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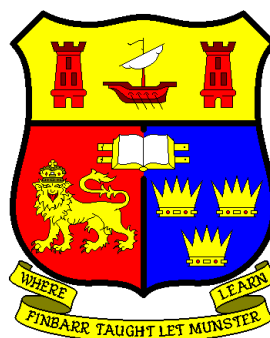University College, Cork

School of Food and Nutritional Sciences

Cereal products for specific dietary requirements

Evaluation and improvement of technological and nutritional properties of gluten free raw materials and end products

Thesis presented by

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Declaration by Candidate

I hereby declare that this thesis is my own work and effort and has not been submitted for another degree, neither at University College Cork nor elsewhere. Where other sources of information have been used, they have been acknowledged.

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Signature of candidate

Abstract

Coeliac disease is one of the most common food intolerances worldwide and at present the gluten free diet remains the only suitable treatment. A market overview conducted as part of this thesis on nutritional and sensory quality of commercially available gluten free breads and pasta showed that improvements are necessary. Many products show strong off-flavors, poor mouthfeel and reduced shelf-life. Since the life-long avoidance of the cereal protein gluten means a major change to the diet, it is important to also consider the nutritional value of products intending to replace staple foods such as bread or pasta. This thesis addresses this issue by characterising available gluten free cereal and pseudocereal flours to facilitate a better raw material choice. It was observed that especially quinoa, buckwheat and teff are high in essential nutrients, such as protein, minerals and folate. In addition the potential of functional ingredients such as inulin, β-glucan, HPMC and xanthan to improve loaf quality were evaluated. Results show that these ingredients can increase loaf volume and reduce crumb hardness as well as rate of staling but that the effect diverges strongly depending on the bread formulation used. Furthermore, fresh egg pasta formulations based on teff and oat flour were developed. The resulting products were characterised regarding sensory and textural properties as well as *in vitro* digestibility. Scanning electron and confocal laser scanning microscopy was used throughout the thesis to visualise structural changes occurring during baking and pasta making.

Acknowledgements

Dedication

To my grandmother Hanne Schichl and my parents Ursula and Franz Hager who emphasised

since my early childhood, how valuable education is.

Chapter 1: Introduction

The gluten free diet was introduced more than 50 years ago, originally as the standard therapy for coeliac disease patients. It remains up to now the only treatment for this life-long autoimmune enteropathy. Classical gluten-induced symptoms of coeliac disease are pubertal growth delays, abdominal pain, diarrhea, nausea and malabsorption deficiencies of vitamins or trace elements. The disease can also manifest outside the gastrointestinal tract (e.g. dermatitis herpetiformis, osteoporosis and neurological disorders) or show no symptoms at all (Kaukinen *et al.* 2010). Damage done to the small intestine of genetically susceptible people is reversed when gluten is excluded. Screening studies have revealed that coeliac disease affects about 1 % of the general population in Western countries (Fasano *et al.*, 2003, Lohi *et al.*, 2007, Riestra *et al.*, 2000, Schapira *et al.*, 2003). A much higher percentage of the general population than this 1 % consider themselves to be suffering from wheat sensitivity and exclude wheat from their diet (Carroccio *et al.* 2012). Purchasers of gluten free products are both diagnosed and undiagnosed individuals of above mentioned conditions as well as their relatives. Furthermore, certain people choose to adhere to a gluten free diet for perceived health benefits or as a life style choice (Packaged Facts Report 2001). Despite technological developments and increased availability over recent years, gluten free products are still very often characterised by poor sensory properties (i.e. dense, crumbly, off-flavors) and reduced shelf-life (i.e. mold spoilage, staling). Due to increased awareness and improved diagnosis, there are a growing number of individuals who want to find a wider choice of better tasting gluten free products and who are willing to pay a premium price (Agriculture and Agri-Food Canada 2011). Hence, the production of high quality gluten free products represents an important socio-economic issue and it is not surprising that the market has experienced significant growth over the past few years.

1.1 Legal standing and Labelling

For regulatory purposes "gluten" is defined as the protein fraction from wheat, rye, barley and oats or their crossbred varieties and derivatives thereof, to which some persons are intolerant. The guidelines proposed by the Food and Drug Administration (FDA) are similar but do not include oat, as the FDA has concluded, based on available research data and clinical trials that the majority of individuals with coeliac disease can tolerate a limited daily intake of uncontaminated oats. From a scientific point of view, using the term "gluten" to describe storage proteins of rye, barley and oats is not completely correct as the coeliac-toxic fractions of these cereals are termed secalin, hordein and avenin, respectively.

To set gluten free standards for international trade purposes, the Codex Alimentarius Commission concluded that gluten free foods can not contain wheat, rye, barley, oats or their crossbred varieties, unless they have been specially processed to reduce the gluten level to below 20 ppm. The standard also states that oats can be tolerated by most but not all people who are intolerant to gluten. Therefore, the allowance of oats that are not contaminated with wheat, rye or barley in foods covered by this standard may be determined at the national level. In the European Union foodstuffs for people intolerant to gluten, that contain a level of gluten not exceeding 100 ppm, may bear the term "very low gluten" (Codex Alimentarius Commission 2009).

1.2 Adherence

Adherence to a gluten free diet, i.e. the lifelong elimination of wheat, rye and barley, results in significant clinical improvements for most patients. Remission of the intestinal mucosa results in a relief of symptoms and a decrease of long-term health risks such as osteoporosis, nutritional deficiencies or gastrointestinal malignancies. Reported levels of strict adherence to the gluten free diet in adult coeliac disease patients vary between 42% and 91% (Hall *et al*. 2009). Studies examining factors related to adherence to a gluten free diet found that factors most strongly associated with compliance are cognitive, emotional and sociocultural influences

as well as dietetic follow-up (Hall *et al*. 2009). One study found that understanding food labelling and affordability were significantly associated with adherence of patients in the UK (Butterworth *et al*. 2004). Conforming to a gluten free diet can have social consequences because normative social rules in relation to meals are not followed when refusing available food (Sobal 2000). Differences in the appearance of gluten free meals, and the generally poor availability and high price of gluten free food not only decreases quality of life but can also result in non-compliance with the gluten free diet. Roma *et al*. (2010) investigated the dietary compliance of children with coeliac disease and reasons provided for nonadherence included dining out, poor availability of products and social pressure. The authors showed that poor palability of gluten free products was the most important reason for non-compliance.

1.3 Availability and cost of gluten free foods

Little is known about availability and cost of gluten free foods, although these two factors certainly influence compliance to a gluten free diet. A study conducted in the UK by Singh and Whelan (2011) demonstrated that, in general, the availability of gluten free foods is limited. The authors also showed that the gluten free version of all 10 wheat-based foods (bread loaf, bread rolls, flaked breakfast cereals, pasta, plain flour, cream crackers, sweet biscuits, fruit pies, pizza base and whole cake) was significantly more expensive, up to 360% higher costs. A Canadian study screened gluten free foods at two grocery stores and found that they were 180% more expensive than standard products (Stevens and Rashid 2008). Lee *et al*. (2007) surveyed 11 gluten free foods in the U.S.A. and found that all products were more expensive than their gluten containing counterparts. These higher prices can be explained by the elevated raw material costs (e.g. alternative cereals, hydrocolloids, etc.), considerable challenges in product development, distribution issues as well as increased analytical and quality control expenses.

1.4 Quality of life

Strict adherence to a gluten free diet represents a difficult challenge and might seriously compromise the quality of life (QoL). Studies on the matter have produced conflicting results. While a Swedish (Roos *et al.* 2006), a Canadian (Zarkadas *et al.* 2006) and U.S.-American study (Green *et al.* 2001) showed an average health related quality of life for adult coeliac disease patients comparable to that of the general population, studies conducted in the UK (Ford *et al.* 2012), Ireland (O'Leary *et al.* 2002), Italy (Fera *et al.* 2003) and Germany (Hauser *et al.* 2006) demonstrated a lower average health related quality of life. Many patients clearly regard their coeliac disease as intrusive and burdensome (Whitaker *et al.* 2009, Barratt *et al.* 2011). Whitaker *et al.* (2009) evaluated patient perceptions of the burden of coeliac disease and its treatment in the UK. In that study, 68% of coeliacs reported that their dietary restrictions reduced their enjoyment of food. About half the subjects (54%) reported doing things they enjoyed less often because of their dietary restrictions, with eating out being the most common activity sacrificed. In the study of Barratt *et al.* (2011), 88% of subjects reported that eating out presents difficulties, while 72% described travelling and 71% socialising as difficult. Even though individuals with coeliac disease show no obvious external signs to indicate their condition to others, interaction in different social contexts produces circumstances in which it is made visible to others (Olsson *et al.* 2009). Feelings associated with social deviance were described as being more pronounced at primary school age, but also made adolescents feel awkward (Olsson *et al.* 2009). De Lorenzo *et al.* (2012) evaluated the quality of life of children adhering to a gluten free diet and found that QoL total scores were comparable between children with and without coeliac disease. However, regarding the leisure dimension, which is related to eating and socialising, significantly lower QoL scores were reported for the coeliac subjects.

In a large Finnish nationwide cohort, Ukkola *et al.* (2012) conducted a prospective study to evaluate the impact of diagnosis and treatment of coeliac disease. Of all patients, only 12%

reported having difficulties with adhering to a gluten free diet and the great majority of patients in all study groups had a positive general attitude towards their disease. However, when asked to indicate in their own words their special wishes and needs, a better availability of gluten free products was frequently mentioned.

1.5 Objectives

The above observations clearly show that research on gluten free raw materials and end products is needed and can potentially make an important difference to people's life. Poor availability and bad quality of gluten free products means that individuals end up balancing health benefits and social sacrifices; often tolerating side effects such as stomach pain or diarrhoea in order to take part in popular activities like eating-out or drinking beer. It is also worth mentioning that despite the benefits of a gluten free diet on symptoms of coelic disease, a number of sequelae such as lower intakes of essential micronutrients (i.e. iron, calcium, folate) and higher sugar intakes have been reported (Kinsey *et al*. 2008; Ohlund *et al*. 2010; Wild *et al*. 2010). Therefore, the aim of this thesis was the evaluation and improvement of technological and nutritional properties of gluten free raw materials and end products. At first two market studies were performed to get an overview of gluten free bread and pasta products currently available on the market (chapters 3 and 4). Hereupon, different approaches for the improvement of gluten free products were taken. Chapters 5 and 6 evaluate the potential of the functional ingredients HPMC, xanthan and β-glucan to improve gluten free breads. A more natural approach is the careful selection of raw materials. Hence, chapter 7 evaluates the nutritional value of different gluten free flours, while chapter 8, 9 and 10 deal with their suitability for the production of bread and pasta. Before all, chapter 2 gives an overview on the formulation of breads for specific dietary requirements.

1.6 References

Agriculture and Agri-Food Canada (2011) Gluten-Free Packaged Foods in the United States. ISSN 1920-6615 Market Indicator Report

Barratt S.M, Leeds J.S and Sanders D.S. (2011) Quality of Life in Coeliac Disease is Determined by Perceived Degree of Difficulty Adhering to a Gluten-Free Diet, not the Level of Dietary Adherence Ultimately Achieved. Journal of Gastrointestinal and Liver Diseases, 20(3), 241-245

Butterworth J.R, Banfield L.M, Iqbal T.H. and Cooper B.T. (2004) Factors relating to compliance with a gluten-free diet in patients with coeliac disease: comparison of white Caucasian and South Asian patients. Clinical Nutrition, 23, 1127-1134

Codex Alimentarius Commission (2009) Commission regulation (EC) No 41/2009 of 20 January 2009 concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten

De Lorenzo C.M, Xikota J.C, Chang Wayhs M, Modesto Nassar S, De Souza Pires M.M. (2012) Evaluation of the quality of life of children with celiac disease and their parents: a case-control study. Quality of Life Research, 21, 77-85

Fasano A, Berti I, Gerarduzzi T, Not T, Colletti R.B, Drago S, Elitsur Y, Green P.H.R, Guandalini S, Hill I.D, Pietzak M, Ventura A, Thorpe M, Kryszak D, Fornaroli F, Wasserman S.S, Murray J.A. and Horvath K. (2003) Prevalence of celiac disease in at-risk and not-at-risk groups in the United States - A large multicenter study. Archives of Internal Medicine, 163, 286-292

Fera T, Cascio B, Angelini G, Martini S. and Guidetti C.S. (2003) Affective disorders and quality of life in adult coeliac disease patients on a gluten-free diet. European Journal of Gastroenterology & Hepatology, 15(12), 1287-1292

Ford S, Howard R. and Oyebode J. (2012) Psychosocial aspects of coeliac disease: A cross-sectional survey of a UK population. British Journal of Health Psychology, 17, 743-757

Green P.H.R, Stavropoulos S.N, Panagi S.G, Goldstein S.L, McMahon D.J, Absan H. and Neugut A.I. (2001) Characteristics of adult celiac disease in the USA: Results of a national survey. American Journal of Gastroenterology, 96(1), 126-131

Gray M. and Papanicolas I.N. (2010) Impact of symptoms on quality of life before and after diagnosis of coeliac disease: results from a UK population survey. BMC Health Service Research, 10, 105

Hall N.J, Rubin G. and Charnock A. (2009) Systematic review: adherence to a gluten-free diet in adult patients with coeliac disease. Alimentary Pharmacology and Therapeutics, 30, 315-330

Hauser W., Gold J., Stein J., Caspary W.F. and Stein J. (2007) Health-related quality of life in adult coeliac disease in Germany: Results of a national survey. European Journal of Gastroenterology and Hepatology, 18, 747-754

Kaukinen K, Lindfors K, Collin P, Koskinen O. and Mäki M. (2010) Coeliac disease – a diagnostic and therapeutic challenge. Clinical Chemistry and Laboratory Medicine, 48, 1205-1216

Kinsey L, Burden S.T. and Bannerman E. (2008) A dietary survey to determine if patients with coeliac disease are meeting current healthy eating guidelines and how their diet compares to that of the british general population. European Journal of Clinical Nutrition, 11, 1333-1342

Lee A.R, Ng D.L, Zivin J. and Green P.H. (2007) Economic burden of a gluten-free diet. Journal of Human Nutrition and Dietetics 20, 423-430

Lohi S, Mustalahti K, Kaukinen K, Laurila K, Collin P, Rissanen H, Lohi O, Bravi E, Gasparin M, Reunanen A. and Maki M. (2007) Increasing prevalence of coeliac disease over time. Alimentary Pharmacology & Therapeutic, 26, 1217-1225

Ohlund K, Olsson C, Hernell O. and Ohlund I. (2010) Dietary shortcomings in children on a gluten-free diet. Journal of Human Nutrition and Dietetics, 23, 294-300

O'Leary C, Wieneke P, Buckley S, O'Regan P, Cronin C, Quigley E.M, Shanahan F. (2002) Celiac disease and irritable bowel-type symptoms. American Journal of Gastroenterology, 97, 1463-1467

Olsson C, Lyon P, Hörnell A, Ivarsson A. and Mattson Sydner Y. (2009) Food that makes you different: the stigma experienced by adolescence with coeliac disease. Qualitative Health Research, 19(7), 976-984

Packaged Facts Report (2011) Gluten-free Foods and Beverages in the U.S. 3rd Edition

Riestra S, Fernandez E, Rodrigo L, Garcia S. and Ocio G. (2000) Prevalence of coeliac disease in the general population of northern spain - Strategies of serologic screening. Scandinavian Journal of Gastroenterology, 35, 398-402

Roma E, Roubani A, Kolia E, Panayiotou J, Zellos A. and Syriopoulou V.P. (2010) Dietary compliance and life style of children with coeliac disease. Journal of Human Nutrition and Dietetics, 23, 176-182

Roos S, Karner A. and Hallert C. (2006) Psychological well-beeing of adult celiac patients treated for 10 years. Digestive and Liver Disease, 38, 177-182

Schapira M, Maisin J.M, Ghilain J.M, De Maeght S, Deltenre P. and Henrion J. (2003) Epidemiology of coeliac disease. Acta Gastro-Enterologica Belgica, 66, 234-236

Singh J. and Whelan K. (2011) Limited availability and higher cost of gluten-free foods. Journal of Human Nutrition and Dietetics, 24, 479-486

Sobal J. (2000) Sociability and meals: Facilitation, commensality, and interaction. In: Meiselman H. (Ed.) Dimensions of the meal. The science, culture, business, and art of eating. Aspen Publishers, Gaithersburg, Maryland

Stevens L. and Rashid M. (2008) Gluten-free and regular foods: a cost comparison. Canadian Journal of Dietetic Practise and Research 69, 147-150

Ukkola A, Mäki M, Kurppa K, Collin P, Huhtala H, Kekkonen L. and Kaukinen K. (2012) Patients' Experiences and Perceptions of Living with Coeliac Disease – Implication for Optimizing Care. Journal of Gastrointestinal and Liver Disease, 21(1), 17-22

Whitaker J.K.H, West J, Holmes G.K.T. and Logan R.F.A. (2009) Patient perceptions of the burden of coeliac disease and its treatment in the UK. Alimentary Pharmacology & Therapeutics 29, 1131-1136

Wild D, Robins G.G, Burley V.J. and Howdle P.D. (2010) Evidence of high sugar intake, and low fibre and mineral intake, in the gluten-free diet. Alimentary Pharmacology and Therapeutics 32, 573-581

Zarkadas M, Cranney A, Case S, Molloy M, Switzer C, Graham I.D, Butzner J.D, Rashid M, Warren R.E. and Burrows V. (2006) The impact of a gluten-free diet on adults with coeliac disease: Results of a national survey. Journal of Human Nutrition and Dietetics, 19, 41-49

Chapter 2: Literature Review on the Formulation of Breads for Specific Dietary Requirements

Anna-Sophie Hager, Emanuele Zannini and Elke Karin Arendt (2012)

In: Breadmaking: Improving quality. Editor: Stanlay Cauvain. Woodhead Publishing

2.1 Introduction

In modern times consumer needs as well as preferences regarding bread have changed substantially. The only purpose of ingestion of bread is not any longer the intake of energy, but moreover consumers expect additional nutritional and functional value. The number of people following a certain diet, if prescribed by the doctor or voluntarily, is increasing. Also the so-called diseases of civilization such as obesity, type-2-diabetes and coronary heart disease as well as colo-rectal cancer are increasing due to changes in life style and eating behaviour of the western population (WHO, 2005). It is becoming apparent that cereals in general have the potential for health enhancement beyond providing macro- and micronutrients required for human growth and maintenance and that their consumption can lower the risk of diet-related diseases quite substantially (Topping 2007). Therefore increasing interest exists in the manufacture of breads for special dietary requirements and breads with increased nutritional value. Certainly one of the biggest challenges in this respect is the production of good quality gluten-free bread. Also the formulation of wholegrain breads, baked goods with low glycemic index or enriched with dietary fibre is of increasing interest. This chapter will commence with the discussion of wheat allergy and coeliac disease and the formulation of wheat/gluten free breads. The concept of glycemic index and glycemic load will also be explained. In addition, an overview of the production of breads with low glycemic index through incorporation of sourdough, whole grains and different dietary fibres will be given.

