorghum malt. In comparison with the malting of maize, a germination time of 2 days was sufficient and the kilning time was 24 h. The resulting sorghum beer was opaque and had a pale yellowish colour. The foam stability was excellent and the taste was good.

10.4.3 Millet beer

Pearl millet and finger millet, like sorghum, have highly resistant endosperm cell walls, high gelatinisation temperatures (pearl millet: 61–68°C; finger millet: 65–69°C) and similar levels of diastatic power, α-amylase, β-amylase and malting loss (Taylor et al., 2006).

Only a small number of studies are available regarding the brewing process when using millet. In one study, it was found that higher extract yields could be obtained using pearl millet malt with an infusion mashing regime, from 45–70°C, than with sorghum malt. With pearl millet malt, the decantation process resulted in higher extract yields than decoction or infusion mashing (Taylor et al., 2006).

10.4.4 Amaranth beer

Amaranth is a foxtail plant which was a basic food in pre-Columbian times. Today, it is an underutilised crop, which is mainly grown in the Andes region in South America. It is a pseudo-cereal with very small seeds; the thousand-kernel weight of approximately 0.3–1 g is very low. The protein content is approximately 16% and its biological value is high. The fat content is 7% and its starch content is approximately 62%. The content of amylase is quite low, the starch granules have a diameter of 1 μm and the temperature of gelatinisation is 72°C (Ternes, 1994).

Zweytick et al. (2005) varied the process described in maize beer production to produce amaranth malt and brew 100% amaranth beer. Due to the small size of the seeds, a steeping time of 1 h was sufficient. Three days of germination were sufficient and the applied kilning time at 80°C was 24 h. The resulting amaranth beer was slightly opaque and had a yellow colour. The foam stability was not good and the taste was deemed to be too bitter.

10.4.5 Quinoa beer

As well as amaranth, quinoa was also a basic food in Latin America in pre-Columbian times. Quinoa is a goosefoot plant, which is rich in saponins. Like amaranth, it is mainly grown in South America and it is a pseudo-cereal. The protein content is about 8–13%, its biological value is also high. The fat content is 4–6% and the starch content is approximately 60–75%. The starch granules have a diameter of 1–2.5 μm and the temperature of gelatinisation is 57–64°C (Ternes, 1994).

Quinoa malt and quinoa beer were produced by Zweytick et al. (2005) using a similar process used for amaranth malt and amaranth beer. Like amaranth beer, quinoa beer was slightly opaque and had a yellow colour. The foam stability was quite good and the taste was very acceptable.

10.4.6 Buckwheat beer

Buckwheat does not belong to the grass and cereal family; it is a modest knotgrass, traditionally grown in Asia and in Central and Eastern Europe. Buckwheat seeds possess a starchy endosperm, and they are also referred to as pseudo-cereals. Buckwheat contains protein of high biological value, high levels of fibre, fagopyritol, rutin, unsaturated fatty acids and minerals. As it is free from gluten, it can be consumed by coeliac patients (Wijngaard and Arendt, 2006).

Wijngaard and Arendt (2006) found the optimum steeping time for the malting procedure of buckwheat to lie in a range of 7–13 h. The optimum germination temperature was 15°C and the germination time 4–5 days. The final kilning process was carried out at 45°C for 5 h in a first step and at 50°C for 17 h in a second step. At the end of germination, the moisture content was approximately 41%. With regard to the mashing profile, the grist size (best: as small as possible), the grist:liquor ratio (best: 1:4), the mashing-in temperature (best from 35 to 45°C), α-amylase activity (best: at 65°C) and mashing-off temperature (best: 78°C) were optimised. Although the gelatinisation temperature of buckwheat was found to be 67°C, the optimal saccharification temperature was 65°C. A single decoction process resulted in a higher degree of starch gelatinisation, but overall enzyme activity was reduced. An infusion method was used as the optimal mashing procedure – mashing-in temperature: 15 min at 35°C or 15 min at 45°C; 40 min at 65°C; 30 min at 72°; and mashing-off temperature: 10 min at 78°C.

Buckwheat malt was produced by Zweytick et al. (2005), applying 2 h of steeping, 4 days of germination and 26 h of kilning at 80°C. Beer produced from this malt was opaque and had a brown colour. The foam stability was poor and the taste too bitter. Wijngaard and Arendt (2006) take into consideration that it is probably not possible to produce a traditional lager beer from 100% buckwheat without the addition of exogenous enzymes.

Fig. 10.5 shows the results of the sensory analysis of five beers produced by Zweytick et al. (2005) from 100% of maize, sorghum, amaranth, quinoa and buckwheat.

10.5 CONCLUSIONS

As described in patents and publications, it is possible to produce beers from gluten-free raw materials such as sorghum, millet, maize, as well as the pseudo-cereals, amaranth, quinoa

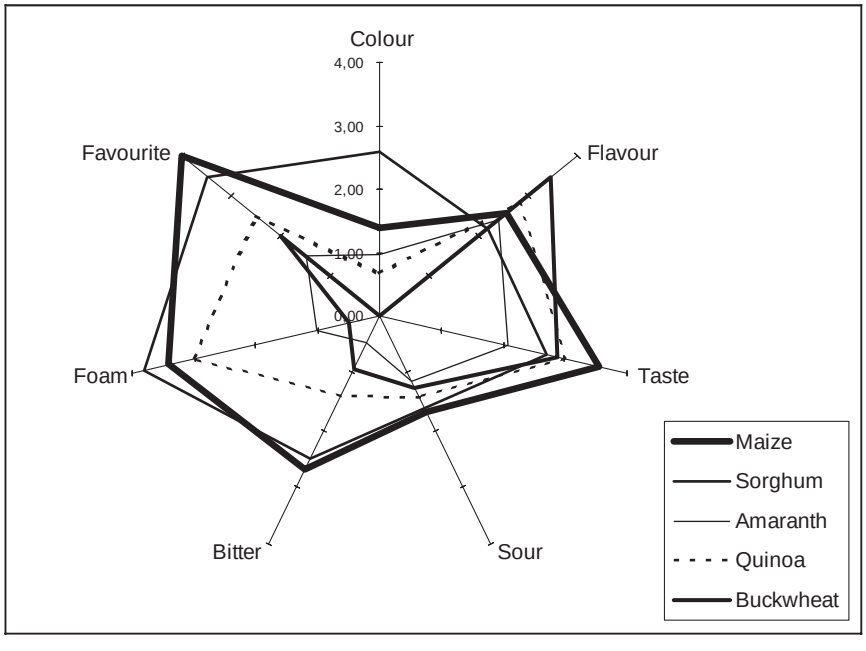

Fig. 10.5 Sensory analysis of gluten-free beers (Zweytick et al., 2005).

and buckwheat. Due to the composition and different physical and chemical properties of the raw materials used, beers differ in foam stability, colour and flavour. As the gelatinisation temperatures of the most gluten-free raw materials are higher in comparison to barley, the decoction or decantation process should be applied. Not only sorghum and maize appear to result in gluten-free beers with the most acceptable flavour, but also other raw materials described in this chapter have great potential for malting and brewing.

For further improvement and optimisation of sensory and technological properties, combinations of different gluten-free raw materials in beer production may be useful. This could result in beers, which are closer to conventionally brewed beers with respect to flavour and appearance.

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11 Functionality of Starches and Hydrocolloids in Gluten-Free Foods

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11.1 INTRODUCTION

Some individuals are intolerant to the storage proteins called prolamins found in wheat, rye and barley and their hybrid varieties. This leads to a health condition known as celiac disease or gluten sensitive enteropathy. Celiac disease affects about 0.2–1.0% of the world population and the number is steadily increasing worldwide (Gallagher et al., 2004a; Mendoza, 2005). In Canada, approximately 1 in 133 persons are affected by celiac disease (CCA, 2007). The world population is currently about 6.7 billion people and according to the UN estimation, it will reach 7.7 billion in 2020. This means that approximately 13.4–67.0 million persons are now suffering from celiac disease. Anticipated increases in those affected highlights a need to establish strategies to find remedies for them. These strategies are likely based on one of three fundamentals: consumption of a gluten-free diet, use of peptidase supplement therapy (able to break down the toxic prolamins) or development of cereal varieties free from the toxic amino acid sequences in the proteins that trigger celiac disease. The last strategy seems to be complicated due to the complexity of the multi-genes associated with the biosynthesis of storage proteins in wheat, rye and barley. The other two approaches present challenges as well, but they seem to be more effective since some accomplishments have already been made in these areas. For instance, a bacterial prolyl endopeptidase was found to cause degradation and loss of the antigenicity of the toxic peptide in celiac disease (Schuppan and Shan, 2002; Shan et al., 2002; Stepniak et al., 2006). This would open the possibility for the use of these enzymes for detoxification of toxic peptides and developing therapeutic strategies to treat celiac disease.

At present, the only effective remedy for celiac patients is a strict lifelong adherence to a gluten-free diet (Mendoza, 2005). This requires exploring alternative food ingredients that can replace gluten in cereal-based food products yet produce high-quality foods. The replacement of gluten in a cereal-based food system would pose a major technological challenge due to its structure-forming capacity. Gluten substitutes must fulfill this function, that is, they need to form cohesive elastic dough that can be baked into a food product with pleasant taste and acceptable texture. Starches and hydrocolloids are common food ingredients that impart texture and appearance properties and they represent key ingredients in the development of gluten-free food products. This chapter describes the functionality of starches and hydrocolloids in gluten-free food systems. It also points out their roles in two common food categories, bakery and extruded products. These food products are staples and popular around the world and they are based on different formulations and processing technologies. Several food examples under each category are presented and discussed. Sources and properties of starches and hydrocolloids are also highlighted.

11.2 SOURCES AND PROPERTIES OF STARCHES

Starchy foods are the world's most abundant staples and include cereals, legumes, potatoes and tubers. They provide about 70–80% of the calories consumed by human worldwide (Thomas and Atwell, 1999). Other forms of starches such as enzyme resistant and modified starches are also found in food applications. The latter starches are not digested and reach the human large intestine and are thus considered as a class of dietary fiber providing physiological health benefits. In addition to the nutritional and health properties, starches are widely used as food ingredients to impart appearance and textural properties. These properties include gelling, texturizing, thickening, adhesion, moisture retention, stabilizing and anti-staling. In a gluten-free food system, starch may be incorporated into the food formula to contribute one or more of these functionalities depending on the type of food product and other ingredients in the formulation.

Commercial starches are obtained from different sources including cereals such as corn and wheat, tubers such as potato and roots such as cassava (tapioca starch). Of the world production of starch (~60 MT), about 83% comes from corn, 7% from wheat, 6% from potato and 4% from cassava. Smaller amounts of rice, sago, arrowroot and pea starches are also commercially available. In addition, novel starches and starchy flours such as canary seed starch (Abdel-Aal et al., 1997; Abdel-Aal and Hucl, 2005), amaranth starch (Marcone, 2005) and quinoa starch (Lopez-Garcia, 2007) (Fig. 11.1) have also been developed for food and non-food uses. These starches have unique properties and would introduce novel functionality in food applications. Most of the cornstarch in the US is converted into sweeteners and ethanol. Approximately one-fifth is used as starch mainly in the paper industry (~60%) and only 15% goes to the food use.

Starch is deposited in the plastid of higher plants in the form of granules. These granules vary in their size and shape depending on their botanical sources. The shape and size of different starch granules obtained from eight sources are illustrated in Fig. 11.1. These starches possess diverse range of pasting, gelling, thermal and textural characteristics depending on their compositional and structural properties (Table 11.1). Starch is composed of two types of glucose polymer, the essentially linear amylose and the highly branched amylopectin, in addition to other minor components such as proteins and lipids. Both amylose and amylopectin polymers are α-glucans that differ in chain configuration and molecular weight. Amylose is relatively intermediate polymer having molecular weights varying from 10^5 to 10^6 Da compared to amylopectin, which is a much larger polymer with molecular weights ranging from 10^7 to 10^8 Da. The structural differences between these two polymers contribute to substantial differences in starch properties and functionality, in particular pasting, gelation and retrogradation properties. It is worth mentioning that based on the content of amylopectin or amylose, three types of starches are now available. These include normal starch having amylose content of about 15–30%, waxy starch almost consisting of amylopectin (i.e. 0 to $<$5% amylose) and high-amylose starch having up to 70% amylose such as high-amylose cornstarch.

Pasting and gelling properties of starches are important in many food applications since texture and eating quality rely on these properties. When starch is heated or cooked in enough water, granules swell due to water absorption, then the granules structure becomes disrupted and amylose leaches out resulting in the formation of viscous slurries or paste depending on concentration. This process is called gelatinization which takes place in thermal processing of starchy foods. The behavior and pasting properties of starch slurries during heating and cooling (e.g. pasting temperature, peak viscosity, trough viscosity, final viscosity, breakdown

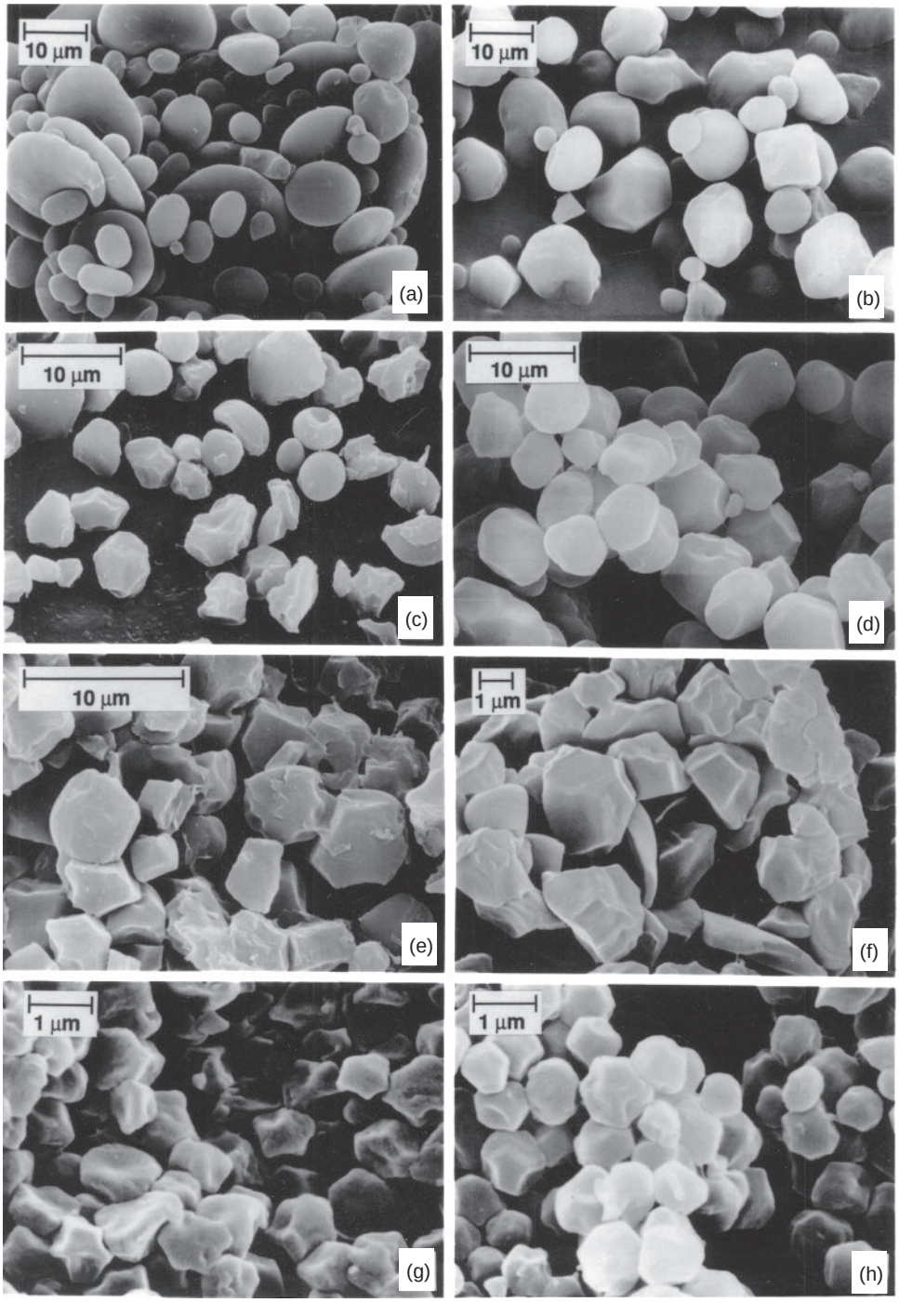

Fig. 11.1 SEM photomicrographs illustrating shape and size of different starches: (a) wheat, (b) corn, (c) oat, (d) buckwheat, (e) rice, (f) canary seed, (g) quinoa and (h) amaranth.

Table 11.1 Properties of common starches and their pastes.

Property	Corn	Waxy corn	Wheat	Waxy wheat	Rice	Potato	Tapioca
Shape	Round, polygonal	Round, polygonal	Round, lenticular	Round, lenticular	Polygonal, spherical compound granules	Oval, spherical	Oval, truncated
Diameter (μm)	3–25	3–25	2–35	2–35	1–3	5–100	4–35
Amylose (%)	21.0	2.9	26.9	3.2	16.4	22.0	17.0
Crystallinity (%)	27	28	20	27	25	24	24
Peak viscosity (BU)	500	1200	210	1100	315	770	620
DSC transition temperatures (T_0–T_p–T_c, °C)	57–70–84	60–72–84	55–63–73	56–66–80	64–68–74	60–66–76	63–71–81
Paste viscosity	Medium	Medium high	Medium low	Medium high	Medium high	Very high	High
Paste texture	Short	Long	Short	–	–	Long	Long
Paste clarity	Low	Medium high	Low	–	–	Very high	High
Retrogradation rate	High	Very low	High	Very low	Medium low	Medium low	Low

Source: Collated from Abdel-Aal et al. (2002); Chaisawang and Suphantharika (2006); and Thomas and Atwell (1999).
BU = Brabender unit; T_0, T_p and T_c = onset temperature, peak temperature and completion temperature, respectively.

and setback) are measured by Brabender viscoamylograph or rapid visco analyzer (RVA) viscometers, while gelatinization transition temperatures (onset, peak and completion temperatures) and enthalpy of gelatinization are determined by differential scanning calorimeter (DSC) (Table 11.1). A study on pasting properties of wheat, barley, rye, sorghum and millet flours and their blends has shown that RVA could be used to assist in formulating starchy flour blends or starch mixtures exhibiting certain levels of paste viscosity as well as in evaluating their heat and shear stability (Ragaee and Abdel-Aal, 2006). Figure 11.2 shows the pasting properties of normal and waxy corn and wheat starches measured by viscoamylograph. Waxy starches swell rapidly at low temperature to peak viscosities which are much higher than normal starches, but then the waxy starch pastes disintegrated quickly and form low gel consistency due to the absence of amylose (Abdel-Aal et al., 2002). In other words, waxy starches show greater degrees of pasting and shear thinning compared with normal starches. In general, pasting properties of starches depend on many intrinsic and extrinsic factors such as source and type of starch, amylose content, amylose/amylopectin ratio, molecular weight, percentage of starch damage, moisture content, shear rate, temperature, time and the presence of other substances such as sugar.

Potato and tapioca starches have weak intermolecular bonding and gelatinize easily to produce high-viscosity pastes that thin rapidly with moderate shear. Potato starches form clear and viscous pastes which are used in products such as extruded cereals and dry soup and cake (Thomas and Atwell, 1999). Corn, wheat and rice starches form opaque, gelled pastes that have a slight cereal flavor, while tapioca starch produces clear, cohesive pastes that gel slowly over time. Cereal starches are usually considered gelling materials, and in baking they significantly contribute to the crumb texture of baked products through gelatinization and gel forming properties. In addition, native starches can also be modified by physical, chemical

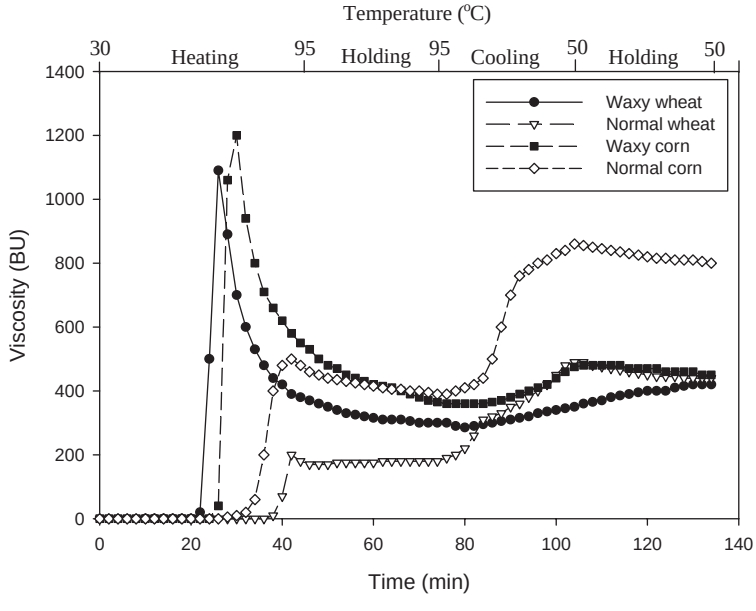

Fig. 11.2 Viscoamylograph pasting curves of normal and waxy starches isolated from wheat and corn. (*Source*: Adapted from Abdel-Aal et al. (2002); with permission of AACC International.)

or genetic means to withstand heat, acids and shear during processing or to enhance their nutritional and health properties.

The rheological properties of starch gels are also important in handling and forming starch-based doughs as well as in determining quality of the end products. The rheological properties of corn, potato and pea starch gels (Ring, 1985) and wheat starch gel (Eliasson and Bohlin, 1982) have been investigated. Corn, potato and pea starches start to form elastic gels at concentrations above 6% w/w, whereas below this concentration only weak viscoelastic pastes with no recoverable deformation on the application of static stress were formed. The rigidity modulus of the gels for a given concentration is in the order pea starch > cornstarch > potato starch. Above 6% concentration, there is a linear relationship between the rigidity modulus of the gel and concentration. Amylose was found to be essential for forming rigid gels by increasing elasticity of the continuous phase. The relaxation modulus and half relaxation time of concentrated wheat starch gels containing water at 30, 50, 60 or 80% (w/w) were found to increase during gelatinization for all gels with enough water. The increase in relaxation modulus could be explained by leaking of amylose that would increase elasticity of the continuous phase and also enhance the adhesion between dispersed and continuous phases.

Another important attribute of starch in food processing is the ability of amylose to form a complex with fats and food emulsifiers such as mono- and diacylglycerols which can shift starch gelatinization temperatures and alter textural and viscosity profiles of the resultant paste and limit retrogradation. This property is used to maintain freshness and to delay staling of baked products. Starches differ in their retrogradation rates in the order of wheat, common corn > rice, tapioca ≫ waxy corn (Jacobson et al., 1997). Waxy starches are commonly known to have lower retrogradation rates and accordingly they can be used in blending with normal starches to delay staling of baked products.

11.3 FUNCTIONALITY OF STARCHES IN FOOD

Bakery products, particularly bread, are staple foods worldwide. They include a wide variety of products such as bread, pizza, cookie, biscuit, cake, muffin, cracker, brownie, pretzel, doughnut, and so on, that have different shape, appearance, texture, taste and flavor. The role of starch in these baked goods varies depending on product type and usually is associated with the native starch in wheat flour, the basic ingredient in bakery products. Wheat flour is unique among cereals in that it is able to form cohesive viscoelastic dough after mixing with water in which the storage proteins are responsible for the formation of this membrane network structure called gluten. Gluten proteins comprise of gliadin and glutenin with various ratios being approximately 1:1.1 in common bread wheat (Abdel-Aal et al., 1995). Additionally, gluten proteins exhibit diverse molecular structures and functions and are classified into several subgroups depending on their molecular structure and electrophoresis properties. For instance, wheat flour contains about 50 different protein subunits: 4–6 high molecular weight glutenins, up to 15 low molecular weight glutenins, and 30 or more α-, β-, γ- and ω-gliadins (Shukla, 2001). This complex structure of gliadin and glutenin subunits determines its ability to form strong or weak gluten and hence its suitability for a specific end use. The formation of dough is a complex process but it could be visualized via three major events: hydration of proteins and other polymers (starch and pentosans) in the flour, alignment and reorientation of glutenin chains through physical and chemical disaggregation and finally interactions of glutenin chains by chemical bonding to form a membrane network with

viscoelasticity and gas retaining properties. These properties make wheat a unique cereal for making bakery products. This process, the formation of viscoelastic dough from wheat flour, may demonstrate that the formulation of gluten-free products especially bread would present a big technological challenge since gluten must be removed and replaced with a non-gluten food ingredient.