2.2 Wheat allergy and coeliac disease

It is a fundamental requirement that all food manufacturers, caterers and retailers ensure that any food that they supply is safe to eat and is labelled in accordance with relevant legislation. However, even when foods are manufactured and marketed under well-controlled conditions,

they can still pose a threat to individuals who are allergic or intolerant to one or more of the ingredients (Dean 2000). In this relation the baking industry has to consider individuals suffering from wheat allergy and/or coeliac disease.

Coeliac disease is one of the most frequent genetically based diseases, affecting approximately 1 % of the western population (Catassi and Yachha 2009). IgE-mediated wheat allergy is relatively uncommon, with a cumulative incidence of 1-2 % in the first six years of life and a reduced prevalence in later childhood and adulthood. It is also responsible for the occupational disease referred to as baker's asthma (Dean 2000). Both coeliac disease and wheat allergy are characterized by a clinically abnormal response induced in susceptible persons by wheat components, which are well tolerated by the vast majority of the population. However, there are important differences between wheat allergy and coeliac disease regarding the mechanism of the immune response as well as nature and severity of the symptoms. Wheat allergy can be characterized by a severe sudden onset of symptoms, which include coughing, breathing difficulties, projectile vomiting and even anaphylactic shock. The symptoms of wheat allergy are not limited to the gastronintestinal tract but are also cutaneous, respiratory and even cardiovascular (Wal and Lovik 2007). These symptoms have been associated with a number of wheat proteins, including gliadins, glutenins, serpins (serine proteinase inhibitors), thioredoxin, agglutinin and a number of enzymes (α- and β-amylases, peroxidase, acyl CoA oxidase, glycerinaldehyde-3-phosphate dehydrogenase and triosephosphate isomerase). However, the predominant wheat proteins responsible for bakers' asthma are a class of α-amylase inhibitors, also known as CM proteins due to their solubility in chloroform:methanol mixtures (Shewry 2009). This allergic reaction is mediated by IgE, generated by antibody-producing B-cells during the phase called sensation. Upon re-exposure, the causal food binds to the IgE molecules specific for it and triggers the rapid release of chemical mediators such as histamines, leukotrienes and prostaglandins that cause the symptoms. In patients suffering from wheat allergy, minute amounts of the allergenic food

can cause serious symptoms and death. Coeliac disease is defined as a delayed-type hypersensitivity (or type IV allergic reaction), with enteropathy being caused by the local cell-mediated immune-response to dietary gluten. Type IV reactions are mediated by CD4 helper T-lymphocytes. Some of the stimulated T-cells produce soluble factors that initiate the hypersensitivity reaction, while others develop cytotoxicity. Tissue damage occurs as a result of persistent antigenic stimulation. Injury results from hydrolytic enzymes and toxic oxidants secreted by macrophages, which are activated by CD4 lymphocytes. Symptoms of coeliac disease include abdominal discomfort, weight loss or gain, tiredness, anaemia and severe diarrhoea (Fasano and Catassi 2001). Later, chronic inflammation and fibrosis dominate the clinical picture (Nicoletti *et al*. 2007). The intestinal mucosa of coeliac patients is flat and therefore they often suffer from malabsorption of essential nutrients such as vitamins and minerals.

In both cases, at present the only successful and safe treatment is the complete avoidance of foods containing the provocative proteins. Therefore, all foods containing wheat, including durum wheat, spelt wheat, kamut, einkorn, triticale, and products thereof, have to be excluded from the patient's diet. Since the protein fraction triggering coeliac disease can also be found in rye and barley, patients cannot tolerate these cereals either. For the production and distribution of gluten free food intended for coeliac disease patients a threshold of <20 ppm was established (based on scientific evidence supporting the safety of the intake of such low amounts) (Deutsch *et al*. 2008). However, setting such a limit is not possible for sufferers of wheat allergy as allergenicity is not an intrinsic property of a food, but allergy is the result of an interaction between the food and the consumer. A threshold that protects the most extremely allergic individuals is likely to be so low, that it will not be meaningful and not possible to implement (Wal and Lovik 2007). While coeliac disease is a lifelong condition, the symptoms of wheat allergy might disappear and wheat can be reintroduced into the diet.

2.3 Wheat free/ gluten free bread

The replacement of wheat in bakery products is a major technological challenge, as the wheat protein gluten is essential for structure-formation. Its removal impairs dough's capacity to properly develop during kneading, leavening and baking. This makes it necessary to add substances that mimic the viscoelastic properties of gluten, in order to provide structure and retain gas. In order to achieve suitable consistency for breadmaking, gluten free batters usually require very high hydration compared to wheat doughs. The addition of large quantities of water leads to considerable improvement of the dough behaviour during mixing. Due to the higher water level required the formulations are usually more fluid than wheat dough and in most cases not formable. Their viscosity is similar to that of cake batters. Therefore, the production of gluten free breads requires a different technology. For the replacement of wheat flour there are many naturally gluten free flours and starches available. The use of a mixture of two or more gluten free flours is often beneficial, as negative sensory or technological properties of the individual flours can be balanced out.

Rice

The most widely used on the market and in literature is certainly rice flour, probably due to its hypoallergenic proteins, bland taste and lack of colour. Three different rice products can be used in gluten free baking: rice paddy, brown and white rice. Rice paddy is the product obtained directly after harvesting and threshing, where the kernel is still within the hull or husk. By de-hulling "brown rice" is produced. To obtain "white rice" the hull is removed, the bran stripped off the endosperm and the germ removed. Finally broken and altered kernels are excluded. However, the removal of the outer bran layer causes a loss of proteins, fats and a large percentage of fibre, vitamins and minerals. The type of rice flour used for the production of gluten free bread is vital, as the properties of the rice have an impact on the quality of the resulting product. A study by Moore *et al*. (2004) suggested that the production of high quality gluten free bread could be obtained from a blend of gluten free rice flour and starches (potato,

corn) in combination with the correct hydrocolloid. Rice flour varieties mainly differ in the amylose content, which determines the gelatinization temperature, their general pasting behaviour as well as viscoelastic properties. Since pasting properties influence the behaviour during baking, careful selection of the rice cultivars used is recommended. Rice proteins have relatively poor functional properties for food processing. Due to their hydrophobic nature, rice proteins are insoluble and unable to form the viscoelastic dough necessary to retain the carbon dioxide produced by yeast during bread making, leading to a product with low specific volume and a very compact crumb (Arendt and Dal Bello 2008).

Pseudocereals

Other important raw materials for the production of gluten free foods are buckwheat, amaranth and quinoa. From a botanical point of view they are dicotyledonous plants and thus not cereals (monocotyledonous), but since they produce starch-rich seeds like cereals they are referred to as "pseudocereals". Pseudocereal flours can be used as nutritious ingredients for the formulation of gluten free products, because these grains have an excellent protein profile and contain considerable amounts of dietary fibre and minerals such as calcium and iron (Alvarez-Jubete 2010). There are two cultivated species of buckwheat for human consumption, i.e. common buckwheat (*Fagopyrum esculentum Moench*) and tartary buckwheat (*F. 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 starch granules are irregular in shape and are packed tightly. Like cereals, buckwheat endosperm has a non-starchy aleurone layer and a starchy endosperm (Aufhammer 2000). Buckwheat is rich in nutrients and the limiting amino acids Threonin and Methionin and can thus be used as a protein supplement together with other crops. Torbica *et al.* (2010) used different mixtures of rice and buckwheat flour and evaluated the resulting rheological, textural and sensory properties of the resulting breads. These authors found that the incorporation of buckwheat flour makes hydrocolloids redundant for the dough structuration effect. Sensory

properties are strongly influenced by the type of buckwheat used. Also Peressini and Sensidoni (2009) produced breads using a rice-buckwheat blend. These authors found that the quality of these breads can be successfully improved by the addition of hydrocolloids. Amaranth is another pseudocereal that may be utilized in the production of gluten free products. The amaranth plant family includes more than 60 species with *A. hypochondriacus, A. cruentus* and *A. caudatus* being the three major grain producing species (Williams and Brenner 1995). With regards to protein content, all amaranthus species have high seed protein contents, from approximately 13-18 % dry weight (Singhal and Kulkarni 1988), with albumins and globulins being the major proteins. Starch is the most abundant carbohydrate component of the amaranth grain. Several studies have investigated the use of amaranth in gluten free food products. Gambus *et al*. (2002) replaced cornstarch with amaranthus flour to enhance the protein and fibre contents of gluten free breads. At a 10 % replacement level, protein and fibre levels increased by 32 and 152 % respectively, while the sensory quality was unaffected. Tosi *et al*. (1996) described the use of amaranth in gluten free products and formulated a gluten free mix using wholemeal amaranthus flour. In contemporary times, quinoa has become highly appreciated for its nutritional value. The protein content of quinoa (13-14 %) is slightly higher than that of most other cereal grains. The fat content, which ranges from 5 % (Risi and Galwey 1989), through 7 % (Guzmán-Maldonado and Paredes-López 1998) to 9.7 % (Ruales 1992) is at least twice as high as in most cereals. The dietary fibre content is slightly higher and the starch content is somewhat lower as compared to other grains such as wheat or rice, ranging from 52 % (Ruales 1992), through 60 % (Risi and Galwey 1989) to 69 % (Guzmán-Maldonado and Paredes-López 1998). Quinoa is a good source of minerals. It contains more calcium, magnesium, iron, and zinc than common cereals, and the iron content is particularly high. Quinoa contains more riboflavin (B_2) and α-tocopherol than rice, barley or wheat and it can be a good source of vitamin E (Valencia-Chamorro 2003). The grain structure of quinoa is complex: In cereals such as maize and wheat, the main starch reserves for embryo development are

stored in endosperm tissue, but in quinoa, the living endosperm tissue is reduced to one or two layers surrounding the hypocotyls-radicle axis (Prego *et al*. 1998). The starch is stored in the non-living, thin-walled perisperm that occupies about 40 % of the volume of the seed (Ruales 1998). Quinoa starch, being high in amylopectin, gelatinizes at a low temperature (Hoseney *et al*. 1981). Of particular interest is the fact that quinoa starch has excellent freeze-thaw stability (Ahamed *et al*. 1996), as starch high in amylopectin is less prone to retrograde and hence less water is expelled from the polymer matrix. Taylor and Parker (2002) discussed the application of quinoa as a novel ingredient in the production of enriched gluten free bakery goods. These authors observed a reduction in loaf volume upon addition of quinoa flour to other non-gluten containing flours.

Millet

Millets are cultivated grasses (cereals) that have small kernels and that are grouped together solely on this basis. There are many different millets such as pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*) and tef (*Eragrostis tef*). Millet grains can contain significant amount of phenolic compounds which give them antioxidant activity. Although millets have huge potential for wider use, these grains remain virtually unresearched and their potential untapped (Taylor *et al*. 2006). Tef is a unique grain, ancient, minute in size, and packed with nutrition. The grain characteristics that influence food, nutritional and technological properties include colour, pericap and seed coat, endosperm texture and hardness (Babatunde Obilana and Manyasa 2002). The colour of the tef grains can be ivory, light brown or dark reddish brown, depending on the variety. Tef has a mild, nutty taste and exhibits a slight molasses-like sweetness. The white tef varieties exhibit a chestnut-like flavour, darker varieties are earthier. The grains of tef are very small, with 2500-3000 grains weighing approximately 1 g. Tef is always consumed in the whole grain form (germ, bran and endosperm). This is the reason for higher nutritive density compared to the major cereals such as wheat, barley and maize, because the bran and

germ are the most nutritious parts of any grain. Tef contains 11 % protein, 80 % complex

carbohydrate and 3 % fat and is an excellent source of essential amino acids, especially lysine,

the amino acid that is most often deficient in grain foods. Tef contains more lysine than barley,

millet, and wheat and slightly less than rice or oats. Tef is also an excellent source of fibre and

iron, and has many times the amount of calcium, potassium and other essential minerals than

other grains.

Sorghum

Sorghum (*Sorghum bicolor*) is often recommended as a safe food for coeliac patients because it

is more closely related to maize than to wheat, rye or barley. However, certain cultivars contain

high amounts of proanthocyanidins, also referred to as condensed tannins, and therefore

exhibit bitter taste (Delcour and Hoseney 2010). The development of so-called "food-grade"

sorghum lines has enabled white, bland-tasting flour to be produced from sorghum grain. This

flour is useful in food products because it does not impart unusual colours or strong flavours

and it may be desired over maize flour for these reasons (Taylor *et al*. 2006). Like other cereal

grains, the primary component of sorghum is carbohydrate in the form of starch (Rooney and

Waniska 2000). Sorghum has a similar chemical composition to maize. However, sorghum is

often reported to have a slightly lower protein and starch digestibility as compared to maize.

This is especially true in cooked sorghum and has been attributed to increased cross-linking of

the proteins during cooking (Bean and Fellers 1982). The average protein content of sorghum is

11-12 %. With regard to amino acids, sorghum is unusually high in glutamic acid, leucine,

alanine, proline, and aspartic acid (Hoseney *et al*. 1981). Only a limited number of studies have

addressed wheat-free sorghum breads and most have used relatively complex recipes

incorporating xanthan gum (Satin 1998), carboxymethyl cellulose and skimmed milk powder

(Cauvain 1998), egg (Keregero and Mtebe 1994)(Cauvain 1998) and/or rye pentosans (Casier *et*

al. 1977). Schober *et al*. (2005) produced gluten free sorghum breads using different varieties

and observed large differences in the visual appearance of the crumb. Kernel hardness and

damaged starch are key elements determining the quality of the resulting bread. Sorghum breads have been repeatedly reported to exhibit a sandy mouthfeel, which is related to the amount of damaged starch present in the flour (Onyango *et al.* 2010; Schober *et al.* 2005).

Corn

Corn (*Zea mays*) is the most widely grown crop in the Americas. Corn flour is composed of the endosperm, which generally contains between 75 and 87 % starch and 6-8 % protein (Shukla and Cheryan 2001). Zeins, the storage proteins of corn, represent 60 % of the proteins and are located in protein bodies (Lending and Larkins 1989). Since corn is considered a gluten free cereal, it can be used in the production of gluten free bread. However, little research is available on the production of breads from maize. This may be due to the distinctive flavour and colour attributes (Arendt and Dal Bello 2008). Ács *et al.* (1996a, b) used binding agents (xanthan, guar gum, locust bean gum and tragant) as a substitute for gluten in a gluten free bread formulation based on cornstarch. These authors found that the binding agents resulted in an increase in loaf volume and loosening of the crumb structure.

Wheat starch

Compared to starches from naturally gluten free sources, wheat starch has outstanding technological properties. According to the Codex Alimentarius specially manufactured wheat starch which complies with the international gluten free standard can safely be included in the gluten free diet (Kaukinen *et al.* 1999). However, the coeliac associations of some countries, such as Canada and U.S., recommend avoiding wheat starch (Thompson 2001). Since it can not be guaranteed that wheat starch is 100 % protein-free, products containing wheat starch are not suitable for people suffering from wheat allergy. In addition, many coeliac disease patients cannot tolerate even the very small amount of this protein remaining in isolated wheat starch. Reports have highlighted that the long-term effects of regular ingestion of small amounts of gliadin (e.g. from wheat starch) were harmful to patients with coeliac disease (Chartrand *et al.* 1997; Lohiniemi *et al.* 2000; Skerritt and Hill, 1992).

Oat

The use of oat for the production of gluten free bread is controversial since the issue of oat toxicity has not been conclusively resolved. However, due to fact that an inclusion of oat would increase diversity and nutritional quality of the gluten free diet, the Codex Alimentarius Commission (2009) reassessed their status in the international gluten free standards and concluded that oats, although kept in the category of gluten-containing cereals, can be tolerated by most but not all people who are intolerant to gluten. Therefore, the use of oats not contaminated with wheat, rye or barley in foods may be determined on national level (Hüttner and Arendt 2010).

Non-cereal starches

Many naturally gluten free, non-cereal starches and flours can be used such as potato, cassava, pea, hemp, plantain, sweet potato, bean and lentil, chestnut, chickpea and coconut flour. Among those potato flour is widely used in the baking industry and has long been associated with the baking of bread. It is well known that the small amounts of added potato solids help to retain the freshness of bread and also impart a distinctive, pleasing flavour and improved toasting qualities (Willard and Hix 1987). Potato starch has desirable characteristics, which differ significantly from those of starch from other plant sources (Madsen and Christensen 1996). Potato starch forms a high-viscosity paste that is susceptible to shear. The potato granules are large and are extremely susceptible to breakage. The high molecular weight amylose and phosphate groups esterified to amylopectin contribute to high transparency, swelling power, water-binding capacity and freeze-thaw stability of potato starches (Craig *et al*. 1989). The pasting characteristics and other physicochemical properties of starches vary with genotype and cultural practices (Barichello and Yada 1991), for instance temperature during tuber growth affects granule size and pasting temperature. In the study of Kaur *et al*. (2006) where 21 different Indian potato cultivars were observed, lower temperature during tubers growth resulted in starch with higher granule size and lower pasting temperatures.

Hopkins and Gormley (2000) reported that the rheological properties of pastes and gels made from starch isolated from different Irish potato cultivars differed significantly.