Native starch in wheat flour plays a less significant role in the formation of dough and most likely starch granules along with other dough ingredients are enclosed in the gluten network. However, starch plays a substantial role during baking in the oven. During baking in the oven, the crumb structure is set as a result of starch gelatinization which is found to influence the expansion volume of the dough and eventually loaf volume and crumb texture. Therefore, gelatinization properties such as gelatinization transition temperatures and pasting viscosities would be crucial in determining texture and appearance of gluten-free baked products. The role of starch in dough rising during baking was investigated using artificial flours made from dry vital wheat gluten and wheat starch, potato starch or topical starch (Kusunose et al., 1999). The three starches were selected due to their diverse gelatinization properties. Breads made from gluten and tapioca starch had the largest loaf volume and the most extensive postbaking shrinkage, while those baked from potato starch and gluten possessed the lowest loaf volume and the least shrinkage. The study indicated that starch granules should not gelatinize early in the baking cycle as potato starch does but should gelatinize later in the baking cycle as it does with wheat starch. This is to prevent early setting of the dough which inhibits expansion during baking. In addition, starch granules should not disrupt and fuse together during gelatinization as tapioca starch does and results in the formation of impermeable gas membrane. The starch granules should gelatinize individually and retain their integrity after gelatinization to provide cracks in the gas cell membranes after the dough is set in the oven like the behavior of wheat starch granules during baking step. Tapioca starch produces an impermeable membrane; the starch granules fuse together forming a gas discontinuous system, which is extensible for a relatively long time. This leads to a larger loaf that collapses during cooling after baking.

Starch granule size was also found to influence baking performance of wheat (Soulaka and Morrison, 1985; Sahlström et al., 1998). Wheat flour contains bimodal starch granules: large A-type starch granules (10–35 μm in diameter, which contribute more than 70% of the total weight of starch and about 3% of the total granule number) and small B-type starch granules (1–10 μm in diameter, which account for more than 90% of the total granule number but less than 30% of the total weight of starch). These granules were found to exhibit different amylose content and gelatinization properties (Peng et al., 1999). The A-type starch granules at the ratio of 75:25 (A-type:B-type granules by weight) and proportion of B-type granules as well as gelatinization temperature of the total starch or their A-type granules had marked effects on loaf specific volume (Soulaka and Morrison, 1985). The optimum proportion of B-type starch granules was 25–35% by weight, but their gelatinization temperature had no effect on loaf specific volume.

Starches from various bread wheat exhibiting good, intermediate and poor baking quality, durum wheat and pastry wheat, in addition to A-type starch granules, B-type starch granules and cornstarch were evaluated in the baking process (D'Appolonia and Gilles, 1971). These starches were blended with gluten (80:20 w/w) with and without the addition of wheat water-solubles and baked into breads. The percentage of wheat water-solubles was 3.2% of the starch–gluten blend. The baking absorption for the starch–gluten and starch–gluten–water-soluble blends containing bread wheat starches were constant (about 74%) except for blends containing small starch granules (84%), durum wheat starches (76–80%) and cornstarch

(88%). The loaf volume of starch–gluten breads containing wheat starches ranged from 137 to 161 cm^3 versus 128 cm^3 for cornstarch–gluten bread. In this study, a 25 g loaf was baked. The loaf volume of the starch–gluten loaves containing the small-granule starch fraction and large-granule starch fraction were at the lower end being 140 and 138 cm^3, respectively. The latter loaf volumes were slightly lower than that of the blends containing poor baking quality wheat starches. The addition of water-soluble fraction to the starch–gluten loaves resulted in an increase in loaf volume in all cases suggesting different types and degree of interaction between certain water-soluble components and the various starches. These interactions between starch and water-soluble components would be taken into consideration when preparing gluten-free formulations. The water-soluble fraction contains about 23% protein. Other water-soluble polymers in wheat flours include pentosans, which have marked effects on rheological properties of dough and gluten (Michniewicz et al., 1991) and are surface active substances (Izydorczyk et al., 1991), which helps stabilize protein foams. In addition to proteins and pentosans, polar lipids in wheat flour may also take part in the air–water interface in doughs.

Modified starches such as partially cross-linked and pregelatinized starches could play an important role in gluten-free bakery formulations due to their ability to form highly viscous slurries and pastes. For example, gelatinized starches would form a matrix in which gas and air bubbles are entrapped, which is a major structural component in bakery products such as bread and cake. In addition, starches are added to batter formulations as tenderizers in order to soften crumb texture. Combinations of native and modified starches can be used to enhance batter consistency during mixing and to control starch gelatinization during the baking process. Cross-linked starches can help provide shear resistance and create viscous batter. In general, starches can have several functionalities in bakery products such as thickeners, tenderizers, gelling ingredients or fat mimics as well as in delaying staling and enhancing water holding properties.

Due to the lack of a gluten network in gluten-free formulations, the gluten-free dough is more fluid than wheat flour dough and is closer to batters in viscosity (Cauvain, 1998). This batter-type dough has to be handled in a way similar to cake batters rather than typical bread doughs (Schober et al., 2005). Starches that form a rigid gel can be used to improve consistency of gluten-free batters. Gas holding capacity would also be more difficult in gluten-free batters and therefore addition of gelling-forming starches such as pregelatinized starches and air cell stabilizers such as gums have been suggested as a means to provide gas occlusion and stabilizing mechanisms. Wheat starch as well as various flours and starches obtained from cereals that do not trigger celiac disease have been used as the basic ingredient in gluten-free bread formulae. For instance, rice starch or flour has been used in making gluten-free bread because it lacks gluten and contains low levels of sodium and high amounts of easily digested carbohydrates (Ylimaki et al., 1988, 1991; Gallagher et al., 2002). Rice flour also has bland taste and hypoallergenic properties (Gujral et al., 2003). However, rice flour or starch contains low amount of proteins that lack viscoelastic properties and addition of other polymeric substances that would help form consistent dough are required. Corn and tapioca starches are also functional ingredients in gluten-free bread products but they can cause some difficulties by imparting unusual taste to bread and require technological improvements (Sánchez et al., 1996). Gums have been combined with starches in gluten-free formulations to help form cohesive dough system and to stabilize air cells.

Starches provide several functionalities in extruded food products such as thickening, gelling, expansion, and so on. Extruded products include a diverse array of foods that could be categorized into three main groups: pasta, ready-to-eat cereals and snack foods. Starches play an important role in each of these products. In the development of a particular

gluten-free extruded product, formulations and extrusion process variables (temperature, pressure and shear) are key factors in determining extrudability and quality of the final products. In general, the amylose fraction is responsible for the strength of starch gels and crispness of the end product. Increasing amylose in the formulation makes starch more resistant to shear degradation during extrusion and can help to improve cutting and shaping during drying or in the final processing steps such as baking and frying (Thomas and Atwell, 1999). The branched amylopectin forms a network-like structure within the food matrix during gelatinization, which increases the product viscosity and forms a structural framework for the expansion process. The setting of the framework aids in providing crispy final products. On the other hand, amylopectin is susceptible to shear degradation during extrusion resulting in the formation of dextrins and short-chain polymers that can cause an increase in stickiness and difficulty during cutting after extrusion (Thomas and Atwell, 1999). Thus, source and type of starch and its content of amylose and amylopectin determine quality and characteristics of extruded products. Waxy starches (almost 100% amyopection) contribute to an increase in the expansion of the extruded product, while high-amylose starches would produce crispy products. Potato starches with high swelling capacity forms dough that is difficult to roll into thin sheets of uniform thickness.

During the extrusion process, starch granules undergo several changes by the action of heat, shear, pressure and moisture. These changes include gelatinization, melting of crystalline starch, chain mobilization, chain interaction, starch expansion and starch degradation (Camire et al., 1990). Such changes are dependent on extrusion process variables (feeding rate, barrel temperature, dwelling time, pressure, shear rate), moisture content of starches or formulations, and starch composition and structure. The presence of other polymeric substances such as protein, fiber or gums also affects characteristics and quality of the extruded product through interaction with starch. These process variables and starch-based formulations are carefully chosen and controlled to produce extruded products having the desired properties, that is, degree of expansion, porous structure, crispy texture, and so on. For more details about physical and chemical changes in starch, protein, dietary fiber and other nutrients, the reader may see the review article by Camire and colleagues (1990).

11.4 SOURCES, STRUCTURE AND FUNCTIONALITY OF HYDROCOLLOIDS

Hydrocolloids are substances that form a gel with water. They include a diverse range of biopolymers (e.g. polysaccharides and proteins) that are derived from natural sources such as plant, animal, seaweed or microbial origin. They are used in a wide range of food applications to impart texture and appearance as well as to improve product stability. Based on their functionality in a food system, hydrocolloids and gums could be classified into three main categories: gelling agents, thickeners and emulsifiers. Data in Table 11.2 show a range of food hydrocolloids and their functionalities.

Structural properties of hydrocolloids (e.g. molecular weight, molecular shape and configuration, chain length, degree of substitution, etc.) and their influences by processing variables (e.g. heat, pH and shearing) determine their functionality. The structure–functional relationships of hydrocolloids and their roles in food processing have been extensively investigated. The effects of guar gums with different molecular weights were found to govern gelatinization performance (Funami et al., 2005a) and retrogradation behavior (Funami et al., 2005b)

Table 11.2 Common hydrocolloids and their functionalities in food products.

Source	Hydrocolloid	Functionality
Plant	Pectin	Gelling, thickening
	β-Glucan	Gelling
	Gum arabic	Thickening
	Guar gum	Thickening
	Locust bean gum	Thickening
	Arabinoxylan	Gelling
Seaweed	Agar	Gelling
	Alginate	Gelling, thickening
	Carrageenan	Gelling
Animal	Milk proteins	Gelling, emulsification
	Egg proteins	Gelling, emulsification
	Gelatin	Gelling, emulsification
Microbial	Xanthan gum	Thickening

Source: Collated from Ward and Andon (2002) and Norton and Foster (2002).

of cornstarch. The weight-average molecular weight (M_w) of the guar gum substances ranged from 4.7×10^5 to 34.6×10^5 g/mol. Guars with M_w values higher than 12.2×10^5 g/mol shifted the onset of viscosity increase for the starch/guar system to lower temperatures and increased its peak viscosity upon heating at a relatively low starch concentration (e.g. 5% w/v). Dynamic mechanical loss tangent for starch with 26% amylose (5% w/v) was increased by the addition of 0.5% guar after storage at 4°C for 24 h, which indicates the reduction of gelled fraction in the starch/gum system, leading to the retardation of short-term retrogradation of starch. The higher the molecular weight of guar, the lower the amount of amylose leached, but this effect of guar became less dependent on its molecular weight at above 15.0×10^5 g/mol.

In addition to the commonly used hydrocolloids, a number of cereal grains contain gums at relatively high levels such as pentosans in rye and β-glucans in oat and barley. These cereal gums can be isolated and used in gluten-free formulations due to their ability to form viscous slurries and their physiological and health benefits. But, like wheat starch, pentosans and β-glucans should meet Codex standards for gluten-free foods. In fact, both pentosan (Casier et al., 1977) and β-glucan (Lazaridou et al., 2007) biopolymers have been used in the development of gluten-free products. Pentosans are found in rye ranging from 6% to 12% of which about 1.5–3.0% are water extractable. Most of the water extractable pentosans are found in the starchy endosperm. Water extractable pentosans play an important role in baking performance of rye due to its high water holding capacity and its ability to form highly viscous solutions (Hoseney, 1984). In other words, water extractable pentosans contribute to the water holding capacity and viscosity of dough and likely produce a film inside the vacuoles of the fermenting dough that contributes to gas retention and loaf volume. The formation of pentosan gel is performed by a special gelation process called oxidative gelation which requires oxidants such as a combination of hydrogen peroxide and the enzyme peroxidase that is native in rye flours. Ferulic acid (hydroxycinnamic acid) that is naturally esterified to the water-soluble arabinoxylans in rye is also involved in the gelation process by cross-linking of arabinoxylan chains. In addition to their role in breadmaking, rye pentosans as being part of the dietary fiber are not digested in the small intestine and contribute to the fecal bulk and physiological health benefits of dietary fiber.

β-glucan is another gum found in barley and oat at a relatively high level compared to other cereals. It constitutes about 4–6% of the oat kernel and makes up to 70–87% of the gums in oat, while it considerably varies in barley ranging from 2–11% depending on type of barley, for example malting, feed or food barley. Due to the adverse effects of β-glucan in malting and brewing industries and in animal feed, immense efforts have been made to lower β-glucan content in malting and feed barley. On the other hand, a high level of β-glucan is required in food barley due to its hypocholesterolemic effect in human nutrition. In addition to the health benefits, β-glucan is able to bind water and form a gel which is a desirable functionality in gluten-free foods.

In a starch-based gluten-free bread formulation, other polymeric substances are required in order to help form elastic dough and to stabilize air cells. The gas holding property is more difficult in gluten-free dough and surface active substances such as hydrocolloids are incorporated in the formulation to provide gas occlusion and stabilizing mechanisms and generally to enhance baking performance of starches or starchy flours. Several hydrocolloids and gums have been investigated to function as gelling and stabilizing agents and to enhance overall quality of final products (Casier et al., 1977; Gujral et al., 2004; Lazaridou et al., 2007).

Functionality of hydrocolloids is usually determined in a single system, but in a real food, functionality of a given hydrocolloid may change to some extent due to interactions with other food polymers and components. Thus, it is important to understand interactions between hydrocolloids and other food polymers such as starches and proteins. These interactions are crucial in designing gluten-free foods in order to make sure hydrocolloids and starches have synergetic interactions. Many studies have been conducted to investigate interactions between gums and starches as well as their impact on the rheological, gelation and retorgradation behavior of starch (Funami et al., 2005a,b; Chaisawang and Suphantharika, 2006; Ojijo and Shimoni, 2007). Similarly, effects of gums on functional properties and stability of food proteins have extensively been investigated (.Ibanoğlu and Ercelebi, 2007; Camacho et al., 2001; Amako and Xiong, 2001). These interactions and their effects on the quality of food products are discussed in the following sections of this chapter.

The level of interaction between xanthan and locust bean gum was found to be dependent on the degree of disordering of the xanthan molecule at the preparation temperature (Zhan et al., 1993). At a specific configuration for the xanthan molecule, it will bind to the locust bean chains, and the level of interaction will determine the rheological and gelation properties of the gum mixture. The molecular interactions between xanthan gum and waxy cornstarch in a ternary solution system were attractive but did not show synergic effects (Wang et al., 2001). The addition of xanthan gum to waxy cornstarch solution resulted in decreased overlap concentration and increased intrinsic viscosity. The study has shown that xanthan gum is a better thickener than waxy cornstarch. Addition of locust bean gum and yellow mustard mucilage resulted in increased pea starch paste viscosity due to synergistic interactions (Liu and Eskin, 1998). Addition of guar and xanthan gums increased RVA peak viscosity, breakdown and final viscosity of native tapioca starch with this effect being more pronounced for guar gum than xanthan gum (Chaisawang and Suphantharika, 2006). RVA setback viscosity was increased by adding guar gum, while xanthan gum had the opposite effect. DSC onset and peak gelatinization temperatures were increased with the addition of gums, while gelatinization enthalpy was decreased.

Guar and locust bean gums were also found to retard bread staling when used at low replacement levels, that is, 2% was the most effective (Schwarzlaff et al., 1996). Addition of low levels of xanthan gum preserved the smoothness characteristics of unfrozen cornstarch and wheat flour pastes and maintained quality characteristics of frozen pastes even at low

freezing rates (Ferrero et al., 1993). The addition of xanthan gum encourages amylose–hydrocolloid interaction which competes with amylose–amylose aggregation and decreases the probability of retrogradation occurrence. Freeze-thaw stability of tapioca starch paste was enhanced by adding xanthan gum (Sae-Kang and Suphantharika, 2006). Xanthan gum was most effective in reducing the syneresis at pH 7 and this effect increased with increasing gum concentration in the starch/gum mixture (6.0/0.0, 5.7/0.3 and 5.4/0.6 ratio).

In general, gluten-free bakery formulations are typically made from non-wheat or non-gluten containing flours and starches that may form dough exhibiting poor viscoelastic and gas retaining properties. Hydrocolloids are included in the gluten-free formulations to enhance these properties and to provide other functionalities. These functionalities may include gelation, thickening, emulsification and/or stabilization of air cells. The interactions between gums and starches may offer novel functionality such as improved rheological and textural properties and/or enhanced product acceptability and stability. The effects of five different hydrocolloids including pectin, CMC, agarose, xanthan, and oat β-glucan (purity 92% and contains 2% non-gluten protein) on dough rheology and bread quality of gluten-free formulations consisting of rice flour, cornstarch, sodium caseinate, fresh yeast, sunflower oil, salt and sugar were investigated (Lazaridou et al., 2007). The hydrocolloids were added at 1% and 2% w/w (rice flour basis). Increasing the addition level of hydrocolloids to 3% resulted in reduced bread quality in most cases. In the study, all the hydrocolloids were obtained from commercial sources except β-glucan, which was isolated from oat whole flour by water extraction as described by Skendi et al. (2003). The amount of added water was 130 g for 100 g rice flour control bread formula (without hydrocolloids), which was adequate to give consistent dough in the presence of other ingredients making up a total of 200 g solids. The amount of water in hydrocolloid-containing formulations increased to 140 and 150 g/100 g rice flour for 1% and 2% hydrocolloid, respectively. Hydrocolloids have different effects on dough rheological properties and quality of bread depending upon the type and supplementation level used.

Addition of xanthan to gluten-free formulae had the most pronounced effect on viscoelastic properties of dough among the five hydrocolloids used. Xanthan helps in strengthening doughs and producing a farinograph curve similar to that of wheat. The hydrocolloids also enhance elasticity and resistance to deformation of gluten-free doughs following the order of xanthan > CMC > pectin > agrose > β-glucan. The rheological properties of gluten-free doughs were based on oscillatory and creep measurements as those methods used in breadmaking but in the case of gluten-free dough no yeast was added. The quality of gluten-free bread products was based on the measurements of loaf volume, bread porosity, crumb elasticity, crumb color and crust color. In general, loaf volume increased with the addition of hydrocolloids at 1% except for xanthan. Increasing the amount of hydrocolloid from 1% to 2% reduced bread loaf volume except for pectin but the loaf volumes were still higher than that of control bread. Similar findings were revealed by McCarthy et al. (2005) who reported a slight decrease in loaf volume of gluten-free breads made from rice flour, potato starch and milk protein with increasing levels of HPMC. Addition of xanthan at 1% had no effect on bread volume, but at 2%, the volume was significantly reduced. The high rigidity of doughs containing xanthan resulted in breads with low volumes and high crumb firmness. Xanthan also increased elasticity and lightness of the bread crumb. Haque and Morris (1994) found no influence of including xanthan into rice flour breads, whereas Schober et al. (2005) observed a decrease in loaf volume of gluten-free breads baked from sorghum with increasing levels of xanthan.

The elasticity, porosity and uniformity and size of gas cells are important parameters in determining quality of bread crumb. Inclusion of xanthan increased elasticity, porosity

and lightness of bread crumb except for 2% xanthan, which resulted in reduced porosity compared to control bread samples (no hydrocolloids) (Lazaridou et al., 2007). CMC and β-glucan produced breads with higher porosity than control bread samples and improved elasticity. Their results showed that CMC (1%) and pectin (2%) gave the best gluten-free bread quality among the five hydrocolloids based on higher values for loaf volume and crumb porosity and elasticity. In general, the gluten-free bread products were acceptable by the consumer panel based on a nine-point hedonic scale test.

11.5 GLUTEN-FREE BAKERY PRODUCTS

11.5.1 Starch-based gluten-free bread

Wheat starch has been used as a functional ingredient in gluten-free bakery products perhaps to mimic its role in wheat flour. In Europe, it has been included in gluten-free bakery formulations. Since it is difficult to remove gluten completely from starch in the wheat fractionation process, gluten testing must be performed on the starch fraction to make sure that wheat starch meets Codex specifications for gluten-free foods. The designation of 'gluten-free' may reflect that the foods or food ingredients contain zero gluten, but this is not necessarily true. The safe gluten threshold set by the Codex Alimentarius Commission of the Joint FAO and WHO Food Standards Programme is 20 mg/kg for foods consisting of or made only from ingredients which do not contain any prolamins, or 200 mg/kg for food consisted of ingredients from wheat, rye, barley, [oats], spelt or their crossbred varieties which have been rendered gluten-free (Codex Alimentarius Commission, 2000). Recently, Catassi et al. (2007) carried out a multi-center, double-blind, placebo-controlled randomized trial in 49 adults with active celiac disease to establish a safe gluten threshold. The study found that the gluten should be kept lower than 50 mg/day in the treatment of celiac disease. Several gluten-testing methods have been developed and validated (Skerritt and Hill, 1990, 1991; Gabrovská et al., 2006; Olexová et al., 2006). Some of these gluten detection methods are commercially available in kits for use by consumers. It also worth mentioning that Kjeldahl nitrogen analysis was found to be not suitable for testing gluten level in wheat starches (Skerritt and Hill, 1992).

In Codex's definition for gluten-free food, oats were put between square brackets because of its uncertain status with regard to celiac disease. The consumption of pure and uncontaminated oat that is free from the offending cereal grains (wheat, barley, rye) (i.e. one offending seed per kg oat) is considered safe for persons with celiac disease (Burrows, 2005). Thompson (2003) reported six in vivo studies published since 1995 that assessed the effects of including moderate amounts of oat in the diet of persons with celiac disease and/or dermatitis herpetiformis (a form of gluten intolerance involving the skin) showing safety of oat with no adverse effects to celiac patients. Nevertheless, this recommendation should be taken with caution because commercial oat might be contaminated with wheat, rye or barley and some individuals may generate positive reaction to oat. Burrows (2005) summarized a strategy to supply pure and uncontaminated oat that includes establishing a dedicated production, cleaning, grading, inspection and processing system for oat. In Canada, a few oat producers have dedicated their system for the production of pure oat. At the present time, the only risk-free choice for celiac patients is to exclude oat from their diet (CCA, 2007).

Non-wheat breads include a variety of bread products that are made from non-wheat cereals (e.g. corn, sorghum, millet and rice) or roots (e.g. cassava). These breads are produced

in many countries around the world for different reasons such as unavailability of wheat, abundance of non-wheat cereals and/or production of breads with special flavor, taste or nutritional properties such as rye or multi-grain bread and gluten-free breads for celiac patients and individuals sensitive to wheat. Several non-wheat breads have been described by Casier et al. (1977), Cauvain (1998) and others in which some of them are free from gluten and suitable for those having celiac disease. The gluten-free bread products include cassava bread (Satin, 1988), rice bread (Ylimaki et al., 1988, 1991), corn bread (Ács et al., 1996a, b) and sorghum bread (Schober et al., 2005; Cauvain, 1998). These bread products are based on non-wheat starchy flours, starches or mixture of both in addition to the basic bread ingredients (yeast, water, sugar and salt) with or without using surface active substances or stabilizers to help stabilize gas and air cells. In general, inclusion of 2–4% rye pentosans as a baking improver in the formulations resulted in enhanced bread quality (Casier et al., 1977).