Hydrocolloids

In order to mimic viscoelasticity, usually provided by the gluten proteins, the addition of hydrocolloids or gums, to the gluten free formulation is critical. These compounds are long-chain, high molecular weight molecules, which in water-based systems produce gels, i.e. highly viscous suspensions or solutions with low dry-substance content (Hoefler 2004). They are derived from seeds, fruits, plant extracts, seaweeds or microorganisms and are usually of polysaccharide and less frequently of protein nature (Norton and Foster 2002). The effects of their addition to bakery products include: altered dough rheological performance, improved sensory properties such as crumb hardness and cell size distribution, increased loaf volume, retarded starch retrogradation, and increased moisture retention. Hence, their use extends overall quality of the product during time (Anton and Artfield 2008; Armero and Collar 1996a, b; Davidou *et al*. 1996; Rojas, 1999). They appear to improve gas retention and water absorbing characteristics usually supplied by gluten (McCarthy *et al*. 2005). Additionally, they effect swelling, gelatinization, pasting properties and staling of starch (Rojas 1999). It was shown that frequently, single hydrocolloid solutions or gels do not deliver all desired product properties. Hence, in many cases two or more different hydrocolloids are used. The most commonly used hydrocolloids in gluten free bread formulations are cellulose, hydroxypropylmethylcellulose (HPMC), pectin, guar gum, xanthan gum and locust bean gum (Onyango *et al*. 2009; Turabi *et al*. 2010). A general quality improvement of gluten free breads can be achieved through the use of gums and hydrocolloids. However, these positive results are reversed if too high concentrations are used. It is important to mention that no general effect can be attributed to the use of hydrocolloids, since the specific properties differ greatly. Especially when derived from natural sources, the hydrocolloids vary widely in their molecular

weight and hence their performance in bakery products. Therefore the effect of each substance has to be evaluated separately (Anton and Artfield 2008).

Proteins

The replacement of gluten with other protein sources such as soy, pea, egg and dairy proteins is another approach used in the production of gluten free products. Egg proteins, part of the soy protein or colloidal solutions such as caseins swell and form viscous solutions in a gluten free bread system (Moore *et al*. 2004). Several studies have been reported where the inclusion of dairy proteins in gluten free systems plays an important role. Dairy proteins have functional properties similar to gluten. They are capable of forming networks and they have good swelling properties. There are two major protein groups in milk, which are referred to as caseins and whey proteins. Caseins in milk exist as large micelles (Nishinari and Takahashi 2003), while whey proteins, on the contrary, are globular in structure, consisting of hydrophobic, compact folded peptide chains. The major whey proteins are β–lactoglobulins and α–lactalbumin, representing about 10 % and 4 % of the total milk proteins, respectively. Dairy proteins are highly functional ingredients and due to their versatility 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; Gallagher *et al*. 2003; Kenny *et al*. 2001; Mannie and Asp 1999). Dairy ingredients contribute to a number of critical characteristics of a food product. These include the emulsifying and stabilizing 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). Gallagher *et al*. (2003) used dairy products in gluten free bread formulations to increase water absorption and therefore enhance the handling properties of the batter. These authors found that the inclusion of dairy powders resulted in improved volume, appearance and sensory aspects of the loaves. However, since coeliac disease can result in lactose intolerance due to

reduction or lack of lactase production, approximately 50 % of coeliac disease patients must avoid cow's milk (Murray, 1999). Therefore the use of low-lactose dairy powders is recommended. Several dairy powders were applied to a gluten free bread formulation by Gallagher *et al*. (2003) and Nunes *et al*. (2009). In general, the powders with high protein/low lactose content (sodium caseinate, milk protein isolate, whey protein isolate, whey protein concentrate) gave breads with an improved overall shape and volume, and a firmer crumb texture. These breads had an appealing dark crust and white crumb appearance, and received good acceptability scores in sensory tests. When optimal water was added to the gluten free formulation, these breads exhibited increased volume and a much softer crust and crumb texture than the controls. These studies suggest that low lactose dairy powders can successfully be used in gluten free recipes, getting the benefit of the functional dairy ingredients without the deleterious effect of lactose.

Soy flour is widely used in the production of gluten free products. Soy beans belong to the plant family *Fabaceae*, also known as "legumes" or "pulses". Members of this plant family are characteristically rich in protein, but deficient in sulfur containing amino acids (Belitz *et al*. 2004). Ranhotra *et al*. (1975) discussed the addition of soy protein to gluten free bread. These authors formulated wheat starch-based gluten free breads with 20, 30 and 40 % soy protein isolate (protein content of 88 %). The breads contained more protein and fat than wheat bread and showed satisfactory baking results. In a different study it was observed that full-fat enzyme active soybean flour improved volume and structure (Ribotta *et al*. 2004). Also particle size and concentration had an effect. Kato *et al*. (1990) studied the gelation of heat treated spray-dried egg white protein (10% solution) and suggested that egg proteins form strong cohesive viscoelastic films, which are essential for stable foaming. Eggs are one of nature's nearly perfect protein foods, being made of albumen (egg white) and egg yolk. The albumen is a 10 % aqueous solution of various proteins, whereas the egg yolk is a fat-in-water emulsion with about 15 % protein. The suitability of egg in bakery products is basically due to three

properties: coagulation when heated, foaming ability and emulsifying properties. Also the colouring ability and aroma of egg has to be mentioned (Belitz *et al.* 2004). These authors also stated that at high egg albumin concentrations, improved gas retention properties were found. Onyango *et al.* (2009) studied the effect of egg white powder, skim milk powder, soybean protein isolate and soybean protein concentrate on crumb firmness, staling rate, specific volume and pore size of gluten free bread baked from pre-gelatinized cassava starch and sorghum. Comparison of the different proteins used showed that gluten free bread prepared with egg white had the least crumb hardness and staling rate. Also specific volume was highest upon incorporation of egg white, due to improved gas retention properties of the resulting batter.

Enzymes

The functions of enzymes are widespread throughout the baking industry. These include decolourising (bleaching) of dough, improvement of the volume and texture of dough, substitution of bromates, and maintenance of shelf-life (Corsetti et al. 2000; Delcros *et al.* 1998; Géllinas *et al.* 1998; Grossman and De Barber 1997; Rossel *et al.* 2001; Sahlström and Brathen 1997; Vemulappali and Hoseney 1998). One advantage of the use of enzymes in the baking industry is their GRAS status (Generally Recognized As Safe). Enzymes used in gluten free baking include transglutaminase (protein-glutamine γ-glutamyltransferase, EC 2.3.2.13), which is an aminotransferase obtained from microbial cultures. Transglutaminase (TGase) has the ability to link proteins of different origins: casein and albumin from milk, animal protein from eggs and meat, soy protein and wheat protein. Several researchers investigated the use of this enzyme for the production of gluten free breads. Onyango *et al.* (2010) investigated the effect of different concentrations of microbial transglutaminase on gluten free breads prepared from pregelatinized cassava starch, sorghum and egg white, showing that increasing enzyme concentration increased crumb firmness and chewiness. Renzetti *et al.* (2008) investigated the network forming potential of TGase on flours from six different gluten free

cereals (brown rice, buckwheat, corn, oat, sorghum and tef). The addition of this enzyme led to significant improvements in terms of increased loaf specific volume and decreased crumb hardness and chewiness. Under the conditions of the study, no effects of TGase could be observed on breads from oat, sorghum or tef. The results of this study show that TGase can be successfully applied to gluten free flours to improve their breadmaking potential by promoting network formation. However, the protein source is a key element determining the impact of the enzyme.

Two other common problems of gluten free breads are the increased staling rate and the crumbly structure due to higher starch retrogradation of certain gluten free flours compared to wheat flour. These have been addressed by the use of the starch-hydrolyzing enzymes α-amylase and cyclodextrin glycosyltransferase. Both enzymes decrease the ability of amylopectin to retrograde during storage and therefore extend shelf life of the bread (Rosell 2009).

Sufferers of wheat allergy and coeliac disease patients have been deprived from good quality cereal products, which is part of the staple diet of the majority of human beings. The wheat protein gluten has unique properties in relation to production of good quality cereal products, which make its replacement so difficult. From the evidence gathered it becomes apparent that a mixture of different flours, gums and proteins is necessary to replace gluten and produce good quality gluten free cereal products. The development of gluten free cereal products still represents a challenge for food technologists, in order to provide the consumer with palatable products of high nutritional value.

2.4 Glycemic index and Glycemic load

The Glycemic index (GI) is a model ranking carbohydrate containing foods from 0-100 based on their postprandial blood glucose response. Not all carbohydrates illicit the same response: highly refined grains have a high GI, whereas wholegrain products tend to have a low GI. The

American Association of Cereal Chemists (AACC) defined the term "glycemic carbohydrate" as the carbohydrate consumed by an individual causing a measurable increase in blood glucose levels (AACC International 2006). The glycemic index is calculated over a period of two hours immediately post consumption and is defined as the area under the glucose response curve when 50 g of a test food is consumed divided by the area under the curve when 50 g of a standard control food is consumed multiplied by 100 (Jenkins *et al*. 1981). The GI represents the relative rate of entry of glucose in the bloodstream compared with a reference carbohydrate source (Bell and Sears 2003). The control or reference food used is generally glucose or white wheat bread and is given the value 100. All other foods are assigned a value relative to this (Foster-Powell *et al*. 2002). Because blood glucose release is higher when glucose is used as reference food instead of white wheat bread, the glycemic index values are much lower (Autio *et al*. 2004).

Low glycemic index carbohydrates are generally considered to be those with a GI below 40 (using white bread as a reference) and include for example pumpernickel bread, pasta, legumes and parboiled rice (Kendall *et al*. 2010). Products like oat porridge, that have a GI between 40 and 70 are considered to have a moderate GI; and those with a GI greater than 70, such as popcorn, chocolate bars and wafers, can be considered high-glycemic index carbohydrates (Bell and Sears 2003; Foster-Powell *et al*. 2002).

The glycemic index takes into account the availability and absorption rate of a fixed sample (50 g). Therefore, one of the main criticisms of the glycemic index over the years has been its failure to take into account the amount of carbohydrate consumed. To overcome this problem the glycemic load was introduced. The glycemic load (GL) is calculated by multiplying the total amount of dietary carbohydrate in a food serving by the glycemic index of that food (Salmeron *et al*. 1997). The glycemic load gives an estimate of the predicted elevation of postprandial blood glucose levels when dealing with mixed meals and varying amounts of carbohydrate

(Autio *et al*. 2004). The higher the glycemic load, the greater the rise in predicted blood glucose level (Foster-Powell *et al*. 2002). The glycemic load strongly influences appetite through hormonal regulation: meals containing carbohydrates with low GL reduce hunger and promote satiety; which in turn favours weight loss and reduces the risk of cardiovascular disease as well as diabetes (Bell and Sears 2003). Carbohydrates with high glycemic indexes and high glycemic loads produce substantial increases in blood glucose and insulin levels after ingestion. Within a few hours after their consumption, blood sugar levels begin to decline rapidly due to an exaggerated increase in insulin secretion. A profound state of hunger is created. The continued intake of high-glycemic load meals is associated with an increased risk of chronic diseases such as obesity, cardiovascular disease and diabetes (Bell and Sears 2003). Therefore, the prediction and control of glucose absorption after the ingestion of bread is of great interest in the context of weight control, diabetes management, as well as the management of other disorders of the carbohydrate metabolism.

2.5 Bread of low glycemic index

Several modifications can be made to the composition of white wheat bread in order to reduce its glycemic index. One approach is to incorporate substances high in dietary fibre such as whole grains or the bran fraction of cereals. Another way is the use of sourdough for bread making. Sourdough can be defined as an intermediate product containing a flour/water mixture that has been fermented by a combination of bacteria, usually heterofermentative strains of lactic acid bacteria and yeast (DeVuyst and Neysens 2005), resulting in an end product with a sour taste. Sourdough fermentation improves nutritional aspects, such as texture and palatability of whole grain, fibre rich or gluten free products. Sourdough delays starch bioavailability and increases the mineral bioavailability. Regarding the latter trait, this increase in especially iron, calcium, zinc and magnesium, is a consequence of phytate degradation exerted by phytases secreted by lactic acid bacteria (Zannini *et al*. 2009). The glycemic index of bread can be drastically reduced by incorporating sourdough to the recipe.

The international table of glycemic index and load compiled by Foster-Powell *et al*. (2002) illustrated that the addition of sourdough to wheat bread reduced the glycemic index from a value of 100 to 77, a drop of 23 points. This effect can be attributed to the presence of organic acids produced during sourdough fermentation which have been shown to reduce postprandial blood glucose and insulin response by ameliorating glucose disposal delaying gastric emptying or suppressing enzymatic activity (Scazzina *et al*. 2009). In general, most medium and low GI foods are those that are known for their high fibre content, such as all-bran, oats and legumes. Therefore, by undertaking a diet that is composed of many low GI foods, one is essentially undertaking a diet that is high in fibre (Kendall *et al*. 2010).

2.6 Bread high in dietary fibre

Dietary fibers do not constitute a defined chemical group, but are a combination of chemically heterogeneous substances such as cellulose, hemicelluloses, pectins, lignins, and gums (Thebaudin *et al*. 1997) and are grouped together solely on the basis of their physiological function. The European Commission defines fiber as fibre carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories: edible carbohydrate polymers naturally occurring in the food as consumed; edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence; edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence (The Commission of the European Communities 2008). Dietary fibre generally falls into the two categories soluble and insoluble. The most soluble dietary fibres are more rapidly fermented in the colon and they are more accessible to hydrolytic enzymes; whereas the less soluble fibres are excreted in the stool and thus have the effect of increasing faecal bulk. Potential health benefits of dietary fibre include, reduction of bowel transit time, prevention of constipation, reduction in risk of colorectal cancer, lowering of

blood cholesterol, production of short chain fatty acids and promotion of the growth of beneficial gut microflora (Brennan and Cleary 2005; Zekovic *et al*. 2005). Viscous fibres account for the majority of the clinical benefits observed. The viscosity of these fibres slows digestion of nutrients by preventing bulk diffusion of foods across the intestinal lumen. This reduction in absorption lowers postprandial blood glucose levels and insulin responses, which has significant implications in prevention and management of insulin-resistance and type-2-diabetes (Kendall *et al*. 2010). The insoluble fibre fraction causes increased faecal weight, bulk and softness, increases the frequency of defecation and reduces the intestinal transit time (Dewettinck *et al*. 2008).

It was shown that high dietary fibre intake can be linked to weight loss due to increased satiety and decreased voluntary food intake. Technological properties of dietary fibres include water holding capacity, oil holding capacity, viscosity, texturizing, stabilizing, gel-forming capacity and antioxidant capacity (Elleuch *et al*. 2010). Dietary fibres can be incorporated into bakery products to prolong freshness, due to their capacity to retain water, thereby reducing economic losses. Fibres can modify loaf volume, its springiness and the softness of the bread crumb, crumb and crust colour (Peressini and Sensidoni 2009; Poinot *et al*. 2010; Wang *et al*. 2002). Hence, the enrichment of baked products with dietary fibres has been a topic of research for various teams of food technologists. Due to the fact that dietary fibers vary widely in chemical composition and structural properties, their nutritional value and technological effects can not be generalized. The following chapter discusses several sources of fibre to be considered for the enrichment of bread.

Wholegrain bread

One important source of dietary fibre is certainly whole grain cereals. Besides providing energy and fibre, cereal grains contain several other important nutrients and therefore many health benefits are achieved through their ingestion. The milling process applied in order to gain refined flour commonly used for baking, reduces the level of these important nutrients or even

removes them completely. In wholegrain products most (or all) of the pericarp-seed coat, aleurone and germ are retained. Therefore the incorporation of whole grains into bread is an important way to improve the nutritional value of this staple food. However, it must be remembered that some processing is essential for palatability, safety and adequate nutrient bioavailability of cereal foods (Topping 2007).

Cereals are grown in over 73 % of the total world harvested area and contribute over 60 % of the world food production providing energy, dietary fibre, nutritious proteins and lipids, vitamins and minerals required for human growth and maintenance (Charalampopoulos *et al*. 2002). Cereals are rich in linolenic, linoleic and palmitic acid. Cereals are a good source of vitamin E and the B-vitamins thiamine, riboflavin, niacin, pyridoxine and folic acid. Potassium levels are high and sodium levels very low. A reduction in dietary sodium intake, together with a simultaneous increase in potassium, leads to an overall reduction in blood pressure levels (Charlton *et al*. 2007). Many grains are also a source of manganese, iron, selenium, zinc and copper as well as moderate amounts of calcium and magnesium. Wholegrain cereals contain a wide range of compounds with antioxidant effect (Fardet *et al*. 2008), including carotenoids and the phenolic acids ferulic, diferulic acid, sinapic acid, *p*-coumaric acid, caffeic acid and benzoic acid derivatives. About 95 % of grain phenolic compounds are linked to cell wall polysaccharides through ester bonds (Vitaglione *et al*. 2008). Cereals are also a good source of phytoestrogens of the lignan family and the lipid fraction of certain cereals may also be rich in phytosterols which are hypocholesterolaemic (Dewettinck *et al*. 2008). However, it is important to mention that attempts identifying the factors in whole grain components which mediate their health benefits have been unsuccessful. While the effects of wholegrain components (e.g. dietary fibre, antioxidants, vitamins and minerals) have been shown to reduce disease risk, the whole seems to be greater than the sum of the parts (Topping 2007).

Wheat (T*riticum aestivum*) is by far the most important cereal for breadmaking because of its supreme baking performance in comparison with other cereals. Wheat contains up to 14.6 %

dietary fibre (Wrigley *et al*. 2004), which is made up of cellulose, arabinoxylans, β-glucan and arabinogalactan. The majority of dietary fibre is found in the bran or outer layers (Sluimer 2005). Therefore the consumption of wholemeal bread is advisable. However, dough from whole wheat is less cohesive than dough from refined wheat flour and the loaf specific volume is usually significantly lower than of corresponding white bread.

Supplementing bread with non-wheat fibres

The nutritive value of bread can be increased through the use of cereals or their isolated fibres other than wheat (Dewettinck *et al*. 2008). One cereal with highly interesting nutritional properties is oats (*Avena sativa*). Oats are a relatively minor crop in terms of world grain production and were traditionally used as an animal feed. However the health claims published in recent years have contributed to their increased human consumption (Brennan and Cleary 2005; Lehtinen *et al*. 2009). Asp *et al*. (1991) carried out an analysis on the nutritional composition of oats in Sweden; and found that samples contained on average 9.7 % dietary fibre, comprising 3.5 % soluble dietary fibre. The main component of soluble dietary fibre in oats is β-glucan. The native mixed linkage β-glucan of cereals is a non-starch polysaccharide, composed of linear chains of glucopyranose with β-(1→3) and β-(1→4) linkages (Tiwari and Cummins 2009). In addition to the function as a dietary fibre, β-glucan has been associated with several other health promoting effects. Clinical studies have shown that oat bran and purified β-glucan from yeast can be used to lower serum cholesterol levels and to reduce the risk of heart disease (Bell *et al*. 1999). Cereal β-glucan has been shown to have an important influence on human glycaemic control by lowering of the postprandial blood glucose level (Jenkins *et al*. 2002; Lazaridou and Biliaderis 2007; Wood, 2007). The European Food Safety Authority (EFSA) granted a health claim regarding the cause and effect relationship between the consumption of beta-glucans and the reduction of blood cholesterol concentrations. The following wording reflects the scientific evidence: "Regular consumption of beta glucans contributes to maintenance of normal blood cholesterol concentrations". In

order to bear the claim, foods should provide at least 3 g/day of beta-glucans from oats, oat bran, barley, barley bran, or from mixtures of non-processed or minimally processed beta-glucans in one or more servings (EFSA Panel on Dietetic Products 2009). Hüttner *et al*. (2010) evaluated different wholegrain oat flours for their bread making ability and found significant differences between the flours. This shows that the choice of flour is critical for the end quality of the produced bread. Bread made from oats is considered to not only have high nutritional quality but has also excellent moisture retention properties that reduces the rate of staling and therefore keeps breads fresh for longer. Also barley grains contain a considerable amount of soluble fibre and can be used for the production of bread with additional health benefits. Barley flours enriched in β-glucan can be produced by air-classification (Ferrari *et al*. 2009). Several authors evaluated the enrichment of bread with fibre-rich barley fractions (Cavallero *et al*. 2002; Jacobs *et al*. 2008; Symons and Brennan 2004; Trogh *et al*. 2004). There is a general consensus between these authors that the enrichment of wheat bread with β-glucan rich flours results in increased crumb hardness and decreased loaf volume.

In addition to cereals there is a great variety of fruit and vegetable raw materials for the isolation of dietary fibre available, which are in many cases processing by-products. Although dietary fibre isolated from cereals is more commonly used than that isolated from fruits or vegetables, the latter are believed to be of better quality due to higher total and soluble fibre content, water and oil holding capacity and colonic fermentability as well as lower phytic acid content and caloric value (Figuerola *et al*. 2005). Apples contain only about 2 % dietary fibre (Souci *et al*. 2004), but apple fibre products for the enrichment of food are available on the market contain about 60-90% dietary fiber (Rettenmaier, product specification). Citrus and apple fibres have better quality than other dietary fibres due to the presence of associated bioactive compounds, such as flavonoids, polyphenols and carotenes (Figuerola *et al*. 2005; Larrauri 1999).

Another interesting source for the isolation of fibres are the vegetables psyllium or chicory. The seed husk of *Plantago ovata* (psyllium) has a long history of use as a dietary fibre supplement to promote the regulation of large bowel function and to lower blood cholesterol levels (Fischer *et al*. 2004). Inulin-type fructans are natural components of several edible fruits and vegetables such as wheat, onion, banana, garlic and leek. The inulin commonly used in the food industry derives from chicory and consists of oligo- and polysaccharides which are composed of fructose units connected by β (2-1) links. Almost every molecule is terminated by a glucose unit (Roberfroid 2007). Standard inulin, as it is extracted from chicory roots, always contains small amounts of monosaccharides (up to 10 %). These are present in the root and are not a result of processing (Coussement 1999). Because of the β-configuration of the linkages, they show prebiotic properties. Inulin resists digestion in the upper gastrointestinal tract and reaches the large intestine virtually intact where it is fermented by the intestinal microflora, stimulating selectively the growth and/or activity of intestinal bacteria associated with health and well-being, especially bifidobacteria (Gibson *et al*. 1995; Tuohy *et al*. 2007). However, at high doses, increased flatulence and osmotic pressure can cause intestinal discomfort. Gallagher *et al*. (personal communication) incorporated inulin into a wheat starch-based gluten free formulation resulting in an improved overall quality of the gluten free bread. Addition of inulin leads to a darkening of the bread crust due to a greater extent of Maillard reaction. This is caused by monosaccharides initially present in the inulin powder as well as the reducing sugars produced by yeast invertase which partially breaks down inulin during bread making (Verspreet *et al*. 2013).

Some of the starch contained in food products escapes digestion and is therefore commonly referred to as "resistant starch" (RS). Therefore the molecule elicits beneficial physiological effects that make it somewhat comparable to dietary fibre constituents (Delcour and Hoseney 2010). Resistant starch is composed of four groups: RS1: physical inaccessible starch, RS2: ungelatinized starch granules, RS3: retrograded starch and RS4: chemically modified starch

(Elleuch *et al.* 2010). Resistant starch provides better appearance, texture, and mouthfeel than conventional fibres (Charalampopoulos *et al.* 2002).

It has to be pointed out that although the enrichment with fibre results in increased nutritional value of bread, quality characteristics of the loaves can be influenced negatively. The incorporation of fibre-rich ingredients into dough is known to dilute and disrupt the gluten network, impairing gas retention and changing texture and appearance of the resulting breads (Dubois 1978). Therefore it might be necessary to adapt the formulation or change processing conditions. The hydration properties of a fibre determine the maximum usage level in a food product because a desirable texture must be retained (Thebaudin *et al.* 1997). Due to the high water-binding capacity of most fibres, gas holding capacity and sensory characteristics of the final bread, such as texture, visual appearance, flavour and staling behaviour, can be negatively influenced. Therefore an adjustment of water level or a pre-soaking of the fibre might be necessary. Considering colour and flavour there are neutral fibres available on the market, such as pea, oat, rice or maize, but also fibres which cause a tinge and/or off-taste owing to other molecules associated with the fibres, i.e. cocoa, apple and citrus (Thebaudin *et al.* 1997). Overall it can be said that it is important to choose from the wide range of dietary fibres, the option that exhibits the desired nutritional and technological properties to achieve the required quality characteristics of the produced breads.

2.7 Trends

The trend for wholesome healthy bread products will certainly continue to be in the focus of the baking industry. Due to the high prevalence of the so called "diseases of civilization" such as obesity, diabetes and coronary heart disease, the incorporation of functional ingredients shown to lower the risk factors of these medical conditions into baked goods is considered. In this respect breads rich in omega-3-fatty acids, antioxidants, vitamins, or minerals such as calcium and iron are of interest. Another approach is to reduce the total energy intake by influencing the feeling of satiety upon bread intake by incorporating dietary fibre or alternative

grains, which are more nutrient dense such as oat, buckwheat or quinoa. The dietary intake of sodium chloride has increased considerably over the last few decades due to changes in the human diet. This higher intake has also been linked to cardiovascular diseases. Numerous international health agencies have now recommended a salt intake level of about 5-6 g daily, approximately half the average current daily intake level. Cereal products, and in particular bread, are a major source of salt in the diet. Therefore, any reduction in the level of salt in bread would benefit the consumer. Another trend is clearly the production of breads using all-natural or even organic ingredients. Also "clean labelling" will certainly continue to be a major concern for producers and consumers. Since the market for "free from" products is still growing, the production of high quality gluten free breads will certainly be in the centre of attention for food technologists as well as the baking industry.

With today's consumer being more health conscious, increasing demand for products with additional nutritional value can be observed. However, the key for successful new bakery products will still be their tastefulness.

2.8 References

AACC International (2006) AACC International Approves Definitions Related to Glycemic Carbohydrates. Available at: http://www.aaccnet.org/news/06glycemicdefinitions.asp.

Ács E, Kovacs Z. and Matuz J. (1996) Bread from corn starch for dietetic purposes. I. Structure formation. Cereal Research Communications, 24, 441-449

Ács E, Kovacs Z. and Matuz J. (1996) Bread from corn starch for dietetic purposes. II. Formation of the visual and technological properties. Cereal Research Communications, 24, 451-459

Ahamed N.T, Singhal R.S, Kulkarni P.R. and Pal M. (1996) Physicochemical and functional properties of Chenopodium quinoa starch. Carbohydr Polym., 31, 99-103

Alvarez-jubete L, Arendt E.K. and Gallagher E. (2010) Nutritive value of pseudocereals and their increasing use as functional gluten- free ingredients. Trends in Food Science & Technology, 21(2), 106-113

Angelis M, Damiano N, Rizzello C.G, Cassone A, Di Cagno R. and Gobbetti M (2009) Sourdough fermentation as a tool for the manufacture of low-glycemic index white wheat bread enriched in dietary fibre. European Food Research and Technology, 229(4), 593-601

Anton A.A. and Artfield S.D (2008) Hydrocolloids in gluten-free breads: a review. International journal of food sciences and nutrition, 59(1), 11-23

Arendt E.K. and Dal Bello F (2008) Gluten-free Cereal Products and Beverages, Academic Press, Elsevier.

Armero E. and Collar C. (1996) Anti-staling additive effects on fresh wheat bread quality. Food Science and Technology International, 2, 323-333

Armero E. and Collar C. (1996) Anti-staling additives, flour type and sourdough process effects on functionality of wheat doughs. Journal of Food Science, 61, 299-303

Asp G, Mattsson B. and Onning G. (1991) Variation in dietary fibre, β-glucan, Starch, protein, fat and hull content of Oats grown in Sweden 1987-1989. European Journal of Clinical Nutrition, 46, 31-37

Aufhammer W. (2000) Pseudo-getreidearten: Buchweizen, Reismelde und Amarant, Herkunft, Nutzung und Anbau., Stuttgart,: Verlag Eugen Ulmer

Autio K, Niskanen L. and Poutanen K. (2004) Starch in food diabetes and coronary heart disease. In Arnoldi A. Functional Foods, Cardiovascular Disease and Diabetes. Cambridge, Woodhead, 376-401

Babatunde Obilana A. and Manyasa E. (2002) Millets. In Belton P. and Taylor J. Pseudocereals and Less Common Cereals: Grain properties and utilization potential. Berlin, Springer-Verlag

Barichello V. and Yada Y. (1991) Starch properties of various potato (Solanum tuberosum L.) cultivars susceptible and resistant to low temperatures sweetening. Journal of Science of Food and Agri, 56, 385-397

Bean M. and Fellers D. (1982) Composite flour breads in Bolivia: technical aspects. In Proceedings of the 7th World Cereal and Bread Congress. Prague, 859-864

Belitz H, Grosch W. and Schieberle P. (2004) Food Chemistry 3rd., Berlin, Springer Verlag.

Bell S, Goldman V, Bistrian B, Arnold A, Ostroff G. and Forse R. (1999) Effect of β-Glucan from Oats and Yeast on Serum Lipids. Critical Reviews in Food Science and Nutrition, 39(2), 189-202

Bell S.J. and Sears B. (2003) Low-glycemic-load diets: impact on obesity and chronic diseases. Critical reviews in food science and nutrition, 43(4), 357-77

Brennan C. and Cleary L. (2005) The potential use of cereal (1→3,1→4)-β-glucans as functional food ingredients. Journal of Cereal Science, 42(1), 1-13

Casier J, de Paepe G, Willems H, Goffings G. and Noppen H. (1977) Bread production from pure millet and sorghum flours, using cereal endosperm-cellwall-pentosan as a universal baking factor. In Dendy D, Proceedings of a symposium on sorghum and millets for human food. London: Tropical Products Institute, 127-131

Catassi C. and Yachha S. (2009) The epidemiology of coeliac disease. In Arendt E. K. and Dal Bello F. The science of gluten-free foods and beverages. AACC International, 1-13

Cauvain S. (1998) Other cereals in breadmaking. In Cauvain S. and Young L. Technology of Breadmaking. London: Blackie Academic & Professional, 330-346

Cavallero A, Empilli S, Brighenti F, Stanca A.M. (2002) High (1→3,1→4)-Glucan Barley Fractions in Bread Making and their Effects on Human Glycemic Response. Journal of Cereal Science, 36, 59-66

Chandan, R. (1997) Properties of Milk and Its Components. In AACC Dairy-Based Ingredients. St. Paul, Minnesota, USA, Egan Press Handbook, 1-10

Charalampopoulos D, Wang R, Pandiella S.S, Webb C. (2002) Application of cereals and cereal components in functional foods: a review. International journal of food microbiology, 79(1-2), 131-41

Charlton K.E, MacGregor E, Vorster N.H, Levitt N.S. and Steyn K. (2007) Partial replacement of NaCl can be achieved with potassium, magnesium and calcium salts in brown bread. International journal of food sciences and nutrition, 58(7), 508-21

Chartrand L, Russo P.A, Dulhaime A.G, Seidman E.G, (1997) Wheat starch intolerance in patients with coeliac disease. Journal of the American Dietetic Association, 97, 612-618

Cocup R. and Sanderson W. (1987) Functionality of dairy ingredients in bakery products. Food Technology, 41, 86-90

Codex Alimentarius Commission 2008 Codex Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten

Corsetti A, Gobbetti M, De Marco B, Balestrieri F, Paoletti F, Russi L, Rossi J, (2000) Combined effects of sourdough lactic acid bacteria and additives on bread firmness and staling. Journal of Agricultural and Food Chemistry, 48, 3044-3051

Coussement P.A. (1999) Nutritional and Health Benefits of Inulin and Oligofructose Inulin and Oligofructose: Safe Intakes and Legal Status. The Journal of Nutrition, 1412-1417

Craig S, Maningat C.C, Seib A.P, Hoseney C.R. (1989), Starch paste clarity. Cereal Chemistry, 66, 173-182

Davidou S, Le Meste M, Debever E, Bekaert D. (1996) A contribution to the study of staling of white bread: effect of water and hydrocolloid. Food Hydrocolloids, 10, 375-383

DeVuyst L. and Neysens P. (2005) The sourdough microflora: biodiversity and metabolic interactions. Trends in Food Science and Technology, 16, 43-56

Dean T. (2000) Food intolerance and the food industry, Cambridge, Woodhead

Delcour J.A. and Hoseney R.C. (2010) Principles of Cereal Science and Technology 3 ed., AACC International Press

Delcros J, Rakotozafy L. and Boussard A. (1998) Effect of mixing conditions on the behaviour of lipoxygenase, peroxidase, and catalase in wheat flour. Cereal Chemistry, 75, 85-93

Deutsch H, Poms R, Heeres H, van Der Kamp J.-W. (2008) Labelling and regulatory issues. In Arendt E. K.and Dal Bello F. Gluten-free products and beverages. Academic Press, 29-46

Dewettinck K, Vanbockstaele F, Kuhne B, Vandewalle D, Courtens T, and Gellynck X. (2008) Nutritional value of bread: Influence of processing, food interaction and consumer perception. Journal of Cereal Science, 48(2), 243-257

Dubois D. (1978) Practical Application of fibre materials in bread production. Bakers Digest, 52(2), 30-33

EFSA Panel on Dietetic Products, (2009) Scientific Opinion on the substantiation of health claims related to beta-glucans and maintenance of normal blood cholesterol concentrations (ID 754, 755, 757, 801, 1465, 2934) and maintenance or achievement of a normal body weight (ID 820, 823) pursuant . EFSA Journal, 7(9), 1254

Elleuch M, Bedigian D, Roiseux O, Besbes S, Blecker C, Attia H, (2010) Dietary fibre and fibre-rich by-products of food processing: characterisation, technological functionality and commercial applications: a review. Food Chemistry, 124(2), 411-421

FDA, (1996) Food Labeling: Health claims; Oat and coronary heart disease. Federal register 61(3)

Fardet A, Rock E. and Remesy C. (2008) Is the *in vitro* antioxidant potential of whole-grain cereals and cereal products well reflected *in vivo*? Journal of Cereal Science, 48(2), 258-276

Fasano A. and Catassi C. (2001) Current Approaches to Diagnosis and Treatment of Celiac Disease: An Evolving Spectrum. Gastroenterology, 120(3), 636-651

Ferrari B, Finocchiaro F, Stanca A.M, Gianinetti A. (2009) Optimization of air classification for the production of β-glucan-enriched barley flours. Journal of Cereal Science, 50(2), 152-158

Figuerola F, Hurtado M, Estevez A, Chiffelle I, Asenjo F. (2005) Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. Food Chemistry, 91(3), 395-401

Fischer M.H, Yu N, Gray G.R, Ralph J. Anderson L, Marlett J.A. (2004) The gel-forming polysaccharide of psyllium husk (Plantago ovata Forsk). Carbohydrate research, 339(11), 2009-17

Foster-Powell, K., Holt, S. and Brand-Miller, J. (2002) International table of glycemic index and glycemic load values. The American Journal of Nutrition, 76, 5-56

Gallagher E, Gormley T. and Arendt E. (2003) Recent advances in the formulation of gluten-free cereal-based products. Trends in Food Science & Technology, 15, 143-152

Gambus H., Gambus F. and Sabat R. (2002) The research on quality improvement of gluten-free bread by amaranthus flour addition. Zywnosc, 9, 99-112

Gibson G.R, Beatty E.R, Wang X, Cummings J.H. (1995) Oligofructose and Inulin. Gastroenterology, 975-982

Grossman M. and De Barber B. (1997) Bread staling. Simultaneous effect of bacterial alpha-amylase and emulsifier on firmness and pasting properties of bread crumbs. Archivos Latinoamericanos Nutric., 47, 229-233

Guzmán-Maldonado S. and Paredes-López O. (1998) Functional products of plants indigenous to Latin America: amaranth, quinoa, common beans, and botanicals. In G. Mazza Functional foods: biochemical and processing aspects. Lancaster, PA: Technomic Publishing, 293-328

Géllinas P, Poitras E, McKinnon C.M, Morin A, (1998) Oxidoreductases and lipases as dough-bleaching agents. Cereal Chemistry, 78, 810-814

Hager, A-S., Wolter, A., Jacob, F., Zannini, E., Arendt, E.K. (2012) Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours, Journal of Cereal Science 56:239-247

Hoefler A. (2004) Hydrocolloids, St. Paul, Minnesota, USA, Egan Press Handbook

Hopkins S. and Gormley R. (2000) Rheological properties of pastes and gels made from starch separated from different potato cultivars. Lebensmittel-Wiss-und-Technol, 33, 388-396

Hoseney R, Varriano-Marston E. and Dendy D. (1981) Sorghum and millets. Advances in Cereal Science and Technology, 6, 71-144

Hüttner E. and Arendt E. (2010) Recent advances in gluten-free baking and the current status of oats. Trends in Food Science and Technology, 21(6), 303-312

Hüttner E.K, Bello F.D. and Arendt E.K. (2010) Rheological properties and bread making performance of commercial wholegrain oat flours. Journal of Cereal Science, 52(1), 65-71

Ikeda K. (2002) Buckwheat: composition, chemistry, and processing. Adv. Food Nutr. Res., 44, 395-434

Jacobs M.S, Izydorczyk M.S, Preston K.R, Dexter J.E. (2008) Evaluation of baking procedures for incorporation of barley roller milling fractions containing high levels of dietary fibre into bread. Journal of the Science of Food and Agriculture 88, 558-568

Jenkins D, Wolever T, Taylor R, Barker H, Fielden H, Baldwin J. (1981) Glycemic index of foods - a physiological basis for carbohydrate exchange. American Journal of Clinical Nutrition, 34(3), 362-366

Jenkins A.L, Jenkins D.J.A, Zdravkovic U, Würsch P, Vuksan V. (2002) Depression of the glycemic index by high levels of beta-glucan fiber in two functional foods tested in type 2 diabetes. European journal of clinical nutrition, 56(7), 622-8

Jonagh G, Slim T. and Greve H. (1968) Bread without gluten. Baker's Digest, 6, 24-29

Kato A, Ibrahim H, Watanabe H, Honma K. and Kobayashi K. (1990) Enthalpy of denaturation and surface functional properties of heated egg white proteins in the dry state. (55), 1280-1282

Kaukinen K, Collin P, Holm K, Rantala I, Vuolteenaho N, Reunala T, Mäki M. (1999) Wheat starch-containing gluten-free flour products in the treatment of coeliac disease and dermatitis herpetiformis. A long-term follow-up study. Scandinavian Journal of Gastroenterology, 34(2), 163-9

Kaur A, Singh N, Ezekiel R. and Singh Guraya H. (2006) Physicochemical, thermal and pasting properties of starches separated from different potato cultivars grown at different locations. Food Chemistry 101, 643-651

Kendall C.W, Esfahani A. and Jenkins D.J. (2010) The link between dietary fibre and human health. Food Hydrocolloids, 24(1), 42-48

Kenny S, Wehrle K, Auty M, Arendt E.K. (2001) Influence of sodium caseinate and whey protein on baking properties and rheology of frozen dough. Cereal Chem., 78, 458-463.