11.5.1.1 *Gluten-free rice bread*

A mixture of rice flour (80%) and potato starch (20%) was evaluated in making gluten-free rice bread and compared with a reference wheat white bread based on sensory properties measured by 16 trained panelists (Ylimaki et al., 1991). The sensory measurements include 15 attributes: yeasty odor, rice odor, firmness, moistness, cohesiveness, yeasty flavor, rice flavor, adhesiveness, graininess, aftertaste, top crust color, crumb color, predominant cell size, cell size uniformity and cell wall thickness. Two types of gum, carboxymethylcellulose (CMC) and hydroxypropyl-methylcellulose (HPMC) were added to the rice flour/potato starch formula to enhance dough-forming properties and rice bread quality. Three different rice flours were assessed in the study based on sensory properties of the resultant bread. A central composite design, consisting of a three-variable (CMC, HPMC and water) five-level pattern with 20 design points, was used to optimize concentration of gum. The study showed that several combinations of CMC, HPMC and water resulted in rice breads with moistness, cohesiveness, yeasty flavor, adhesiveness, aftertaste, crust color, crumb color, cell size uniformity and predominant cell size comparable to a reference wheat bread. The development of rice bread formulae that are comparable to control wheat bread based on sensory properties is dependant upon type of rice flour and levels of CMC, HPMC and water. Rice bread made from medium grain was more comparable to the control wheat bread than that baked from long-grain rice flour.

The staling properties of two gluten-free breads (rice bread and low-protein starch bread) and two gluten-containing breads (standard wheat and added-protein wheat) were compared over a 120-h period (Ahlborn et al., 2005). The rice bread formulation contained rice flour (800 g), potato starch (200 g), tapioca starch (150 g), yeast (25 g), sucrose (90 g), salt (20 g), vegetable oil (60 g), non-fat dry milk (45 g), (200 g), xanthan gum (18 g), HPMC (5 g) and water (650 g). Low-protein starch bread formula consisted of wheat starch (600 g), potato starch (180 g), cornstarch (140 g), yeast (25 g), sucrose (90 g), salt (20 g), vegetable oil (60 g), non-dairy creamer (40 g), xanthan gum (18 g), HPMC (5 g) and water (850 g). The wheat control bread was made from flour and the basic ingredients (yeast, salt, sucrose and oil), while the added-protein wheat bread contains non-fat dry milk, egg, xanthan gum and HPMC in addition to the basic ingredients. Staling was assessed based on sensory properties by quantitative description analysis (QDA) and critical stress values measured by mechanical compression testing and scanning electron microscopy (SEM).

The gluten-free rice bread had the highest QDA scores for moistness and overall freshness, whereas the low-protein starch bread possessed the lowest scores for both sensory attributes.

This indicates that gluten-free rice bread maintains its moisture over the staling period. With regard to crumb texture, the gluten-free rice bread had the greatest resistance to mechanical collapse indicating the least structural damage, whereas the low-protein starch bread exhibited the least resistance to mechanical collapse. Both wheat breads possessed QDA moistness and freshness scores and critical stress values that ranged between the gluten-free rice and low-protein starch breads. The SEM examinations showed that gluten-free recipes containing rice, egg and milk proteins, xanthan gum and HPMC had a bicontinuous matrix with starch fragments similar to that of gluten. It seems that a combination of starch and small amounts of hydrocolloid and protein are essential for the formation of crumb texture that would mimic the texture of wheat bread.

Chapatti is unleavened flat bread made from whole-wheat flour and it has served as the staple food of the Indian subcontinent and parts of the Middle East. For example, over 85% of the wheat consumption in India is in the form of chapatti. Gluten-free chapatti made from rice flour containing hydrocolloids and α-amylase was investigated (Gujral et al., 2004). Several hydrocolloids including guar gum, xanthan, locust bean gum and HPMC were added to the rice flour at levels of 0.25–0.5% flour basis resulting in improved chapatti texture by keeping it more extensible during storage. Fungal α-amylase was also included in the rice flour alone and in blend with the hydrocolloids which results in further improvements in the texture. Chapatties containing hydrocolloids and/or α-amylase had also lower retrogradation after storage.

Enzymes have been used in the baking industry to improve rheological and handling properties of dough, crumb texture and staling properties of wheat bread. These enzymes include amylases, lipases, hemicellulases, pentosanases, proteases and oxidases with α-amylases being the most frequently used in bakeries because of their desirable effects on loaf volume, crumb grain, crust texture, crumb color, bread flavor and staling properties. Transglutaminase (TGase), an enzyme that catalyzes acyl-transfer reactions to cross-link proteins, was used to improve the structure of gluten-free bread made from a formula containing white rice flour (relative amount 35), potato starch (30), corn flour (22.5), xanthan gum (1) and various protein sources (skim milk powder, soy flour and egg powder) (Moore et al., 2006). The enzyme was added at different concentrations 0, 0.1, 1 and 10 units of TGase/g protein to study its impact on bread quality including percent bake loss, specific volume, color, texture, microstructure and total moisture content. There were no significant effects in the soy breads between 1 and 10 units of TGase/g protein; however, a protein network was formed within the skim milk and egg powder gluten-free breads. The formation of protein network structure was shown by a confocal laser scanning microscope. It was concluded that the formation of this protein network may improve loaf volume, crumb characteristics, appearance and overall quality of gluten-free breads. In addition, the efficiency of the enzyme is dependent on both the protein source and the level of enzyme concentration. Glucose oxidase was also used to modify rice flour proteins by lowering thiol and amino group concentration for making rice bread (Gujral and Rosell, 2004). The addition of glucose oxidase resulted in an increase in the elastic and viscous modulus and produced rice bread with better specific loaf volume and texture with using small amount of HPMC.

11.5.1.2 *Gluten-free corn bread*

Gluten-free bread has also been made from cornstarch with the addition of binding agents such as gums and hydrocolloids. The incorporation of xanthan, guar gum, locust bean gum or tragant at levels of 1%, 2%, 3%, 4% and 5% into cornstarch formulations gave breads

with significantly higher loaf volume (212–419 cm^3) compared to control breads (125 cm^3) made from cornstarch without gums (Ács et al., 1996a). The volume increasing effect was in the order of xanthan > guar gum > locust bean gum > tragant. Addition of gums also enhanced crumb softness. The optimal levels of xanthan based on increased loaf volume were 1–3%. In a subsequent study by the same authors to optimize baking ingredients and to study their effects on sensory properties and overall quality of gluten-free corn breads (Ács et al., 1996b), it was necessary to add 5–10% powdered sugar to achieve pleasant bread flavor. Addition of δ-glucono-lactone at the appropriate level would give breads with fine crumb and crust texture. Use of margarine is not recommended in systems that are low in protein and free from gluten due to its unfavorable effect on crumb properties, that is, crumb with large pores, brittle and bright cell walls.

A mixture of cornstarch, rice flour and tapioca starch was optimized for making gluten-free bread using a central composite design containing two variables: cornstarch/tapioca starch ratio and rice flour/tapioca starch and second order (quadratic function) of three responses: specific volume, crumb-grain score and bread score without soy flour and with 0.5% soy flour, respectively (Sánchez et al., 2002). Soy flours and protein concentrates at low levels were incorporated in the bread formulae to improve grain texture from a rough crumbly open-faced interior to a more tender, close grain and even texture. In addition to the individual functions, synergistic effects through polymer–polymer interactions are extremely important. In this study, the optimal gluten-free bread was achieved from 74.2% cornstarch, 17.2% rice flour and 8.6% tapioca starch based on the response maxima for crumb-grain score and bread score values. In addition, incorporation of 0.5% soy flour improves crumb-structure quality and enhances dough properties.

Response surface methodology was used to optimize the levels of methylcellulose, gum arabic and dried egg albumin in a gluten-free pocket-type flat bread formula based on sensory properties (Toufeili et al., 1994). The study used gluten-free formula made from pregelatinized rice flour (100 g), pregelatinized cornstarch (50 g), corn flour (50 g), sugar (6 g), salt (3 g), yeast (4 g), sodium stearoyl-2-lactylate (0.5 g) and varied amounts of three polymeric ingredients, methylcellulose, gum arabic and egg albumin. The study showed that sensory properties were most influenced by changes in methylcellulose and egg albumin levels and to a lesser extent by gum arabic levels. Inclusion of 3 g of gum arabic, $\geq$2.10 g to $\leq$4.12 g methylcellulose and $\geq$2.18 g to $\leq$4.10 g egg albumin in the bake mix resulted in gluten-free breads that are comparable to regular wheat bread in the frequency of cracks, separation of layers, rollability, tearing quality, hardness, adhesiveness and cohesiveness. Addition of lower levels (1.37 g) of gum arabic produced loaves that were less cohesive and inferior to wheat bread in rollability. All breads exhibited a perceptible corn flavor, a light yellow crumb with apparent waxy patches and a faster staling rate than that of regular wheat bread.

Gluten-free bread formulations have also been developed from multi flour and starch ingredients to provide additional nutrients such as protein and dietary fiber in addition to starch. Two gluten-free bread formulations, the first (non-dairy formula) contained cornstarch (54), brown rice flour (25), soy flour (12.5) and buckwheat flour (8.5) and the second formulation (dairy formula) comprised of brown rice flour (50), skim milk powder (37.5), whole egg (30), potato starch (25), cornstarch (12.5) and soy flour (12.5) (% fwb) were evaluated and compared with wheat bread and gluten-free bread made from a commercial gluten-free flour mix in terms of loaf volume, textural properties and shelf life (Moore et al., 2004). Xanthan gum at 1.3% fwb was added to the non-dairy formula and a mixture of xanthan (0.9% fwb) and konjac (1.5% fwb) gums was incorporated in the dairy formula to enhance rheological

properties of doughs. The commercial gluten-free flour mix, according to the supplier information, is based on wheat starch (Codex Alimentarius quality), milk solids, modified cornstarch, soy flour and HPMC. The total nitrogen content in the first formula is 0.62% fwb and 1.50% fwb in the second dairy formula which was higher than that in the gluten-free commercial mix (0.32% fwb) but lower or higher than wheat flour (1.20% fwb) depending on the formulation.

The batter consistency of the two formulae, gluten-free commercial mix and wheat, were compared based on extrusion and penetration tests. Based on the extrusion test, significant differences were observed between batters, while no significant differences were found among batters using the penetration test. Breads baked from wheat and commercial gluten-free flour mix had significantly higher loaf volume than those baked from the gluten-free two formulae. In addition, all the gluten-free breads were brittle after 2 days of storage. This observation is based on the occurrence of fracture, and the decrease in springiness, cohesiveness and resilience derived from textural profile analysis. These changes were generally less pronounced for the dairy formula showing a better keeping quality. Confocal laser scanning microscopy showed that the dairy-based gluten-free bread crumb has a network-like structure resembling the gluten network in wheat bread crumb. The study suggests that the formation of a continuous protein phase is critical for an improved keeping quality of gluten-free bread.

In another study, seven dairy powders including Molkin (sweet whey, 6.5% protein), demineralised whey powder (11.0% protein), Kerrylac (fresh milk solids, 18.0% protein), skim milk replacer (26.0% protein), skim milk powder (35.0% protein), sodium caseinate (89.0% protein) and milk protein isolate (90.0% protein) were found to have variable effects on the quality of gluten-free bread baked from commercial gluten-free flour (Gallagher et al., 2003). The dairy powders with a high protein content (skim milk powder, sodium caseinate and milk protein isolate) resulted in breads having lower loaf volume and increased crumb and crust hardness, but the breads have appealing dark crust and white crumb appearance and received good acceptability. When additional water was added to the gluten-free formula supplemented with Molkin, Kerrylac and milk protein isolate, the resulting breads exhibited increased loaf volume and a much softer crust and crumb texture than the control bread. The dairy powders were added at three levels 3%, 6% and 9%, and in a second trial additional water was added at 10% and 20% to breads containing 6% diary powder.