Keregero M.M. and Mtebe K. (1994) Acceptability of wheat-sorghum composite flour products: An assessment. Plant Food Hum. Nutr., 46, 305-312

Larrauri J. (1999) New approaches in the preparation of high dietary fibre powders from fruits by-products. Trends in Food Science and Technology, 10(1), 3-8

Lazaridou A. and Biliaderis C. (2007) Molecular aspects of cereal β-glucan functionality: Physical properties, technological applications and physiological effects. Journal of Cereal Science, 46(2), 101-118

Lehtinen P, Kaukovirta-Norja A, Sibakov J, Myllymaki O, Poutanen K, (2009) Functional Oat Ingredients - Opportunities and Challenges for Food Technology. Cereal Foods World, 54(6), 267-271

Lending C. and Larkins B. (1989) Changes in the zein composition of protein bodies during maize endosperm development. The Plant Cell, 1, 1011-1023

Lohiniemi S, Maeki M, Kaukinen K, Laippala P. and Collin P. (2000) Gastrointestinal Symptoms Rating Scale in Coeliac Disease Patients on Wheat Starch-based Gluten-free Diets. Scandinavian Journal of Gastroenterology, 9, 947-949

Madsen M. and Christensen D. (1996) Changes in viscosity properties of potato starch during growth. Starch, 48, 245-249

Mannie E. and Asp E. (1999) Dairy ingredients in baking. Cereal Foods World, 44, 143-146.

Mazza G. (1993) Storage, processing, and quality aspects of buckwheat seed. In Janick J. and Simon J. New Crops. New York, Wiley, 251-254

McCarthy D, Gallagher E, Gormley T.R, Schober T.J, Arendt E.K. (2005) Application of Response Surface Methodology in the Development of Gluten-Free Bread. Cereal Chemistry, 82(5), 609-615

Moore M.M, Schober T. J, Dockery P, Arendt E. K. (2004) Textural comparison of gluten-free and wheat based doughs, batters and breads. Cereal Chemistry, 81, 567-575

Murray J. (1999) The widening spectrum of coeliac disease. American Journal of Clinical Nutrition, 69, 354-365

Nicoletti C., Temblay J. and Ivory K. (2007) The immune system and allergic responses to food. In C. Mills, H. Wichers, and K. Hoffmann-Sommergruber Managing allergens in Food. Cambridge, Woodhead, 29-46

Nishinari K. and Takahashi R. (2003) Interaction in polysaccharide solutions and gels. Current Opinion in Colloid and Interface Science, 8, 396-400

Norton I. and Foster T. (2002) Hydrocolloids in real food systems. In Gums and stabilizers in the food industry II. Royal Society of Chemistry, 187-200

Nunes M.H, Ryan L.A. and Arendt E.K. (2009) Effect of low lactose dairy powder addition on the properties of gluten-free batters and bread quality. European Food Research and Technology, 229(1), 31-41

Onyango C, Mutungi C, Unbehend G, Lindhauer M.G. (2009) Creep-recovery parameters of gluten-free batter and crumb properties of bread prepared from pregelatinised cassava starch, sorghum and selected proteins. International Journal of Food Science and Technology, 44(12), 2493-2499

Onyango C, Mutungi C, Unbehend G, Lindhauer M.G. (2010) Rheological and baking characteristics of batter and bread prepared from pregelatinised cassava starch and sorghum and modified using microbial transglutaminase. Journal of Food Engineering, 97(4), 465-470

Onyango C., Unbehend G. and Lindhauer M.G. (2009) Effect of cellulose-derivatives and emulsifiers on creep-recovery and crumb properties of gluten-free bread prepared from sorghum and gelatinised cassava starch. Food Research International, 42(8), 949-955

Peressini D. and Sensidoni A. (2009) Effect of soluble dietary fibre addition on rheological and breadmaking properties of wheat doughs. Journal of Cereal Science, 49, 190-201

Poinot P, Arvisenet G, Grua-Priol J, Fillonneau C, Le-Bail A, Prost C. (2010) Influence of inulin on bread: Kinetics and physico-chemical indicators of the formation of volatile compounds during baking. Food Chemistry, 119(4), 1474-1484

Prego I, Maldonado S. and Otegui M. (1998) Seed structure and localization of reserves in Chenopodium quinoa. Ann Bot., 82, 481-488

Ranhotra G, Loewe R. and Puyat L. (1975) Preparation and fortification of soy-fortified gluten-free bread. Journal of Food Science, 40, 62-64

Renzetti S, Dal Bello F. and Arendt E.K. (2008) Microstructure, fundamental rheology and baking characteristics of batters and breads from different gluten-free flours treated with a microbial transglutaminase. Journal of Cereal Science, 48(1), 33-45

Ribotta P.D, Ausar S.F, Morcillo M.H, Pérez G.T, Beltramo D.M, León A.E. (2004) Production of gluten-free bread using soybean flour. Journal of the Science of Food and Agriculture, 84(14), 1969-1974

Risi J. and Galwey N. (1989) Chenopodium grains of the Andes: a crop for temperate latitudes. In Wickens G., Haq N., and Day P. New crops for food and industry. London, Chapman and Hall, 222-234

Roberfroid M.B, (2007) Inulin-type fructans: functional food ingredients. The Journal of nutrition, 137(11), 2493S-2502S

Rojas J, (1999) Pasting properties of different wheat flour-hydrocolloid systems. Food Hydrocolloids, 13(1), 27-33

Rooney L. and Waniska R. (2000) Sorghum food and industrial utilization. In C. Smith and R. Frederiksen Sorghum: Origin, History, Technology, and Production. New York, John Wiley and Sons, Inc, 689-750

Rosell C.M. (2009) Enzymatic manipulation of gluten-free breads. In E. Gallagher Gluten-Free Food Science and Technology. Chichester, United Kingdom: Blackwell Publishing Ltd, pp. 82-98.

Rossel C, Haros M, Escriva C, Benedito De Barber C. (2001) Experimental approach to optimise the use of alpha-amylases in breadmaking. Journal of Agricultural Food Chemistry, 49, 2973-2977

Ruales J. (1992) Development of an infant food from quinoa (Chenopodium quinoa, Willd): technological aspects and nutritional consequences. University of Lund, Thesis

Ruales J. (1998) Quinoa (Chenopodium quinoa, Willd): nutritional quality and technological aspects as human food. In Increasing the utilisation of sorghum, buckwheat, grain amaranth and quinoa for improved nutrition. Norwich, UK, Institute of Food Research, 49-64

Sahlström S. and Brathen E. (1997) Effects of enzyme preparations for baking, mixing time and resting time on bread quality and bread staling. Food Chemistry, 58, 75-80

Salmeron J, Manson J, Stampfer M, Colditz G, Wing A, Willett W. (1997) Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. JAMA-Journal of the American Medical Association, 277(6), 472-477

Satin M. (1998) Bread without wheat. Novel ways of making bread form cassava and sorghum could reduce the Third World's dependence on imported wheat for white bread. New Sci., 28, 56-59

Scazzina F, Del Rio D, Pellegrini N, Brighenti F, (2009) Sourdough bread: Starch digestibility and postprandial glycemic response. Journal of Cereal Science, 49(3), 419-421

Schober T, Messerschmidt M, Bean S.R, Park S.H, Arendt E.K. (2005) Gluten-Free Bread from Sorghum: Quality Differences Among Hybrids. Cereal Chemistry, 82(4), 394-404

Shewry P. (2009) Wheat. Journal of Experimental Botany, 60(6), 1537-1553

Shukla R. and Cheryan M. (2001) Zein: the industrial protein from corn. Industrial Crops and Products, 13, 171-192

Singhal R. and Kulkarni P. (1988) Review: amaranthus an under-utilized resource. Int J Food Sci Technol, 23, 125-140

Skerritt J. and Hill A. (1992) How 'free' is 'gluten-free'? Relationship between Kjeldahl nitrogen values and gluten protein content for wheat starches. Cereal Chemistry, 69, 110-112

Sluimer P. (2005) Principles of Breadmaking, Functionality of Raw Materials and process Steps, St. Paul, Minnesota: American Association of Cereal Chemists

Souci, S.W., Fachmann, W., Kraut, H. (2004) Lebensmitteltabelle für die Praxis. Wissenschaftliche verlagsgesellschaft mbH, Stuttgart, Germany

Symons L. and Brennan C. (2004) The Influence of (1→3) (1→4)-B-D-Glucan-rich Fractions from Barley on the Physicochemical Properties and *In Vitro* Reducing Sugar Release of White Wheat Breads. Food Chemistry and Toxicology, 69(6), 463-467

Taylor J. and Parker M. (2002) Quinoa. In P. S. Belton and J. Taylor Pseudocereals and less common cereals, grain properties and utilization potential. Berlin, Springer Verlag, 93-122

Taylor J, Schober T. and Bean S. (2006) Novel food and non-food uses for sorghum and millets. Journal of Cereal Science, 44(3), 252-271

Thebaudin J, Lefebevre A.C, Harrington M. and Bourgeois C.M. (1997) Dietary fibres: Nutritional and technological interest. Trends in Food science and Technology, (1978), 41-48

The Comission of the European Community (2008) Council Directive 90/496/EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions

Thompson T. (2001) Wheat Starch, Gliadin, and the Gluten-free Diet. Journal of the American Dietetic Association, 101(12), 1456-1459

Tiwari U. and Cummins E. (2009) Factors influencing β-glucan levels and molecular weight in cereal based products. Cereal Chemistry, 86, 290-301

Topping D. (2007) Cereal complex carbohydrates and their contribution to human health. Journal of Cereal Science, 46(3), 220-229

Torbica A, Hadnađev M. and Dapčević T. (2010) Rheological, textural and sensory properties of gluten-free bread formulations based on rice and buckwheat flour. Food Hydrocolloids, 24(6-7), 626-632

Tosi E, Ciappini M. and Masciarelli R. (1996) Utilisation of whole amaranthus (Amaranths cruentus) flour in the manufacture of biscuits for coeliacs. Alimentaria, 34, 49-51

Trogh I, Courtin C.M, Andersson A.A.M, Aman P, Soerensen J.F, Delcour J.A. (2004) The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley flour breads with increased arabinoxylan and. (1-3)(1-4)-β-D-glucan levels. Journal of cereal Science, 40(3), 257-267

Tuohy K.M, Kolida S, Lustenberger A.M, Gibson G.R, (2007) The prebiotic effects of biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides – a human volunteer study. British Journal of Nutrition, 86(03), 341

Turabi E, Sumnu G. and Sahin S. (2010) Quantitative analysis of macro and micro-structure of gluten-free rice cakes containing different types of gums baked in different ovens. Food Hydrocolloids, 24(8), 755-762

Valencia-Chamorro S. (2003) Quinoa. In Caballero and B Encyclopedia of Food Science and Nutrition. Amsterdam, Academic Press, 4895–4902

Vemulappali V. and Hoseney R. (1998) Glucose oxidase effects on gluten and water solubles. Cereal Chemistry, 75, 859-862

Verspreet, J., Hemdane, S., Dornez, E., Cuyvers, S., Delcour, J.A., Courtin, C.M. (2013) Maximizing the Concentrations of Wheat Grain Fructans in Bread by Exploring Strategies To Prevent Their Yeast (Saccharomyces cerevisiae)-Mediated Degradation. Journal of Agriculture and Food Chemistry 61, 1397-1404

Vitaglione P., Napolitano A. and Fogliano V. (2008) Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. Trends in Food Science and Technology, 19(9), 451-463

WHO (2005) Preventing chronic diseases: a vital investment. Geneva, World Health Organisation

Wal J. and Lovik M. (2007) Integrative approaches to assessing the allergenicity of foods. In C. Mills, H. Wichers, and K. Hoffmann-Sommergruber Managing allergens in food. Cambridge, Woodhead, 207-227

Wang J, Rosell C. and Beneditodebarber C. (2002) Effect of the addition of different fibres on wheat dough performance and bread quality. Food Chemistry, 79(2), 221-226

Willard M. and Hix V. (1987) Potato flour. In W. Talburt and O. Smith Potato processing. New York, Van Nostrand Reinhold Co., 665-681

Williams J. and Brenner,D. (1995) Grain amaranth (Amaranthus species). In J. Williams Cereals and pseudocerals. New York, Chapman and Hall, 129-186

Wood P. (2007) Cereal β-glucans in diet and health. Journal of Cereal Science, 46(3), 230-238

Wrigley, C., Corke, H., and Walker C.E. (2004) Encyclopedia of grain science, Elsevier Academic Press

Zannini E, Garofalo C, Aquilanti L, Santarelli S, Silvestri G, Clementi F. (2009) Microbiological and technological characterization of sourdoughs destined for bread-making with barley flour. Food microbiology, 26(7), 744-53

Zekovic D, Kwiatkowski S, Vrvic M.M, Jakovljevic D, Moran C.A. (2005) Natural and modified (1->3)-beta-D-glucans in health promotion and disease alleviation. Critical reviews in biotechnology, 25(4), 205-30

Chapter 3: Status of carbohydrates and dietary fibre in the gluten-free diet

Anna-Sophie Hager, Claudia Axel and Elke K. Arendt (2011)

3.1 Summary

Coeliac disease is one of the most common food intolerances worldwide and at present the gluten free diet remains the only suitable treatment. Since the life-long avoidance of this cereal protein means a major change to the diet, it is important to consider its effect on the nutritional status of adhering individuals. This study reviews the available publications on the total carbohydrate as well as the fibre intake of coeliac disease patients compared to that of the non-coeliac general public. In addition, 95 gluten free breads currently available on the market have been purchased and evaluated with regard to their nutritional value. Several fibre enriched gluten free breads are available on the market. This leaves the responsibility to the consumer to choose nutritionally valuable products. In conclusion it can be said that the dietary fibre intake in coeliac patients, as well as the general public is below recommendations.

3.2 Introduction

Coeliac disease is an immune-mediated enteropathy triggered by the ingestion of the cereal protein gluten, present in wheat, rye and barley. Since life-long avoidance of gluten is currently the only treatment for coeliac disease, it is important to consider the effect of such a gluten free diet on nutrient intake and nutritional status of adhering patients. Concerns have been raised over the long term dietary habits and food choices of individuals on a strict gluten free diet, as a result from a number of studies indicating unbalanced intake of carbohydrates, proteins, and fat, as well as limited intake of certain essential nutrients in coeliac patients (Lohiniemi *et al*. 2000; Thompson *et al*. 2005). One highly important nutrient, repeatedly shown not to be consumed in sufficient amounts, is dietary fibre. Even though adherence to a gluten free diet might also lead to an insufficient consumption of other nutrients such as

calcium or certain vitamins, this review focuses on carbohydrate, sugar and fibre consumption in coeliac patients as well as on the content of these macronutrients in commercially available products. According to the European Food Safety Authority (EFSA) the intake of total carbohydrates - including starch and simple carbohydrates such as sugars - should range from 45 to 60% of total energy intake for both adults and children. Insufficient evidence was found to set an upper limit for sugars. This is because the possible health effects are mainly related to patterns of food consumption – i.e. the types of foods consumed and how often they are consumed – rather than to the total intake of sugars itself (Ruxton *et al*. 2010; Anderson *et al*. 2009). Although dietary fibres are in terms of chemical structure also carbohydrates, they are per definition not included in this macronutrient group, but stated separately on the nutrition label. The European Commission defines fiber as carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories: edible carbohydrate polymers naturally occurring in the food as consumed; edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence; edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence (The Commission of the European Communities 2008). Dietary fibre generally falls into the two categories soluble and insoluble. The most soluble dietary fibres are more rapidly fermented in the colon and they are more accessible to hydrolytic enzymes; whereas the less soluble fibres are excreted in the stool and thus have the effect of increasing faecal bulk. EFSA as well as the Food and Drug Administration (FDA) recommend a daily total dietary fibre intake of 25 g per day, of which 6 g should be soluble fibre. The role of dietary fibre in contributing to a healthy intestine has long been recognised. Potential health benefits of dietary fibre include, reduction of bowel transit time, prevention of constipation, reduction in risk of colorectal cancer, lowering of blood cholesterol, production of short chain fatty acids

and promotion of the growth of beneficial gut microflora (Brennan and Cleary 2005). From a technological point of view, fibre addition can modify texture and sensory characteristic as well as prolong shelf-life, due to their water binding capacity, gel forming potential, fat mimetic properties and thickening effects (Sabanis *et al*. 2009; Thebaudin *et al*. 1997). One important source of dietary fibre is cereals, contributing to about 50% of the fibre intake in western countries (Nyman *et al*. 1989). In the gluten free diet, the consumed cereal products are considerably different to the gluten containing foods of this category, which might influence their nutritional quality. Several studies show that the dietary fibre intake of coeliac patients is too low (Grehn *et al*. 2001; Lee *et al*. 2009; Lohiniemi *et al*. 2000; Mariani *et al*. 1998; Öhlund *et al*. 2010; Thompson *et al*. 2005). It was proposed that this is due to the fact that gluten free breads are very often made from starches and/or refined flours and that these products are rarely enriched with fibres (Thompson 2000). Therefore they might contain less fibre than their gluten containing counterparts. The purpose of this study was to evaluate gluten free breads currently available on the market. For this purpose, 95 gluten free breads have been purchased in supermarkets and health shops of 7 European countries (France, Ireland, Italy, Finland, Germany, Austria, and Sweden) as well as the United States of America. Their nutritional value regarding intake of calories, carbohydrates and sugar as well as dietary fibre content, as stated on the packaging, was summarised and compared.

3.3 Dietary fibre intake by coeliac patients as well as non-coeliac subjects

The results of several studies reported in the literature on the consumption of carbohydrates, sugars and dietary fibre of coeliac disease patients are summarised and compared in Table 3.1 and Table 3.2. Table 3.1 summarises studies on the intake of these nutrients by the non-coeliac general public. Wild *et al*. (2010) analysed the data from 93 validated 5-day food diaries, observing that only 42 % of men and women got more than 47 % of their total energy intake from carbohydrate sources. These authors reported an intake of nonstarch polysaccharides of

13.7 g/day. Öhlund *et al.* (2010) performed a study of 25 children, at the age of 4-17 years, with confirmed coeliac disease and living on a gluten free diet. Using 5-day food records, it was shown that dietary fibre intakes were lower than the recommendations. The mean intake of carbohydrate met the recommendations. However, quality of carbohydrate was characterised by a high intake of sucrose and a low intake of dietary fibre. After recording the 3-day usual nutritional intakes of fifty randomly selected patients, Lee *et al.* (2009) reported that the standard gluten free diet did not meet the recommended intake for fibre. Hopman *et al.* (2006) evaluated the 3-day food records of 111 adolescent members of the Dutch coeliac society. They showed that the coeliac patients had an intake of fibre significantly lower than the recommended daily allowance (RDA). Thompson *et al.* (2005) concluded from a study employing 3-day estimated self-reported food records that seven out of eight males (88 %) and 18 out of 39 women (46 %) had estimated dietary fibre intakes that met or exceeded recommended daily intakes of 20-35 g/day. Seven out of eight males (88 %) and 34 out of 39 women (87 %) had estimated carbohydrate intakes within the acceptable range of 45-60 % from total calories. There is no information available on how much of these carbohydrates were sugars. From a study examining 47 adolescents, aged 10-20 years, with coeliac disease and 47 healthy age-matched control subjects using 3-day alimentary records, it was concluded that diets contained low amounts of carbohydrates and fibre. It was shown that fibre consumption was significantly reduced in subjects consuming a gluten free diet as compared to the healthy control subjects (Mariani et al. 1998). Grehn *et al.* (2001) published a study assessing the dietary habits of 49 Swedish adult coeliac patients as well as those of a control group. Using a 4-day dietary record it was shown that coeliacs as well as controls had too low an intake of fibre. Comparing the two groups, the fibre intake was significantly lower for the coeliac patients. The relative contribution of dietary fibre from bread was generally lower in coeliac disease sufferers (28 %) than in the controls (38 %). The energy intakes as well as the relative contributions of protein, fat and carbohydrate were in the same range in coeliac

patients and controls. Lohiniemi *et al*. (2000) used a 4-day food record of 58 adult coeliac disease patients, concluding that the daily fibre intake (13 g) was lower than the average consumption level in Finland (24 g).

All the above mentioned studies show too low an intake of dietary fibre among coeliac patients. Hence, more emphasis should be placed on the nutritional quality of the gluten free diet. However, regarding the dietary fibre uptake of the general non-coeliac population, intakes are as well commonly lower than recommended (Table 3.2). Yet, they are higher than in the coeliac patients. Fukuda *et al* (2007) used one day food records to evaluate dietary fibre intake among the Japanese general population, showing an average consumption of 18.4 g per day. Castetbon *et al.* (2009) used three 24 h recalls to describe the dietary intake of 2734 adults in France, concluding that compared to current recommendations, the intake of carbohydrates and total fibre was frequently unsatisfactory. Galvin *et al* (2001) used a 7-day food diary to collect food intake data of 1379 respondents. The resulting average fibre consumption was 20.2 g per day. Elmadfa and Freisling (2003) evaluated the macronutrient intake of the Austrian general population, concluding a too low fibre intake among all groups of the population. As a result of too high intake of protein and fat, the average consumption of carbohydrates was too low. Among children and adolescents, the intake of carbohydrates was sufficient. However, 12 to 19 % of carbohydrates consumed were sugars. Only the subjects of one study, including 4237 subjects and using food-frequency questionnaires, met the recommendations for dietary fibre intake. The determined average fibre consumption was 26.8 g per day. The average intake of carbohydrates was 41.3 % with 19.1 % coming from mono- and disaccharides (Van de Vijver et al. 2009).

Overall it must be concluded that the dietary fibre intake is too low in a substantial proportion of the population. This is likely to contribute to impaired bowel function and constipation, increased risk of chronic gastrointestinal diseases as well as coronary heart diseases and diabetes. The intake of carbohydrates as a percentage of the total energy consumed was

within the acceptable range in most studies. However, the intake of sugars was frequently very

high, especially in children and adolescents.

Table 3.1 Results of several studies on the dietary fibre intake of coeliac disease patients

Nutrient	Recommendations	Mariani (1998)	Lohiniemi (2000)	Grehn (2001)	Thompson (2005)	Hopman (2006)	Lee (2009)	Öhlund (2010)	Wild (2010)
Carbohydrates/Sucrose [%E]	Children, Adolescents: 45-65*	43.2/N.I.A.				54.0/N.I.A.		54.2 /14.7	
	Adults: 45-65*		N.I.A.	49/N.I.A.	53.5/N.I.A.		N.I.A.		58.5/N.I.A.
Fibre [g/day]	Children, Adolescents: 19-26*	8.5				17.0		9.9	
	Adults: 25-38*		13.0	10.8	22.3		5.0		13.7

*Dietary reference Intakes: Macronutrients; USDA United States Department of Agriculture; http://fnic.nal.usda.gov/nal_display/index.php?info_center=4&tax_level=3&tax_subject=256&topic_id=1342&level3_id=5140; accessed 18/06/2010

Table 3.2 Results of several studies on the dietary fibre intake of non-coeliac subjects

Nutrient	Recommendations	Mariani (1998)	Grehn (2001)	Galvin (2001)	Elmadfa (2003)	Fukuda (2007)	Castetbon (2009)	Van de Vijver (2009)
Carbohydrates/Sucrose [%E]	Children, Adolescents: 45-65*	48.4/N.I.A.			52.0/13.5			
	Adults: 45-65*		46.5/N.I.A.	N.I.A.	43.5/9.6	N.I.A.	45.7/20.1	41.9/19.1
Fibre [g/day]	Children, Adolescents: 19-26*	11.2			13.7			
	Adults: 25-38*		16.7	20.2	19.3	18.4	17.5	26.8

*Dietary reference Intakes: Macronutrients; USDA United States Department of Agriculture; http://fnic.nal.usda.gov/nal_display/index.php?info_center=4&tax_level=3&tax_subject=256&topic_id=1342&level3_id=5140; accessed 18/06/2010

3.4 Nutritive value of gluten free breads currently on the market

The cereal group provides important amounts of most nutrients and plays a major role in human nutrition. Carbohydrates have a special significance in cereal products such as breads. In the course of this study, 95 gluten free bread products were purchased and their nutritive value with respect to calories, carbohydrate and dietary fibre content was evaluated. The products were grouped into white breads, brown breads, multi-seed breads and pumpernickel style breads. The average values for provided energy as well as carbohydrate and fibre content were calculated for each bread group and compared to the values for a standard wheat bread of the same category. The data for the reference wheat breads was sourced from the United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference. Energy is required to sustain the body's various functions, including respiration, circulation, physical work, and maintenance of core body temperature. In the gluten free products reviewed, the energy content (kcal/100g) varies from 178 kcal/100g for a multi-seed gluten free bread to 311 kcal for white rolls as well as a sunflower containing bread. In this respect the origin of the calories is interesting. Appendix Ia shows the calorie content as well as the amount of carbohydrates and the amount of sugar of the gluten free products. The primary role of carbohydrates (sugars and starches) is to provide energy to cells in the body. They can be classified into two broad categories: the available and unavailable ones. Available carbohydrates are those digested and absorbed by humans, which include starch and soluble sugar. Dietary fibres are carbohydrates which are not digested by the endogenous secretion of the human digestive tract (Southgate 1995). Starch is the most abundant cereal polysaccharide and is a major food reserve providing a bulk nutrient and energy source in the human diet (Dewettinck 2008). The carbohydrate content of most gluten free products is lower than the standard gluten-containing ones. This is probably due to the fact that in addition to flour or starch, nearly all gluten free formulations contain oil and a protein source such as milk or whey powder, soy protein concentrate, egg albumin, rice or lupine protein.

Another value to be considered is the content of simple carbohydrates or "sugars". On food labels "sugars" is defined as all monosaccharides and disaccharides present, excluding polyols. The intake of mono- and disaccharides should be limited as much as possible since they increase the caloric value of a product without providing vitamins or minerals. Several products (21 out of 95) did not give information on the sugar content. In the other products the sugar content varied widely from trace amounts to substantial amounts of 8.8 g/100g for white rolls (of 46.7 g/100g carbohydrates) and 12.4 g/100g sugar for a sweet white bread (of 52.2 g/100g carbohydrates). The white wheat bread included into the study as a wheat referecence contained 4.3 g of sugar per 100g bread.

The importance of a sufficient dietary fibre intake has long been recognized. This substance group includes resistant starch, cellulose and other complex polysaccharides, such as arabinoxylans, β-glucans, pectins, arabinogalactans and lignin. Dietary fibre can be divided into soluble and insoluble portion. Soluble dietary fibre slows down glucose absorption, reduces plasma cholesterol concentrations and is useful in the management of diabetes and heart diseases. Insoluble fibre increases faecal weight, bulk and softness as well as the frequency of defecation and reduces the intestinal transit times. These effects probably play a role in preventing colon cancer and other bowel disorders (Dewettinck et al. 2008). The analysis of ingredients of the gluten free breads showed that a high number of them are fibre enriched. The substances used for this purpose include sugar beet fibre, psyllium husk, citrus fibre, pea fibre, cellulose and derivates (e.g. Hydroxypropylmethylcellulose HPMC), inulin, apple fibre, and bamboo fibre. Table 3.3 shows the amount of total, soluble and insoluble fibre as well as colour and the water holding capacity of some commercially available fibres. Regarding the dietary fibre content of the products, only 74 out of 95 stated the levels on the packaging. As shown in appendix Ia, big variations of this value could be observed. As expected, the white breads showed the lowest fibre contents, ranging from 0.1 g to 9.7 g per 100 g bread, with an average of 4.0 g per 100 g. However, compared to its gluten containing counterparts, the

majority of gluten free white breads (25 out of 32) contained a higher amount of fibre. The brown breads showed a similar average fibre content to the white bread category, with an average of 4.8 (1.3 g minimum and 9.3 g maximum). This is probably due to the fact that in several cases brands market the same product formulation twice. In many cases the white bread is transformed into brown bread, simply by adding food colouring, i.e. molasses or caramel. Seventeen out of the 54 products (brown, multi-seed or pumpernickel style breads) contained caramel, dark syrup or treacle among the ingredients. The pumpernickel style breads show a higher fibre content between 4.0 g and 7.0 g per 100 g with an average of 5.3 g per 100 g. Therefore these breads lie only slightly below the average for gluten containing pumpernickel style bread (6.5 g). The highest fibre content showed the breads containing multi-seeds, with values ranging from 3.9 g to 14.2 g and an average of 7.7 g per 100 g. The standard reference for this type of bread, containing gluten, is 7.4 g per 100 g. Summarising this data, it can be seen that the dietary fibre content of the gluten free breads is generally higher than that of the gluten-containing counterparts. However, there is some uncertainty to the data; since there is a substantial amount of samples (22 %) that did not give any information on the fibre content. It is possible that these samples were very low in dietary fibre.

Table 3.3 Several commercially used fibres and some of their physiologically and technologically important properties

Fibres	Total dietary fibre [g/100 g]	Soluble [g/100 g]	Insoluble [g/100 g]	Colour	WHC [g water/g solid]	References
Apple fibre	60-90	8-15	45-81	brownish	6.1	Product Specification sheet Rettenmeier (Germany)
Bamboo fibre	97	0	97	white	4.8	Rossel, Santos, Collar (2009)
Cellulose	98	1	97	white	5.6	Rossel, Santos, Collar (2009)
HPMC	100	100	0	white	n.a.	Rossel, Santos, Collar (2009)
Citrus fibre	44-68	5-10	38-62	white - light yellow	n.a.	Figuerola *et al*. (2005)
Inulin	97	97	0	white	11.1	Rossel, Santos, Collar (2009)
Pea fibre	65-75	>0.5	>65	white - beige	6-9	Product Specification sheet Rettenmeier (Germany)
Psyllium husk	77-80	>75	2-5	brownish	20	Product Specification sheet Rettenmeier (Germany); Goni *et al.* (1998)
Sugar beet fibre	67-75	>45	>22	white	26	Sabanis *et al.* (2009); Javidipour *et al.* (2005)

3.5 Possible ways of improving fibre uptake by coeliac individuals

As discussed above, the dietary fibre intake is too low in a substantial proportion of the population, especially in coeliac patients. Therefore possible ways of increasing the uptake of this highly important nutrient have to be considered. Lee *et al*. (2009) suggested that the use of grains with high nutritional value can be used in the gluten free diet and has therefore the potential to improve the nutritional profile of the diet for individuals with coeliac disease. Many alternatives to common gluten containing grains exist, such as the pseudocereals amaranth, quinoa, and buckwheat (Kupper 2005). These grains are characterised by an excellent nutrient profile and therefore the availability of palatable pseudocereal-containing gluten free products would represent a significant advance towards ensuring an adequate intake of nutrients in subjects with coeliac disease (Alvarez-Jubete *et al.* 2010). In particular, dietary fibre content is significantly higher in buckwheat seeds in comparison with common cereals. Therefore, the incorporation of these seeds in the diets of coeliac patients should help alleviate, at least in parts, the deficit in fibre intake in this sector of the population (Alvarez-Jubete *et al*. 2009).

Thompson (2000) suggested that a further measure to increase intakes of fibre is to encourage patients to consume enriched/fortified gluten free flours, breads and pastas whenever possible. Hopman *et al.* (2006) compared the intake of coeliac patients using non-enriched gluten free products and patients who use enriched gluten free foods. The latter had a significantly higher intake of fibre. However these patients still did not reach the RDA for fibre. Hence, patients should also increase their consumption of non-cereal naturally gluten free fibre sources such as fruits, vegetables, legumes, nuts and seeds.

The comparison of several studies on the carbohydrate intake showed that this macronutrient is consumed in reasonable amounts. Frequently, the intake of simple sugars is high. Regarding the dietary fibre uptake, the studies showed that the intake of this nutrient is insufficient among coeliacs as well as non-coeliac subjects. In comparison the dietary fibre uptake in

coeliacs is even lower. However, the link between an inadequate dietary fibre consumption and low nutritional quality of gluten free products cannot be confirmed. In general, it can be said that the necessity of fibre enrichment in gluten free breads was recognized by the food industry. Many products are available that contain a sufficient amount of fibre. Although several products contain high amounts of simple carbohydrates, gluten free breads with reasonable sugar content are available. This leaves the responsibility with the consumer, to choose nutritionally valuable products.