11.5.1.3 *Gluten-free sorghum bread*

Sorghum is closely related to corn and is recommended as a safe food for celiac patients (Kasarda, 2001). Sorghum and other grains were also used to replace wheat flour in baked goods (Lovis, 2003). Decorticated sorghum flours obtained from 10 white or red sorghum samples were evaluated in gluten-free breadmaking (Schober et al., 2005). In previous investigations, sorghum breads have been made with the addition of other polymeric substances such as xanthan gum (Satin, 1988), CMC and skim milk powder (Cauvain, 1998) or rye pentosans (Casier et al., 1977). Gluten-free bread baked from 70% sorghum and 30% tapioca starch has also been developed by Olatunji et al. (1992). The gluten-free sorghum formulation used by Schober and others (2005) includes 70 parts (relative mass) of sorghum flour (14% moisture), 30 parts cornstarch, 105 parts water, 1.75 parts salt, 1 part sugar and 2 parts dried yeast. The sum of sorghum flour and cornstarch (100) was interpreted as flour weight basis. Additional polymeric ingredients such as xanthan gum and skim milk powder were added to the basic recipe to enhance bread quality. The sorghum flours used in the study have significant differences in the content of protein, starch and pentosans. The consistencies of sorghum

batters measured by an extrusion test showed considerable differences among the sorghum samples. Depending on whether the batters from the individual sorghum samples were too soft or too firm, 5% less or more water was added, respectively. With regard to bread quality, no significant differences were observed for specific loaf volume, loaf height, bake loss and water activity. However, all crust and crumb color values were significantly different. Bread crumb properties measured by digital image analysis such as pore size and number of pores and texture profile analysis such as crumb hardness exhibited the largest variations indicating variability among sorghum hybrids in their potential to produce gluten-free leavened bread.

11.5.2 Starch-based gluten-free pizza

Pizza is a popular, convenient and pleasant food product that is widely consumed in North America and Europe. In order to produce high-quality pizza products, the dough should be elastic and sheetable, rises on proving and holds the gas produced by the yeast, as well as having good crust and textural properties. Most of the formulations previously discussed in gluten-free bread could be used in making pizza crust products with slight modifications. In addition to the viscoelastic property of pizza dough, it should be firm enough to hold toppings without fallen apart. It should also demonstrate good stability in freezing storage for frozen pizza. By combining different types of starches and proteins with microencapsulated high-fat powder, it was possible to produce quality gluten-free pizza (Arendt et al., 2002; Gallagher et al., 2004b). In the study, several measurements were performed to assess quality of dough and pizza. These include dough stickiness and hardness, pizza volume, color and texture. The effects of the various ingredients on dough rheology of the optimized formulation were also examined using an oscillation test to measure phase angle, elastic modulus and viscous modulus. Based on these measurements, it was observed that the cornstarch system achieved the highest increase in elasticity when combined with guar gum and high-fat powder.

11.5.3 Starch-based gluten-free cookies/biscuits

A wide array of gluten-free non-bread bakery products are commercially available. These include chocolate chip cookies, chocolate chip snacks, chocolate wafers, crackers, cake mixes, pretzels, bagels, muffins, and so on. Recipes for all these products are available at www.celiac.com. However, only a few scientific articles have been published on gluten-free pastry products. Pastry products such as biscuits and cookies are made from short doughs (i.e. gluten is not fully developed) using soft wheat flour and high content of sugar and fat and relatively low amount of water. In cookies and biscuits, only a small amount of gluten development is needed so that the dough can be sheeted and cut. The retardation of gluten development is achieved by the high content of sugar, high pH because of the baking powder and low amount of water. In other words, the cookie and biscuits batters do not possess a developed gluten network and have a short fracture (i.e. break easily). Therefore, gluten-free biscuits and cookies may be made from pregelatinized starches, or starches having good gelling properties with or without the addition of small amount of gums. A variety of starches from corn, soy, millet, rice and potato were combined with different types of fat including palm oil, cream powder, microencapsulated high-fat powder and low-fat dairy powder (Arendt et al., 2002; Gallagher et al., 2004b). The resulting biscuits were evaluated. Combinations of rice, corn, potato and soy starches with high-fat powders resulted in biscuit batters that can be sheeted and produced biscuits that were comparable to that of wheat biscuit.

11.6 GLUTEN-FREE EXTRUDED PRODUCTS

11.6.1 Starch-based gluten-free pasta

Extruded food products such as pasta, snacks and ready-to-eat cereals differ from bakery products (bread, cakes, cookies) in the way their texture is formed. In the extrusion process, the ingredients are fed into a continuous high-capacity extruder that is equipped with a variety of dies to produce products having different shapes and appearance. The structure of the extruded food products is determined by the physical and chemical changes that occur within the extruder barrel and at the die such as starch gelatinization and protein denaturation. These changes are dependent on the extrusion process variables, that is, cold extrusion versus hot extrusion. Durum pasta products are made by a cold extrusion process to protect gluten from heat damage. The pasta extruder barrels are equipped with a water cooling jacket to dissipate the heat generated during the extrusion process and to help maintain a constant extrusion temperature around 35–50°C to avoid gluten damage. In processing non-durum or starch-based gluten-free pasta formulations, higher temperatures are required to form a consistent dough that can be extruded into a uniform pasta product. For instance, enriched starch pea pasta processed by a high-temperature extrusion process was superior in quality attributes such as integrity, flavor and texture compared to that made by a conventional pasta cold extruder (Wang et al., 1999). Starches and non-gluten proteins can be employed when making gluten-free pasta in which these polymeric substances become reactive molecules in the extruder barrel and form new linkages to form paste. Hydrocolloids (see Table 11.2) may be included in the extrusion formula to enhance paste uniformity and stability during extrusion process.

Pea flour (which is gluten-free) was used to make a pasta-like product by a high-temperature twin-screw extrusion process (Wang et al., 1999). The pea flour is starch-enriched, air-classified yellow pea flour that contains 69% starch, 12% protein and 5% total dietary fiber. The extrusion processing variables such as dough moisture, barrel temperature and screw speed had considerable effects on physical, textural and cooking properties of pasta-like products. The resulting product had shorter cooking times, was firmer and less sticky but had higher cooking losses compared to commercial wheat spaghetti. It had a compact structure with relatively few swollen starch granules deeply embedded in a gelatinized starch and protein matrix and aligned in the direction of flow through the extruder barrel. The texture was also close to that of the commercial spaghetti as it was dense and compact and coated with a smooth protein film with few openings. However, after the product was wetted with water, frozen in liquid nitrogen and freeze dried, both products had different microstructures. For commercial spaghetti, the compact structure becomes a more porous with starch granules loosely held within a discontinuous protein matrix, whereas the pea pasta still had a compact structure with swollen starch granules embedded in a gelatinized starch and protein matrix. Formulation and processing of pasta from non-traditional materials using cereal and non-cereal materials was reviewed by Marconi and Carcea (2001).

Gluten-free pasta formulae were developed using response surface methodology based on optimal sensory properties (Huang et al., 2001). The formulations contained seven different polysaccharides including five independent variables, locust bean gum (10–40 g), xanthan gum (25–40 g), modified potato starch (30–40 g), tapioca starch (63–90 g) and potato starch (32–45 g), and two fixed variables, yellow corn flour (250 g) and rice flour (50 g). The total amount of the seven ingredients was 500 g (100%). Each pasta formula was blended with distilled water in a single screw pasta extruder and extruded through a 1.5 mm noodle shape.

Fifteen treatment combinations were run, and the resultant pasta products were evaluated by a sensory test. The gluten-free pasta was assessed on a 10-cm (0–10) line scale for smoothness of surface, hardness of first bite, adhesiveness of chew down, cohesiveness of chew down and off-flavor against control pasta. Fresh pasta was dried at a controlled temperature 90°C for 5 h, and the dried pasta was boiled in tap water for 13 min and served to panelists. The gluten-free pasta that had the most desirable properties was the product made from locust bean gum (40 g), xanthan gum (40 g), modified potato starch (35 g), tapioca starch (113 g) and potato starch (57 g), corn flour (250 g) and rice flour (50 g).

Homemade gluten-free pasta containing rice flour (350 g), cornstarch (150 g), potato starch (150 g) and eight whole eggs (420 g) was compared with gluten-containing pasta in terms of palatability, glycaemic response, gastric distension and emptying and hydrogen production using 20 healthy medical students less than 30 years of age, and having body mass index less than 25 kg/m^2 (Clemente et al., 2001). The above formula yielded about 1 kg of fresh pasta. The pasta was easier to handle when Asian glutinous rice flour was used: this is rice flour containing rice glue (i.e. prolamins) and is traditionally used in Asia to make noodles and cookies. No differences were observed in taste between the gluten-free pasta and durum pasta, and it was perceived to be better in texture than the control pasta. The sense of fullness and postprandial satiety were less with the gluten-free pasta. The gluten-free pasta elicited a significantly higher postprandial plasma glucose response at 60 and 120 min; the response decreased at 180 and 240 min, so that at 240 min the response was similar for the two meals. The postprandial insulin response was similar after the two meals, but the gluten-free pasta caused a significant increase of plasma insulin area up to 240 min. Hydrogen production was minimal after both meals.

Another approach for the manufacture of pasta that could be tolerable by celiac patients was developed based on a prefermentation of durum semolina (di Cagno et al., 2002, 2005). In this method, durum wheat semolina is fermented by selected lactic acid bacteria to break down toxic durum gliadins and the fermented semolina with reduced level of gluten intolerance is processed into pasta and compared with the control pasta. The fermented pasta had slightly lower scores for stickiness and firmness than control pasta, but no significant differences were found with regard to odor and flavor between the two types of pasta.

Pasta was also made from a corn/durum semolina blend in the range of 70:30–0:100 ratio to study the potential of reducing the amount of wheat material in corn pasta, while retaining high-quality pasta by applying high-heat treatment after conventional drying at 55°C (Abecassis et al., 1989). When the corn/durum (66:34 ratio) pasta, previously processed and dried under the usual industrial conditions, was treated at high temperature (90–120°C) for 90–180 min, the product quality was substantially improved. There was a distinct improvement in the cooking quality (reduced cooking and improved surface condition such as stickiness and swelling) of the pasta product. In addition, the viscoelasticity of cooked pasta was significantly modified when the temperature of treatment was 120°C, but the color was unacceptable. In general, heat treatment at high temperature permits the use of a higher rate of incorporation of corn flour.

11.6.2 Starch-based gluten-free snack foods

Today, snack foods comprise a sizeable portion of the diet, and contribute considerably to the daily intake of energy, protein and other nutrients. The majority of snack foods are based on grain flours or starch mixes, which are fabricated by extrusion into a variety of products with different texture, shape and nutritional properties. An extrusion cooking process is used to

mix, modify, restructure and shape these starchy raw ingredients. This processing technology can be used to produce a wide array of gluten-free extruded snack foods based on blends of starches, non-gluten proteins or flours, dietary fiber materials and other ingredients which offer another avenue for persons with celiac disease. Unlike bakery products, these materials can be mixed and extruded into palatable snack foods with good appearance and desirable texture. Recently, a nutritionally balanced gluten-free extruded snack food was developed and evaluated based on physical and sensory properties (İbanoğlu et al., 2006). The formula used in the extrusion process was composed of rice flour (30% w/w, wb), chickpea flour (30%), corn flour (20%), tomato powder (5.0%), carrot powder (5.0%), onion powder (2.0%), gum arabic (2.0%), vegetable oil (2.0%), oregano (1.5%), basil (1.5%), salt (0.5%) and dried yeast (0.5%). The study showed that the extrusion variables (feed rate and screw speed) did not affect color, flavor and overall acceptability of the final product. However, increasing screw speed resulted in increased expansion and firmness of the product, while increasing feed rate gave less hard and more expanded products.

In another study, mixtures of starch and cellulose fiber were extruded at moisture contents of 14–22%, barrel temperature (140°C) and screw speed (140 rpm) to investigate their physicochemical and macromolecular properties (Chinnaswamy and Hanna, 1991). The maximum expansion ratio was obtained with 2% of 60 μm cellulose fiber and 14% moisture content. Increasing fiber concentration and moisture content generally reduced the expansion volume. The extrusion cooking process caused starch molecular degradation with and without fiber addition, but the degradation was higher in the presence of fiber due to its effect on velocity gradient (shear flow) within the extruder barrel. The maximum starch degradation occurred when 10% fiber was added. Extrusion of mixtures of cornstarch and guar gum (2%, 5% or 10%) with and without citric acid (2%) at barrel temperature of 150°C, screw speed of 180 rpm and moisture content 25% (db) was found to increase content of resistant starch from 6.2% for extruded starch only to 14.2% for starch/gum extrudate, and an additional increase to 16.2% occurred with the addition of 2% citric acid to the starch/gum extrudate (Wang et al., 2007). Resistant starch increased with increasing gum additions and decreased with increase of starch/gum concentration from 7.5% to 12.5%. These studies show the possibility of making nutritious and acceptable gluten-free snack foods by extrusion process. However, more extensive research is still required in this area.