3.6 Acknowledgements

3.7 References

Alvarez-Jubete L, Arendt E.K, Gallagher E. (2009) Nutritive value and chemical composition of pseudocereals as gluten free ingredients. International Journal of Food Science and Nutrition, 60, 240-257

Alvarez-Jubete L, Arendt E.K. and Gallagher E. (2010) Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. Trends in Food Science and Technology, 21, 106-113

Anderson C.A, Curzon M.E, Van Loveren C, Tatsi C. and Duggal M.S. (2009) Sucrose and dental caries: a review of the evidence. Obesity Reviews, 10, 41-54

Brennan C. and Cleary L. (2005) The potential use of cereal $(1\rightarrow3,1\rightarrow4)$-β-glucans as functional food ingredients. Journal of Cereal Science, 42, 1-13

Castetbon K, Vernay M, Malon A, Salanave B, Deschamps V, Roudier C, Oleko A, Szego E. and Hercberg S. (2009) Dietary intake, physical activity and nutritional status in adults: the French nutrition and health survey (ENNS, 2006-2007). British Journal of Nutrition, 102, 733-743

Dewettinck K, Bockstaele F.V, Kuhne B, Van de Wallea D, Courtensa T.M. and Gellynckc X. (2008) Nutritional value of bread: Influence of processing, food interaction and consumer perception. Journal of Cereal Science, 48, 243-257

Elmadfa I, Freisling H. (2003) Zufuhr von Makronaehrstoffen bei verschiedenen Bevoelkerungsgruppen in Oesterreich. Ernahrungsumschau, 50, 464-468

Figuerola F, Hurtado M, Estevez A, Chiffelle I. and Asenjo F. (2005) Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. Food Chemistry, 91(3), 395-401

Fukuda S, Saito H, Nakaji S, Yamada M, Ebine N, Tsushima E, Oka E, Kumeta K, Tsukamoto T. and Tokunaga S. (2007) Pattern of dietary fiber intake among the Japanese general population. European Journal of Clinical Nutrition, 61, 99-103,

Galvin M.A, Kiely M, Harrington K.E, Robson P.J, Moore R. and Flynn A. (2001) The North/South Ireland Food Consumption Survey: the dietary fibre intake of Irish adults. Public Health and Nutrition, 4, 1061-1068

Goni I. and Martin-Carron N. (1998) *In Vitro* fermentation and hydration properties of commercial dietary fiber-rich supplements. Nutrition Research, 18, 1077-1089

Grehn S, Fridell K, Lilliecreutz M. and Hallert C. (2001) Dietary habits of Swedish adult coeliac patients treated by a gluten-free diet for 10 years. Scandinavian Journal of Nutrition, 45, 178-182

Hopman E.G, Cessie S., von Blomberg B.M. and Mearin M.L. (2006) Nutritional management of the gluten-free diet in young people with celiac disease. Journal of Pediatric Gastroenterology and Nutrition, 43, 102-108

Javidipour I, Vural H, Ozbas O.O. and Tekin A. (2005) Effects of interesterified vegetable oils and sugar beet fibre on the quality of Turkish-type salami. International Journal of Food Science and Technology, 40(2), 177-185

Kupper C. (2005) Dietary Guidelines and Implementation for Celiac Disease. Gastroenterology, 128, 121-127

Lee A.R, Ng D.L, Dave E, Ciaccio E.J. and Green P.H. (2009) The effect of substituting alternative grains in the diet on the nutritional profile of the gluten-free diet. Journal of Human Nutrition and Dietetics, 22, 359-363

Lohiniemi S, Maeki M, Kaukinen K., Laippala P. and Collin P. (2000) Gastrointestinal Symptoms Rating Scale in Coeliac Disease Patients on Wheat Starch-based Gluten-free Diets. Scandinavian Journal of Gastroenterology, 9, 947-949

Mariani P, Viti M, Montouri M, La Vecchia A, Cipolletta E, Calvani L. and Bonamico M. (1998) The Gluten-Free Diet: A Nutritional Risk Factor for Adolescence with Coeliac Disease? Journal of Pediatric Gastroenterology and Nutrition, 27, 519-523

Nyman M.I, Björck I, and Siljeström M. (1989) Dietary fibre in cereals – composition, fermentation and effect of processing. In: Proceedings from an international symposium on cereal science and technology, Lund University, 40–54

Rossel C, Santos E. and Collar C. (2009) Physico-chemical properties of commercial fibres from different sources: A comparative approach. Food Research International, 42, 179-184

Ruxton C.H, Gardner E.J. and McNulty H.M. (2010) Is sugar consumption detrimental to health? A review of the evidence 1995-2006. Critical Reviews in Food Science and Nutrition, 50, 1-19

Sabanis D, Lebesi D. and Tzia C. (2009) Effect of dietary fibre enrichment on selected properties of gluten-free bread LWT – Food Science and Technology, 42, 1380-1389

Southgate D. (1995) Dietary Fibre Analysis. Cambridge, UK: The Royal Society of Chemistry

The Comission of the European Community (2008) Council Directive 90/496/EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions.

Thebaudin J., Lefebevre A., Harrington M. and Bourgeois C.M. (1997) Dietary fibres: Nutritional and technological interest. Trends in Food Science and Technology, 8, 41-48

Thompson T, Dennis M, Higgins L.A, Lee A.R. and Sharrett M.K. (2005) Gluten-free diet survey: are Americans with coeliac disease consuming recommended amounts of fibre, iron, calcium and grain foods? Journal of Human Nutrition and Dietetics, 18, 163-169

Thompson T. (2000) Folate, iron and dietary fibre contents of the gluten-free diet. Journal of the American Dietetic Association, 100, 1389-1396

Thompson T. (2010) Nutritional Requirements for Gluten-Free Foods. In Second International Symposium on Gluten-Free Cereal Products and Beverages – Book of Abstracts, 41-42

Van de Vijver L.P, van den Bosch L.M, Brandt P.A. and Goldbohm R.A. (2009) Whole-grain consumption, dietary fibre intake and body mass index in the Netherlands cohort study. European Joural of Clinical Nutrition, 63, 31-38

Wild D, Robins G.G, Burley V.J. and Howdle P.D. (2010) Evidence of high sugar intake, and low fibre and mineral intale, in the gluten-free diet. Alimentary Pharmacology and Therapeutics, 32, 573-581

Öhlund K, Olsson C, Hernell O. and Öhlund I. (2010) Dietary shortcomings in children on a gluten-free diet. Journal of Human Nutrition and Dietetics, 23, 294-30022

Chapter 4: Gluten free Pasta—Advances in Research and Commercialization

Hager, A.-S., Zannini, E., Arendt, E.K. (2012)

4.1 Abstract

Due to ease of preparation, palatability, versatility, cheapness of the product, nutritive value, and long shelf life, pasta is an extremely popular product. Commonly, it is produced by extrusion of durum wheat semolina and water. However, coeliac disease, an immune mediated disease triggered by the ingestion of the protein composite gluten, makes it necessary for a significant part of the human population to exclude wheat, rye and barley permanently from the diet. Therefore, the availability of high quality cereal products from alternative grains is important. Recently, research on the development and amelioration of gluten free pasta has intensified. Also the number of gluten free pasta products on the market has increased strongly. The question arises if the findings of food scientists are well reflected in the available products. Hence, this review presents an overview on the outcomes of recent studies and the composition and quality of commercial gluten free pasta samples. The 33 samples considered in this study were products, sold as "gluten free pasta" and were sourced from eight different European countries (Austria, Finland, France, Germany, Ireland, Italy, Portugal, and Sweden). For comparison, an Italian brand for wheat pasta (DeCecco) was included into the study.

4.2 Selection of raw materials

Coeliac disease is a lifelong immune disorder where parts of the small intestine are damaged or destroyed by the immune system due to a reaction upon ingestion of gluten. Currently, the only available treatment for coeliac disease is the gluten free diet. This creates a demand for products made from raw materials other than wheat, rye and barley. Especially the utilisation of rice (Lai 2002) and corn (Da Silva *et al*. 2008; Mastromatteo *et al*. 2011) for the production of pasta is well researched. These are also the most commonly used gluten free

ingredients in industry: 22 out of the 33 commercial products considered in this study contain rice and 20 out of 33 products contained corn. Less researched raw materials for pasta production include the pseudocereals quinoa (Caperuto 2001), buckwheat (Alamprese *et al*. 2007) and amaranth (Schoenlechner 2010). Only one of the commercial products contained buckwheat. Despite the high nutritional quality of buckwheat, its use is limited due to a higher price in comparison to maize and rice as well as the dark colour and strong flavour. One product contained quinoa, but amaranth was not found in the commercial products screened. Chillo *et al*. (2008) reported that the use of only amaranth flour presented remarkable difficulties in the extrusion phase. However, the incorporation of quinoa, chickpea and broad bean flour improved amaranth pasta and the resulting products demonstrated excellent cooking performance as well as sensory properties (Chillo *et al*. 2009a). The majority of screened products were made up by a mixture of the above mentioned gluten free raw materials. In this way, sensorial deficits of the single flours can be balanced out and technological shortcomings can be compensated.

Apart from gluten free grains, also non cereal sources have been considered for the production of pasta. The commercial pasta products contained chickpea and lupin flour as well as potato flour. Response surface methodology was used by Singh *et al*. (2004) for the development of a formulation based on sweet potato and soy flour. Schoenlechner *et al*. (Schoenlechner 2010) replaced 3% of amaranth, quinoa or buckwheat flour by egg white powder, soy protein isolate and casein. Regarding textural characteristics and cooking loss, egg white powder was shown to be superior, as soy protein and casein addition led to products that disintegrated faster during cooking indicating a weaker dough matrix. Also Chillo *et al*. (2009b) found a negative effect of casein on pasta quality. One of the commercial products contains soy flour (Semper), while egg products or milk proteins could not be found. Pea protein was used in two products (Gerblé, Schär).

A satisfactory pasta product is characterised by uniform colour and a smooth surface, mechanically strong strands and a low matter loss during cooking. As these properties are often not satisfactory when using gluten free cereals as opposed to wheat semolina, additives such as emulsifiers are employed. The surface acitvity of emulsifiers enables them to act as a lubricant in the extrusion process, resulting in less nozzle wear and tear and thus making production easier. They can also provide a firmer consistency, a less sticky surface and better starch retention properties during cooking (Lai 2002). Regarding the samples of this study, 39% contained mono- and diglycerides of fatty acids (E471). Several authors report that pre-gelatinisation of starch rich ingredients can improve the functional properties and give body and texture to the product (Mastromatteo *et al*. 2011). While the production of pasta based solely on oat or quinoa flour failed, Chillo *et al.* (2007) got acceptable results upon addition of pregelatinized starch as structuring agent. Parts of the flours were heated to about 80°C, cooled and added to the flour-water mixture. Among the brands reviewed, only one (Gerblé) stated precooked maize flour as an ingredient. Other products may also contain modified starches, as starches altered by physical means or enzymes can simply be labelled as "starch" rather than the specific name. However, this designation must be complemented by the indication of origin if the source may contain gluten (The Commission of the European Communities 2008). Another well researched group of ingredients, hydrocolloids, could not be found in commercial products. However, studies showed that the incorporation of polysaccharides such as xanthan, carboxy methyl cellulose, locust bean or guar gum can result in pasta with improved sensory characteristics (Huang *et al.* 2001; Raina *et al.* 2005).

4.3 Challenges during production

Pasta is a collective term used to describe products such as macaroni, spaghetti, lasagne sheets or fettuccine. Its production involves mixing, kneading, extrusion, shaping and drying. While the application of rice for the production of noodles is well studied, literature on the production of pasta from gluten free raw materials is scarce. The difference is mainly, that

the traditional process for making noodles involves several heating and cooling steps aimed at the reorganisation of the starch matrix (Mariotti *et al.* 2011), while pasta processing consists of a simple extrusion step. Conventional wheat pasta is usually produced from durum semolina, a granular product achieved through a special grain milling process. According to the publications screened, gluten free pasta is usually made from flours. This can be explained by the fact that the availability of gluten free raw materials in general is limited and the production of gluten free semolina is underdeveloped. The significantly smaller particle size can cause thermal stresses during pasta manufacture, which may cause protein denaturation (Antognelli *et al*. 1980) and also hydration during the mixing step is different. A mixing stage prior to extrusion allows starch and proteins to hydrate. Slightly elevated water temperature is used to speed up the mixing process. This is a particularly important step during the manufacture of pasta as incomplete and uneven hydration of flours impairs the quality of the resulting product (e.g. tendency to crack, uneven colour) (Antognelli *et al*. 1980). Semolina is usually mixed with water in an approximate ratio of 30:100 (water : semolina) (Wrigley *et al*. 2004). For the production of gluten free pasta, water levels are usually higher: Schoenlechner *et al*. (2010) produced pasta from amaranth, quinoa and buckwheat flour with dough moisture of 30-35%. Too high dough moisture toughens the dough, which then adheres to the screw and leads to sticky pasta with low texture firmness, whereas too low dough moisture results in pasta with surface cracks (Schoenlechner *et al*. 2010). If the formulation contains additional protein or fibre ingredients, the required water level is even higher. The dough is forced at high pressure through a dye to obtain the desired shape and size. The process conditions during extrusion, i.e. dough moisture and extrusion temperature are controlled carefully. Hence, in uncooked pasta most of the starch is ungelatinized and proteins are largely undenatured. In order to transform the relatively unstable extrudate into a highly convenient product with a long shelf life, a drying step is necessary. The moisture content of fresh pasta as it emerges from the die lies at around 30%. During drying, the moisture content has to be reduced to below

12.5% in such a way that the complex protein and starch structure remains unchanged and cracking and other physical defects are avoided (Antognelli 1980).

The production of cereal-based products from non-wheat sources presents a major technological challenge. To overcome this hurdle, researchers and product developers focus mainly on the search for appropriate ingredients and additives suitable for the production of a cohesive structure. However, more focus should be placed on the role of adequate processing conditions in order to promote starch organisation able to substitute for the gluten network in the final product. Marti *et al*. (2010; 2011) for instance compared conventional extrusion at 50°C with extrusion-cooking (115°C) and found that the latter improved cooking quality of rice-based pasta. Cooling cycles as involved in rice noodle making, can lead to starch retrogradation which in turn results in decreased stickiness and reduced cooking loss.

4.4 Sensory and textural properties of gluten free pasta

The two main features of pasta quality are texture and colour/visual appearance. Regarding the latter property it is possible to create products similar to wheat counterparts (e.g. Seitz, Roma and Tesco). These products appear smooth with a glossy surface and a clear, bright yellow colour. However, many of the screened commercial products show undesirable colouring. So are products from maize frequently too orange and rice products often too white or even translucent. Several of the products screened were greyish, had an inhomogeneous surface or showed black spots. Getting textural properties equal to wheat products is challenging. A high degree of firmness and elasticity, termed as "al dente" is considered a sign of good quality pasta (Antognelli 1980). Reaching this consistency is difficult when using gluten free raw materials. Cooked gluten free pasta is often too soft and the mouth feel is not comparable to wheat counterparts. While many of the commercial samples show firmness values equal to or higher than wheat pasta, the elastic limit is often significantly lower (Table 4.1).

Table 4.1 Mechanical properties of gluten free pasta samples compared to durum semolina pasta (in bold) as determined using a TA.XT2i texture analyser system (Stable Micro Systems, Surrey, UK) and approved method 66-50.01 (AACC 1999)

		Firmness [g]	Stickiness [g]	Elastic Limit/Tensile Strength [g]
DeCecco	**No 12 Wheat pasta**	**503 ± 16**	**22.93 ± 7.27**	**45.03 ± 1.15**
3 Pauly	Spaghetti, Glutenfreie Maiswaren	271 ± 92	29.30 ± 3.01	21.12 ± 1.60
Biofair	Organic Rice Spaghetti	372 ± 28	40.26 ± 2.95	30.15 ± 2.49
Biofair	Organic Rice Quinoa Spaghetti	551 ± 132	13.23 ± 2.83	36.91 ± 1.83
Bioryza	Spaghetti de Riz Sans Gluten	249 ± 30	54.27 ± 3.11	22.11 ± 1.29
Bioryza	Spaghetti de Riz Complet	515 ± 18	19.56 ± 0.95	28.64 ± 0.60
Carrefour	Spaghetti No Gluten	296 ± 17	12.49 ± 2.00	20.85 ± 1.19
DIET Radisson	Pasta de Maiz	682 ± 46	13.57 ± 2.28	59.64 ± 2.73
Doves Farm Organic	Organic Spaghetti (Maize and Rice)	297 ± 12	37.41 ± 3.21	23.40 ± 1.61
Doves Farm Organic	Organic Spaghetti (Brown Rice)	249 ± 47	34.27 ± 9.28	30.13 ± 2.54
Ellen's allergy friendly	Spaghetti mais	554 ± 59	12.69 ± 1.31	41.54 ± 3.63
Gallo DAL 1856	3Cereali Riso, Mais, Grano Saraceno	415 ± 54	15.36 ± 2.36	26.52 ± 2.03
Gerblé	Spaghetti	166 ± 14	17.73 ± 1.69	16.26 ± 2.16
Glutano	Spaghetti glutenfrei	476 ± 27	13.30 ± 1.78	29.46 ± 0.53
Hammermühle	Spaghetti (lupinus)	403 ± 47	13.13 ± 1.22	24.02 ± 2.07
Hammermühle	Spaghetti (chickpea)	541 ± 64	12.73 ± 1.22	32.18 ± 2.92
Kelkin	Gluten free Spaghetti	742 ± 31	16.10 ± 2.41	47.27 ± 1.19
Le Veneziane	gli Spaghetti	1019 ± 58	11.50 ± 0.71	54.87 ± 2.40
Moilas	Wholemeal Rice Spaghetti	210 ± 12	37.13 ± 3.58	19.36 ± 0.70
Organ Gluten Free	corn & rice spaghetti	746 ± 61	15.51 ± 2.17	71.26 ± 4.47
Primeal	Spaghetti mais & riz	149 ± 23	20.81 ± 1.16	10.59 ± 1.10
Probios	Rice&Rice	772 ± 81	35.25 ± 5.91	42.95 ± 6.17
Probios	Viva Mais	1264 ± 121	12.54 ± 1.93	55.60 ± 4.94
Rapunzel	Reis-Spaghetti	464 ± 33	14.29 ± 1.17	49.81 ± 1.00
Riso Scotti	Pasta Riso, Spaghetti	518 ± 53	12.38 ± 0.85	37.77 ± 1.67
Rizopia	Organic Brown Rice Pasta Spaghetti	623 ± 60	38.75 ± 9.11	45.06 ± 8.82
Roma	Gluten free Spaghetti	151 ± 14	13.79 ± 1.77	23.04 ± 1.13
SamMills	Pasta d'oro	963 ± 197	15.63 ± 2.39	35.50 ± 2.73
Schär	Spaghetti, naturally gluten free	789 ± 31	10.02 ± 0.13	70.31 ± 3.49
Seitz	Gluten frei Spaghetti (maize/chick peas)	433 ± 44	12.97 ± 0.61	23.76 ± 0.43
Semper	Spaghetti	288 ± 45	14.19 ± 0.47	27.74 ± 2.68
SPAR free from	Spaghetti	298 ± 40	21.92 ± 0.75	19.82 ± 0.38
Tesco	Free from Spaghetti	188 ± 16	19.45 ± 1.20	21.26 ± 2.13
Tinkyada	Pasta Joy Ready	200 ± 12	18.88 ± 1.17	27.44 ± 1.88