11.7 CONCLUSIONS

Starches play substantial roles in gluten-free foods. They provide several functionalities that affect product quality and acceptability. In bakery products, gelatinization of starch during baking in the oven controls expansion of dough and characteristics of crumb texture. Furthermore, freshness and staling of bakery products are dependent on starch retorgradation. Expansion and crispness of extruded products also rely on properties and functionality of starch. Blending different types of native and/or modified starches (normal, waxy, high-amylose starch) could be optimized to obtain novel functionalities and properties. In addition, properties of starches or starchy flours can be enhanced by mixing them with hydrocolloids to achieve certain functionality in a gluten-free food system, that is, the formation of cohesive and elastic dough or stabilization of air cells. Molecular interactions between these polymeric substances influence their rheological and gelling properties and eventually quality of final products. The use of response surface methodology to study and optimize levels of hydrocolloids and their interactions with starches and other biopolymers is common and

helpful. In this respect, more research is required to better understand polymeric interactions and to find optimal combinations of starches and gums for specific gluten-free food(s).

Proteins from animal (e.g. milk proteins) or plant (e.g. rice flour, corn flour, soy proteins) sources can also be incorporated in the starch/gum formulations to form a network-like structure resembling that of gluten network and to enhance characteristics of bread crust and crumb. The formation of protein network is influenced by source and concentration of protein. Proteins that are naturally occurring in rice, corn or sorghum flour or added proteins could also be modified by enzymes to enhance the formation of protein network. Without a doubt, the key in the development of gluten-free foods is to understand formulation and interactions between biopolymers in the formula. Gluten-free foods, like conventional foods, should be palatable, nutritious and convenient to manufacture, and should possess the characteristics of their gluten-containing equivalents.

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12 Coeliac Disease and Gluten-Free Research: What Does the Future Hold for the Physician, the Patient and the Scientist?

Eimear Gallagher

12.1 INTRODUCTION

We have seen in the previous chapters many views and reviews concerning coeliac disease and the field of gluten-free research. The area may be divided into two broad themes: coeliac disease (CD) and the gluten-free diet (GFD) from a medical viewpoint, and gluten-free ingredients and interactions from the view of the food scientist. Both are of great importance for the coeliac patient and the general scientific and commercial world. This chapter aims to recap on some of the most recent advances in gluten-free research, as well as highlighting the strides made in recent years in medical research. The chapter will also hypothesise about the future of this area, signalling important research that remains to be done, and putting forward some novel ideas that could enhance the knowledge required to succeed in this difficult and complicated topic.

12.2 COELIAC DISEASE AND THE GLUTEN-FREE DIET: PAST, PRESENT AND FUTURE?

In recent years, a large amount of medical work has been published in the area of CD. Our understanding of its epidemiology, clinical manifestations and diagnostic procedures has broadened hugely, and today the ability of the physician to identify potential cases has greatly improved. Despite this, it is thought that there are still more questions than answers remaining, and Hill (2003) claims 'the story that is coeliac disease is never ending!'

12.2.1 Diagnosis

Until the 1950s, the diagnosis of CD was made when a child or adult showed malabsorption in the absence of infection (Mulder and Cellier, 2005). The 1960s saw the introduction of the small intestinal biopsy, and today this technique is still considered necessary for the confirmation of the diagnosis. However, in the last decade, reliable serological tests have been developed that can help in identifying those who need further biopsy tests. These blood tests are particularly useful for persons who do not present with gastrointestinal symptoms. Initially, anti-gliadin tests were used, but due to their highly variable sensitivities and specificities, they may no longer be warranted (Hill, 2003). Identification of tissue transglutaminase (tTG) as the autoantigen in CD has been a significant breakthrough in further understanding the disease (Dieterich et al., 1997). The detection of antibodies to tTG

has proven very promising in CD screening (Mulder and Cellier, 2005). The test is quick to perform and is becoming the method of choice for many physicians (Vitoria et al., 2001).

Although these serological tests have greatly aided in the detection of CD, the clinical presentation of the disease is still diverse. Symptoms vary from severe, debilitating malabsorption states to mild symptoms such as fatigue, abdominal complaints and isolated iron deficiency (Mulder and Cellier, 2005). However, due to increased publicity and more intensified teaching practices in this area, the growing knowledge of CD has certainly effected reporting on incidence and prevalence of the disease. As a result, the ability to identify potential cases has improved, and its prevalence has been cited as high as 0.3%–1% of the general population of the United States and Europe (Fasano et al., 2003). The age of onset of CD is also very variable. Reports suggest that 60% of newly diagnosed patients are adults, with 15%–20% being over 60 years old (Jansen et al., 1993). CD is also common in children, with a prevalence of 1:80–1:300 (Rodrigues and Jenkins, 2006). Blood tests (tTG) are reported as being 90–95% sensitive; however, a small intestine biopsy is still mandatory in childhood. A meta-analysis by Akobeng et al. (2006) revealed that breast feeding may offer protection against the development of CD. The authors found that breast feeding whilst introducing gluten into the diet, as well as increasing the duration of breast feeding was associated with reduced risk of developing CD. They were unsure, however, if the onset of CD was merely delayed, or whether this was a permanent solution.

12.2.2 Treatment

We know that successful treatment of CD requires lifelong adherence to a strict GFD, eliminating all products containing wheat, barley and rye. Failure to comply with the diet can result in long-term adverse health consequences. However, as well as following a GFD, other precautions should also be taken. Woodward (2007) proposed that until intestinal recovery has occurred, it would be appropriate to supplement identified deficiencies such as iron, folate and calcium. For example, calcium absorption may be reduced despite GF dietary compliance. Therefore, a higher than normal intake should be recommended (15mg/day). Case and Zarkadas (2004) studied the compliance to a GFD amongst Canadians, and its impact on the quality of life. Ninety percent of diagnosed coeliacs were following a GFD. Amongst these, 40% found the diet very or moderately difficult to follow, with the main difficulties occurring in the following areas: determining whether foods were actually GF, finding GF foods in stores, avoiding restaurants and travelling. However, Mayer et al. (1991) reported that a poor dietary compliance was evident in adolescents, where only 50–65% of individuals were adhering to a GFD. Bramall (2000) described the difficulty of adhering to a GFD when eating out, at social events and while travelling. Some patients reported a large proportion of their luggage consisted of gluten-free products. Gluten-free products currently on the market are still not of equal quality to their gluten-containing counterparts. The majority are more expensive, and Mulder and Cellier (2005) has proposed that coeliacs be reimbursed in some way both for the gluten-free foods they purchase and also for any counselling and dietetic services utilised.

12.2.3 What does the future hold for the coeliac patient?

As previously mentioned, complying with a GFD is difficult, and today new treatment strategies are being actively pursued. Sollid and Khosla (2005) suggest that the quality of life of CD patients would improve if there was a treatment that would allow some gluten to

be consumed over a short period of time (e.g. while travelling) through the use of therapeutic agents. These authors also stressed the need in the future for alternative treatments for refractory sprue (when symptoms of malabsorption persist or regress after an initial good response despite a strict adherence to a GFD). Refractory CD is currently treated only with harsh immunosuppressive drugs. The issues of alternative therapeutic strategies to the GFD, as well as possible gluten detoxification techniques have been raised in a few publications. For example, Shan et al. (2002) proposed to detoxify gluten within the intestine by cleaving the gliadin through the use of a bacterial endoprolyl protease (PEP) to degrade the proline-rich polypeptides into short peptides that lose their activity. Animal trials were completed, but the efficacy of this therapy in patients remains to be assessed. Other studies have focused on putting the coeliac patient on a normal diet and add a drug designed to abolish the T-cell stimulatory capacity of the gluten (Molberg et al., 2005). The idea of an oral administration of a therapeutic dose of a suitably formulated PEP was put forward by Hausch et al. (2002), to possibly counter the effects of moderate quantities of ingested gluten. This is supported by in vivo, in vitro and ex vivo studies by other authors (Piper et al., 2004; Shan et al., 2004; Marti et al., 2005). A very recent and intriguing development has been described by Gianfrani et al. (2007). Her collaborative study showed that transamidation of wheat flour with a food grade enzyme and an amine group donor can be used to block gliadin activity. An added bonus from this study was that the functionality of the wheat protein was preserved. In fact, the baking properties of breads from this treated flour were enhanced by the addition of the enzyme.

Cytokine therapies are currently being developed for the treatment of chronic inflammation (Sollid and Khosla, 2005). The authors suggest that such an approach may yield a compound in the future that would be well tolerated, with a mode of action relevant to CD.

Hill (2003) emphasised the need to identify the specific peptide(s) in wheat, barley and rye that are responsible for CD in genetically predisposed people. Some studies have been carried out in the development of wheat grains that have a low (or no) content of immunotoxic sequences, but maintaining their functional properties. In-depth studies in this area were not easy during the 1970s–1990s, as molecular knowledge relating to T-cell stimulatory gluten sequences was not available. However, some studies have been more successful in the new millennium. For example, Vader et al. (2003) studied the repertoire of T-cell stimulatory peptides in barley, rye and oats, and investigated a strategy to destroy T-cell stimulatory epitopes on the basis of T-cell cross-reactivity with gluten proteins. They showed that subtle changes in gluten genes would eliminate some of the T-cell stimulatory properties of gluten molecules. This study provides a rational step to an approach for gluten detoxification via targeted mutagenesis at the genetic level. However, whether it will be applicable for the generation of safer wheat strains in the future still remains to be determined. Through their trials, Molberg et al. (2005) proposed the selective breeding of ancient wheat varieties. They suggest from their results that screening primitive wheat cultivars, followed by breeding and selection based on the absence of certain gluten protein sequences may have a future potential in producing wheat cultivars that are lacking in sequences which would be harmful to those with CD.

The prolamins of rye (secalins) release a family of Pro- and Gln-rich polypeptides that are responsible for the autoimmunological response that triggers coeliac enteropathy (Silano and De Vincenzi, 1999). De Angelis et al. (2006) studied the ability of selected sourdough LAB to hydrolyse these polypeptides. Using several complementary techniques (including 2D electrophoresis, MALDI-TOF mass spectrometry, reversed-phase HPLC and R5-Western blot), they showed that the selected pool of four *Lactobacillus* strains had the capacity to

extensively hydrolyse the rye prolamins. Their potential for degrading these prolamins in high concentrations of rye flour remains to be proven, but the authors are hopeful that future studies could prove the potential of sourdough Lactobacilli strains to eliminate toxic rye peptides in foods. The same pool of selected LAB was used to preferment durum wheat semolina (Di Cagno et al., 2005). After fermentation, the dough was freeze-dried, mixed with buckwheat flour and 'fusilli' type pasta was produced. 2D electrophoresis and MALDI-TOF mass spectrometry showed that the durum wheat gliadins were almost completely hydrolysed during fermentation by the sourdough lactobacilli. However, complete elimination of toxic proteins has not yet been achieved by scientists, and this is where future research in the area must be focused. Gobbetti et al. (2007) are currently focused on decreasing the levels of gluten to below 20 ppm, through the use of the above sourdough strain, coupled with the addition of fungal proteases. Their results are eagerly awaited.

Although much research is actively ongoing in the area of CD and improvement in the quality of life for CD patients, nevertheless, a definitive alternative to a GFD still does not exist. It will be interesting to see if any of the proposed alternative therapies and strategies will come to fruition in the near future.

12.3 GLUTEN-FREE INGREDIENTS, FORMULATIONS AND PROCESSING: WHAT'S IN STORE FOR THE FUTURE?

As the importance of research into alternatives to the GFD is heightened and currently making good progress, the surge in research by food scientists is also evident, as witnessed by the growing number of scientific publications in this area in recent years. Despite such rigorous research and new information relating to gluten-free alternatives and ingredient interactions, the selection of products on the market (in particular breads) is still not of a comparable quality to that of wheat-based baked goods, and the ultimate polymer blend has still to be established. This is not the fault of the cereal scientist or industrial baker; it merely reflects the extent of the challenge presented. As already mentioned many times in the previous chapters, gluten is an integral constituent of the breadmaking process. Its absence leads to a complete breakdown of dough structure and elasticity, and the ingredients used in its place (starches, gums, hydrocolloids, proteins, enzymes) have so far not completely replaced the functional properties of gluten. Some of the most recent gluten-free research publications have focused on diverse ideas: the use of sorghum in breads and cookies (Ciacci et al., 2007; Schober et al., 2007), alternative protein isolates in breads (Gallagher et al., 2007; Marco and Rosell, 2008), enzymes in breads (Marco et al., 2007; Renzetti et al., 2008), fortification of bread through iron compounds (Kiskini et al., 2007), hydrocolloids in breads (Lazaridou et al., 2007), pseudocereals and legumes in spaghetti (Chillo et al., 2008) and bread (Gallagher et al., 2008), amaranth in spaghetti (Chillo et al., 2007), hydrocolloids in pastry dough (Lorenzo et al., 2008).

12.3.1 New formulations

In recent years, studies have diversified to incorporate gluten-free ingredients and formulations, coupled with old processing techniques applied in a novel way. Cellulose derivatives (e.g. HPMC, CMC) are regularly used in gluten-free formulations (McCarthy et al., 2005; Chillo et al., 2007; Lazaridou et al., 2007). They can have anti-staling properties, control

water absorption and dough rheology (Mandala et al., 2008) and also can enhance the crumb texture of bread (Sivaramakrishnan et al., 2004). However, they are expensive ingredients, and concerns have been voiced by industry about their cost. This section will focus not on ingredient combinations which may be used to replace gluten (this has been covered extensively in previous chapters), but on possible alternative *processing* methods which might be considered in the future.

12.3.2 Extrusion

This has been described by Acs et al. (1996a,b) as a suitable process for producing snack foods for patients suffering from CD, as starch, which is the main component in gluten-free formulations, provides a desirable expanded structure in the end product. Extruders provide both thermal and shear energy to the food material undergoing physical and chemical changes. In more recent studies, İbanoğlu et al. (2006) showed how the effect of extrusion screw speed and also the feed rate significantly affects the expansion properties of the cereals used, and hence the firmness of the product.

12.3.3 Resistant starch

Strategies to manufacture resistant starch (RS) were discussed by Thompson (2000). Starch which is not hydrolysed in the small intestine is considered RS in the sense that it has resisted digestion/hydrolysis, and is considered to have beneficial effects similar to those of some forms of dietary fibre (Rabe, 1999). Thompson's review focused on physical and chemical treatments which starch may be subjected to for altering the level of RS in a food or ingredient. Methods described involve chemical modification, heating and the use of moisture. As the majority of gluten-free products are based predominantly on starches, these processes would be worth considering for gluten-free producers. However, the type of RS formed may be lost upon baking, so care needs to be taken during the processing step.

12.3.4 Light pulses

The use of light pulses (PL) is a potential novel technology for food preservation. It is a technique used to decontaminate surfaces and packaging materials through the use of intense and short duration pulses of UV light (Gómez-López et al., 2007). As PL penetrate certain packaging materials, wrapped items such as bread can also be treated. Butz and Tauscher (2002) proposed that light pulses are able to extend the shelf life of bread, cakes and pastries. Knowledge of the mechanism of inactivation by PL and the number of reports on food applications have increased considerably during the last 15 years (Gómez-López et al., 2007). The most important technological problems are to find ways to control food heating and to homogeneously treat foods. Dunn et al. (1989) described how PL may be used as a preservation method for white bread rolls. More research is, however, needed on the nutritional consequences and possible formation of toxic by-products. However, this method may provide a possible avenue of much needed shelf life extension in gluten-free research.

12.3.5 Heat transfer

During the baking process, a range of chemical and biochemical reactions occur that include starch gelatinisation, protein denaturation, Maillard reactions and starch-sugar and

starch-lipid interactions (Yong et al., 2002). Conduction, convection and radiation heat transfer mechanisms are present, changing the physical properties of the products. No reports have been published on gluten-free research which relates the heat transfer characteristics during baking to the properties of the baked products. It is something that surely should be considered for bakers especially when dealing with complex gluten-free systems, as changes in one physical property (e.g. moisture loss, changes in internal temperature, change in size due to bubble expansion, changes in internal and external structure) directly affects other properties. Yong et al., (2002) concluded from their results that the contribution of heat transfer mechanisms in an industrial processing environment could be manipulated to achieve a desired quality in the final baked products.

12.3.6 Nanotechnology

The principle of nanotechnology is that materials with known properties and functions at their normal sizes take on different and often useful properties and functions at their nanosizes (Nickols-Richardson, 2007). In its simplest definition, nanotechnology refers to the process of fabricating and/or controlling matter that is 1 to 100 nm in size (Nickols-Richardson, 2007). Therefore, the understanding of materials at the atomic, molecular and macromolecular levels is crucial. It has a major potential to generate new products with numerous benefits. A greater surface area per mass compared with larger particles of the same chemistry renders nanosized particles biologically more active (Oberdörster et al., 2005). Therefore, the use of nanotechnology has potential in nutraceuticals and functional foods for delivering bioactive compounds to improve health. In their comprehensive review, Sanguansri and Augustin (2006) discuss how concepts in nanoscience and nanotechnology provide a sound framework for developing an understanding of the interactions and assembly of food components into microstructure, which influence food structure, rheology and functional properties at the macroscopic level. A limited number of studies involving nanoscience exist in food research, and none in the gluten-free area. However, in recent years nanotechnology has been employed in food production and food packaging (Kuzma and VerHage, 2006), and food and nutrition products containing nanoscale additives are commercially available (Siegrist et al. (2007). This is, however, in comparison to the biomedical, manufacturing and information technology sectors, where nanotechnology has been utilised extensively. The food industry should reflect on advances made in these fields and how these opportunities could be explored in food product and process development. Although the focus on food formulation, textural and nutritional aspects should remain, attention also needs to be directed towards the targeted release of food components during gastrointestinal transit, while at the same time maintaining high quality texture, structure and sensory appeal (Sanguansri and Augustin, 2006). Such an approach to foods is set to become increasingly more relevant as the relationships between genetics, diet and health are further established.

In relation to coeliac patients and the work of food scientists and bakers, nanotechnology has the potential to enhance flavours, aromas and recipe formulations. It also has potential to alter nutrient intake by broadening the number of enriched and fortified food products available for those following a GFD. Such foods will be particularly beneficial for those who suffer the malabsorption effects of CD. For example, Macdermott (2007) surmised how the resolution of inflammatory bowel disease through nanopharmaceuticals enabled the sufferers to expand their limited food tolerances and choices.

Moraru et al. (2003) suggested that areas in the food industry which would benefit from nanotechnology are the development of new functional materials, micro and nano scale

processing methods and new product development. The use of nanoscience may control interactions between proteins, lipids and polysaccharides to achieve the desired structural and rheological properties of novel foods (Dickinson, 2004). Proteins are regularly used in gluten-free research for structure formation (e.g. emulsification, gelation, water binding properties) and nutritive enhancement, particularly in bread formulations. Recent publications have described the use of dairy proteins, soybean, egg, pea proteins and a range of legumes in gluten-free research, and their effects have already been reported in previous chapters. Chen et al. (2006) report how food proteins can be used to prepare a wide range of matrices in the form of nanoparticles, which can be tailored for specific applications in the development of innovative functional foods. Also, modifications of these nanoparticles will allow them to form complexes with polysaccharides, lipids or other biopolymers, and a wide variety of nutrients can be incorporated by relatively non-specific means. The use of proteins as encapsulants for the microencapsulation of bioactive substances is also a form of nanotechnology. Bioactives are generally unstable; therefore, they are protected both from oxygen and from other food components in the food matrix during processing (e.g. mixing, high baking temperatures) until their targeted release at the desired site of action in the body.

As the potential of nanotechnologies is gradually being realised, the area is now being recognised as an important area of scientific investment (Linton and Walsh, 2007). It should be recognised, however, that nanotechnology is a new area of science and the advantages and limitations of its use in the food industry are still not fully understood (Sanguansri and Augustin, 2006).

12.3.7 Bioprocessing and biotechnology

The use of bioprocessing and biotechnology is another avenue that needs to be researched in greater depth. It has been proven that certain bioprocessing methods, through the application of fermentation procedures or lactic acid bacteria strains, have enhanced certain properties of breads (Luikkonen et al., 2003; Jägerstad et al., 2005).

12.3.8 High pressure processing (HPP)

High pressure processing is a non-thermal processing technique for inactivating vegetative microorganisms and many harmful enzymes in liquid and solid foods (Knorr, 1998). However, besides the destruction of microorganisms, HPP can also be used for protein denaturation or modification, or for changing the properties of carbohydrates and fats. Foods treated by HPP have been shown to keep their freshness, colour, flavour and taste (Butz and Tauscher, 2002). No research has been reported on protein modification for gluten-free purposes. Although HPP is not a cheap technology, its ability to alter the structure of ingredients and ultimately the shelf life of the baked product must surely render it as a viable option for research in the near future. Chapleau and de Lamballerie-Anton (2003) improved the emulsifying properties of lupin proteins by HPP. HPP also improved the emulsifying properties of soy protein isolate (Denda and Hayashi, 1992).

12.3.9 Dairy proteins

Many studies already exist which focus on the inclusion of dairy proteins (Gallagher et al., 2003; Moore et al., 2006) in gluten-free bread formulations. The value of dairy ingredients (such as whey proteins, casein isolates) stem from their inherent ability to impart a range of

desirable attributes to bakery products in general, while at the same time contributing to the nutritional quality of the products. However, the possibility of utilising improved forms of these ingredients through appropriate processing methods certainly opens up new avenues of exploration in the gluten-free field. In a recent paper, Krešić et al. (2008) have promoted the use of HPP for dairy ingredients, as it has the potential to extend their use as novel ingredients in bakery, dairy and meat product applications. Clubbs et al. (2005) investigated the potential for HPP of tortillas to reduce the level of spoilage microflora and decrease or eliminate off-flavours associated with antimicrobial and anti-staling agents. Tortillas, like bread, are high moisture foods.

12.3.10 Analysis and testing procedures

It is timely and pertinent that the actual testing and analysis procedures of the doughs and finished gluten-free products should also receive attention. In recent years, many accounts of research in the gluten-free field have been published, highlighting the importance of this area, and revealing significant applications of gluten alternatives in wheat-free products such as breads and cookies. However, it should also be noted that fundamental methods to study the molecular interactions between the constituents of gluten-free formulations have not been thoroughly explored to date. Therefore, we are still lacking in basic knowledge in this area. Many studies have been carried out in relation to wheat dough mixing, bubble formation, gas production and retention during proofing and a whole range of mechanical properties relating to bread crumb and crust formation both during and after baking. However, such an understanding in relation to gluten-free batter/dough/bread characteristics is not widespread, and future studies should focus on these attributes. As well as empirical tests and procedures such as rheology, and image analysis, more fundamental techniques should also be employed where feasible. Studies involving ultrasonics or nuclear magnetic resonance (NMR, MRI), which are being used routinely in wheat dough and bread research (Elmehdi et al., 2003; Grenier et al., 2003; Garcia-Alvarez et al., 2006; Doona and Baik, 2007) should now be introduced to extract more information for gluten-free doughs and crumb structures.

12.4 CONCLUSIONS

An increased awareness of CD has, in recent years, led to improved methods of diagnosis. Although lifelong adherence to a GFD remains the key treatment for the disease, research is ongoing for alternative treatments to improve the quality of life for the coeliac patient. Food scientists, also, are striving to improve the palatability and texture of gluten-free products. However, this remains a formidable challenge, and for future research in this area to be successful, new approaches such as nanotechnology and bioprocessing techniques need to be addressed.

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