Firmness of wheat control lay at 503 g, while the gluten free samples ranged from 149 – 1264 g. Regarding elasticity, gluten free samples ranged from 11-71 g and only eight of 33 samples showed values higher or equal to wheat pasta (45 g). A common problem regarding gluten free pasta is stickiness of the cooked product. During production of wheat pasta, a gluten layer is formed, entrapping the starch granules (Resmini and Pagani 1983). However, a weak or discontinuous protein matrix as found in gluten free products permits starch to

leach out during the cooking process and the resulting pasta becomes sticky. Figure 4.1 shows commercial wheat and gluten free spaghetti. While the first shows a continuous outer layer of several micrometers, this cannot be observed in the gluten free sample resulting in increased cooking loss and stickiness. Stickiness values for the commercial pasta samples vary widely from 10 to 55 g. The value for commercial wheat pasta was at 23 g. Gluten free products tend to disintegrate during cooking, thus not allowing enough time to achieve well cooked pasta (Schoenlechner *et al*. 2010). Due to the insufficient cooking time, resulting pasta often has poor sensory characteristics (earthy, musty, malty, bitter, and/or germ-like flavour) and poor digestibility. Strong corn/popcorn flavours can be observed in products made with a high proportion of maize flour. Also intense rice or milk rice flavour was attributed to several samples.
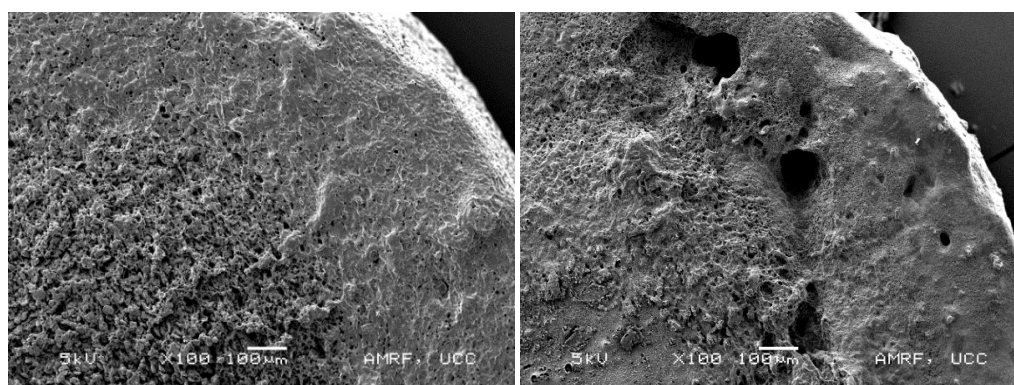
Figure 4.1 Micrographs taken with JEOL JSM-5510 Scanning Electron Microscope (working distance of 8 mm, accelerating voltage of 5 kV) at a magnification level of x100: Cooked commercial DeCecco wheat pasta (left) and gluten free pasta (right)

4.5 Nutritional value

There have been concerns that gluten free products based on white rice, maize flour and potato starch have sub-optimal levels of nutrients compared to standard wheat products. When on a gluten free diet, a higher percentage of the daily energy intake is obtained from fat when compared to individuals on a standard diet (Zannini *et al*. 2012). While energy contents (calories) of the commercial gluten free products are similar or higher to the wheat counterpart, the composition is different (Table 4.2). So contain about half the samples more

fat than the wheat counterpart incuded into the study (DeCecco pasta). Wheat pasta contains 1.5 g/ 100 g fat, while the gluten free pasta samples contain up to 3 g/ 100g. All products show lower protein contents than the wheat containing counterpart (3.0-10.7 g/ 100 g and 13.0 g/ 100 g, respectively). Hence, carbohydrate content is higher in all samples (70-86 g/ 100 g compared to 70 g/ 100g). Most of the commonly used gluten free flours naturally have lower protein contents than their wheat containing counterparts (Hager *et al*. 2012). Therefore the use of grains such as quinoa, buckwheat or teff or the addition of ingredients such as pulses or milk proteins can be recommended. An obvious ingredient to increase the protein content of pasta is egg. Eggs are traditionally used to achieve flavour effects. They can be added fresh, frozen or as dried powders (Antognelli 1980). Soybean flours are a good source of vegetable proteins (38-40 %) and hence were used in several studies to improve the nutritional value of gluten free pasta (Mastromatteo 2011).

Dietary fibre content of the majority of screened gluten free products is unsatisfactory and generally lower than in wheat pasta (2.9 g/ 100 g). However, 5 out of 36 show higher fibre contents (up to 4.6 g/ 100 g). These contain less refined flours such as brown rice flour. None of the products contain additional fibre ingredients. As the dietary fibre intake of the general population and especially among coeliac disease patients is often too low (Hager *et al*. 2011), the inclusion of non-starch polysaccharides such as inulin, β-glucan, bamboo or pea fibre can be recommended. Apart from the nutritional value, fibre incorporation also has an effect on the texture. Its incorporation into the starch matrix has been reported to reduce extreme firmness in pasta from white rice flour (Marti *et al*. 2011).

In order to make food labelling more useful to consumers, packaging in the U.S.A. gives daily values for each nutrient. These recommendations are shown in the bottom line of Table 4.2. The percentage contribution of 100 g of each sample considered in this study was calculated and compared. Regarding the contribution of gluten free pasta to the daily value for protein, it is worthy to mention, that 100 g of the samples on average deliver only about 13 % of the

required amount, percentages ranging from as low as 6 % for a maize pasta product up to 21 % for quinoa spaghetti, while 100 g of wheat pasta contribute to 26 % of the daily value. As pasta is generally low in fat, 100 g of the commercial wheat pasta contains 2.5 % of the daily value. However, 100 g of gluten free spaghetti contain up to 4.6 %. The contribution of wheat pasta to the daily value for total carbohydrates is lower than that of the gluten free samples (23 % as opposed to 23 %-29 %). It is worthy to mention, that the majority of gluten free samples contribute to a much lower extent to the daily dietary fibre value than the wheat pasta (11 %). However, products made from brown rice contain up to 18 % of the daily fibre value.

Table 4.2 Nutritional values per 100 g of gluten free pasta compared to durum semolina pasta (in bold) as stated on the packaging of the respective products.

Brand	Name	Country of purchase	Energy [kcal/100g]	Protein [g/100g]	Carbohydrates [g/100g]	of which sugars	Fat [g/100g]	of which saturates	Dietary Fibre [g/100g]	Sodium [g/100g]
DeCecco	**No 12 Spaghetti**	**Italy**	**352**	**13**	**70.2**	**3.4**	**1.5**	**0.3**	**2.9**	**0.004**
3 Pauly	Spaghetti, Glutenfreie Maiswaren	Germany	366	3.0	85	0.5	1.6	0.3	2.8	<0.02
Biofair	Organic Rice Spaghetti	Ireland	384	6.9	85		0.5			
Biofair	Organic Rice Quinoa Spaghetti	Ireland	369	10.7	75		0.7			
Bioryza	Spaghetti de Riz Complet	France	345	8.3	71.5		2.8		4.6	
Bioryza	Spaghetti de Riz Sans Gluten	France	365	7.5	76.5		3			
Carrefour	Spaghetti No Gluten	France	357	6.7	79	0.2	1.3	0.5	1.1	0.001
DIET Radisson	Spaghetti	Portugal	358	7.5	79	0.5	1.3	0.6	1.6	0
Doves Farm Organic	Organic Spaghetti (Maize and Rice)	Ireland	347	7.0	76	0.4	0.9	trace	2.4	trace
Doves Farm Organic	Organic Spaghetti (Brown Rice)	Ireland	338	7.9	70.3	0.5	1.5	trace	4.1	trace
Gallo DAL 1856	3Cereali Riso, Mais, Grano Saraceno	Italy	353	7.0	77	0.5	1.6	0.4	1.4	0.0019
Gerblé	Spaghetti	France	365	7.3	79.2	0.2	2.1	1.1	1.3	0.1
Glutano	Spaghetti glutenfrei	Finland	359	6.4	78.4		2.2			
Hammermühle	Spaghetti (chickpea)	Germany	370	3.3	86		1			
Hammermühle	Spaghetti (lupinus)	Germany	358	3.9	82		1.6			
Kelkin	Gluten free Spaghetti	Ireland	356	6.5	78.5	0.5	1.5	0.5	1.2	0
Le Veneziane	gli Spaghetti	Italy	345	7.8	77.8		0.71			
Moilas	Wholemeal Rice Spaghetti	Finland	339	7.9	70.3	0.5	2.1	0.4	4.1	
Organ Gluten Free	corn & rice spaghetti	Italy	348	5.6	79.8	0.9	0.9	0	2.7	0.01
Primeal	Spaghetti mais & riz	France	340	7.0	76		0.9			
Probios	Viva Mais	Finland	358	7.5	79	0.5	1.3	0.6	1.6	0.0012
Probios	Rice&Rice	Finland	345	7.0	79	0.9	0.3	0.1	2.5	0.0011

Rapunzel	Reis-Spaghetti	Germany	351	7.1	79		0.6			
Riso Scotti	Pasta Riso, Spaghetti	Italy	360	6.2	80.6		1.4			
Rizopia	Organic Brown Rice Pasta Spaghetti	Ireland	360	7.9	76.8	0.6	2.4	0.4	3.2	
Roma	Gluten free Spaghetti	Ireland	355	7.0	78.4	0.5	1	0.2	2	0.03
SamMills	Pasta d'oro	Germany	350	8.0	72	3.5	1.1	0	3.5	<0.001
Schär	Spaghetti, naturally gluten-free	Italy	358	9.0	73.7		2.5			
	Gluten frei Spaghetti (maize/chick									
Seitz	peas)	Germany	371	5.2	81	<0.5	2.4	0.6	2.1	0.002
Semper	Spaghetti	Sweden	350	8.5	76	0.7	1.8	1	3	0.01
SPAR free from	Spaghetti	Austria	348	5.9	76.7	0.2	1.5	0.5	2.2	0.001
Tesco	Free from spaghetti	Ireland	355	7.0	78.4	0.5	1.0	0.2	0.2	trace
Tinkyada	Pasta Joy Ready	Ireland	375	7.14	76.8	0	3.6	0.89	3.57	0.027
Ellen's allergy friendly	Spaghetti Mais	Italy	358	7.5	77	0.7	1.8	0.4	1.7	<0.02
Daily values based on a 2000 calorie diet*			2000	50	300		<65	<20	25	<2.4

*Titel 21: Food and Drugs; §101.9 Nutrition labelling of food; Electronic code of federal regulations (http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=%2Findex.tpl; accessed April 16th, 2012)

4.6 Conclusion

Literature on the optimisation of gluten free pasta is scarce. Further research should be carried out especially on finding the right processing conditions. The pasta products on the market should be improved in terms of colour, matter loss during cooking and stickiness as well as elasticity. Regarding the nutritional value, protein and fibre contents have to be increased. A better synergistic cooperation between research centres and food industry will allow a profitable knowledge-transfer process and hence allow the production of high quality gluten free pasta in terms of texture, sensory and nutritional properties.

4.7 Acknowledgements

4.8 References

Alamprese C, Casiraghi E, and Pagani M.A. (2007) Development of gluten-free fresh egg pasta analogues containing buckwheat. European Food Research and Technology, 225(2), 205-213

Antognelli C. (1980) The manufacture and applications of pasta as a food and as a food ingredient – a review. Journal of Food Technology, 15(2), 125-145

Caperuto L.C, Amaya-Farfan J. and Carmargo C.R.O. (2001) Performance of quinoa (Chenopodium quinoa Willd) flour in the manufacture of gluten-free spaghetti. Journal of the Science of Food and Agriculture 81(1), 95-101

Chillo S, Civica V, Iannetti M, Suriano N, Mastromatteo M, and Del Nobile M.A. (2009b) Properties of quinoa and oat spaghetti loaded with carboxymethylcellulose sodium salt and pregelatinized starch as structuring agents. Carbohydrate Polymers, 78(4), 932-937

Chillo S, Laverse J, Falcone P.M, and Del Nobile M.A. (2007) Effect of carboxymethylcellulose and pregelatinized corn starch on the quality of amaranthus spaghetti. Journal of Food Engineering, 83(4), 492-500

Chillo S, Laverse J, Falcone P.M, and Del Nobile M.A. (2008) Quality of spaghetti in base amaranthus wholemeal flour added with quinoa, broad bean and chick pea. Journal of Food Engineering, 84(1), 101-107

Chillo S, Suriano N, Lamacchia C, and Del Nobile M.A. (2009a) Effects of additives on the rheological and mechanical properties of non-conventional fresh handmade tagliatelle. Journal of Cereal Science, 49(2), 163-170

Da Silva E.M.M., Ascheri J.L.R., Ascheri D.P.R. and De Carvalho L.M.J. (2008) Effect of extrusion parameters in the characteristics of pasta viscosity and water absorption index of precooked

pasta prepared by mixed flour of brown rice and maize obtained by extrusion cooking. Boletim Do Centro De Pesquisa De Processamento De Alimentos, 26(2), 239-254

Hager A.S, Axel C. and Arendt E.K. (2011) Status of Carbohydrates and Dietary Fiber in Gluten-free Diets. Cereal Food World, 56(3), 109-114

Hager A.S, Wolter A, Jacob F, Zannini E. and Arendt E.K. (2012) Nutritional properties of ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours. Journal of Cereal Science, 56, 239-247

Huang J.C, Knight S. and Goad C. (2001) Model prediction for sensory attributes of nongluten pasta. Journal of Food Quality, 24(6), 495-511

Lai H.M. (2002) Effects of rice properties and emulsifiers on the quality of rice pasta. Journal of the Science of Food and Agriculture, 82(2), 203-216

Mariotti M, Iametti S, Cappa C, Rasmussen P. and Lucisano M. (2011) Characterisation of gluten-free pasta through conventional and innovative methods: Evaluation of the uncooked products. Journal of Cereal Science, 53(3), 319-327

Marti A, Pagani M.A. and Seetharaman K. (2011) Understanding starch organisation in gluten-free pasta from rice flour. Carbohydrate Polymers, 84(3), 1069-1074

Marti A, Seetharaman K, and Pagani MA. (2010) Rice-based pasta: A comparison between conventional pasta-making and extrusion-cooking. Journal of Cereal Science 52(3), 404-409

Mastromatteo M, Chillo S, Iannetti M, Civica V, and Del Nobile M.A. (2011) Formulation optimisation of gluten-free functional spaghetti based on quinoa, maize and soy flours. Int J Food Sci Tech 46(6), 1201-1208

Raina C.S, Singh S, Bawa A.S. and Saxena D.C. (2005) Formulation of pasta from rice brokens: optimization of ingredient levels using response surface methodology. European Food Research and Technology, 220(5-6), 565-574

Resmini P. and Pagani M.A. (1983) Ultrastructure studies of pasta. A review. Food Microstructure, 2, 1-12

Schoenlechner R, Drausinger J, Ottenschlaeger V, Jurackova K. and Berghofer E. (2010) Functional Properties of Gluten-Free Pasta Produced from Amaranth, Quinoa and Buckwheat. Plant Foods for Human Nutrition, 65(4), 339-349

Singh S, Raina C.S, Bawa A.S. and Saxena D.C. (2004) Sweet potato-based pasta product: optimization of ingredient levels using response surface methodology. International Journal of Food Science and Technology, 39(2), 191-200

The Commission of the European Community (2008) Council Directive 90/496/EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions.

Wrigley C, Corke H. and Walker C.E. (2004) Encyclopedia of grain science, Elsevier Academic Press

Zannini E., Pontonio E., Waters D. and Arendt E.K. (2012) Applications of microbial fermentations for production of gluten-free products and perspectives. Applied Microbiology and Biotechnology, 93(2), 473-485

Chapter 5: Influence of the soluble fibres inulin and oat-β-glucan on quality of dough and bread

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5.1 Summary

Bread represents a suitable food product for the addition of functional ingredients, such as the cholesterol lowering dietary fibre oat β-glucan and the prebiotic inulin. Therefore, these soluble fibres were incorporated into wheat as well as gluten free bread and their effects on dough rheological properties, on bread quality and on crumb microstructure were compared. The level of remaining β-glucan as well as its molecular weight was determined using an enzyme kit and size exclusion chromatography. The addition of oat β-glucan resulted in a higher water addition level, whereas incorporation of inulin had the opposite effect. Rheological testing showed that the incorporation of oat β-glucan results in a more elastic dough. The baking characteristics mainly affected by fibre addition were volume and crust colour; with inulin increasing and oat-β-glucan decreasing loaf specific volume in the gluten free breads. Inulin led to a darkening of the crust of both bread types, whereas addition of oat-β-glucan resulted in a lighter crust of gluten free bread. Oat β-glucan softened the crumb of gluten free bread, but had the opposite effect on wheat bread. Inulin resulted in an increased crumb hardness as well as rate of staling. Beta-glucan breakdown was more pronounced in wheat bread than in gluten free bread. The results show that the use of β-glucan to increase the nutritional value of wheat bread is limited due to negative influences on technological properties. However, this soluble fibre is highly suitable for incorporation into gluten free bread.

5.2 Introduction

Demand for food products with additional health benefits continues to increase. In this respect the enrichment of bread with the functional fibres β-glucan and inulin is of interest to the consumer as well as the cereal industry. Wheat bread is a major component of people's diet all over the world. However, it can not be consumed by all parts of society. Patients suffering from coeliac disease, an immune mediated enteropathy triggered by the ingestion of the cereal protein gluten, can not tolerate products made from wheat, rye and barley and therefore rely on gluten free alternatives.

Dietary fibre is an important component of human nutrition with health benefits including reduction of bowel transit time, prevention of constipation, reduction in risk of colorectal cancer, lowering of blood cholesterol, production of short chain fatty acids and promotion of the growth of beneficial gut microflora (Brennan and Cleary 2005). Among the possible sources of fibre, the natural polysaccharide β-glucan has been considered due to outstanding functional and nutritional properties. The polysaccharide consists of long, linear chains of β (1→4) and β (1→3) linked D-glucopyranosyl residues. On average, about 30 % of glucose residues are linked by β (1→3) and 70 % by β (1→4) bonds. One important characteristic is the high viscosity-forming potential of β-glucans. In addition to the function as dietary fibre, oat β-glucan has been associated with several other health benefits. For instance clinical studies have shown that oat bran can be used to lower serum cholesterol levels and to reduce the risk of heart disease (Bell *et al*. 1999). The European Food Safety Authority (EFSA) states that a cause and effect relationship has been established between the consumption of beta-glucans and the reduction of blood cholesterol concentrations. Therefore a health claim has been granted and the following wording reflects the scientific evidence: "Regular consumption of beta glucans contributes to maintenance of normal blood cholesterol concentrations". In order to bear the claim, foods should provide at least 3 g/day of beta-glucans from oats, oat bran, barley, barley bran, or from mixtures of non-processed or minimally processed beta-glucans in

one or more servings (EFSA Panel On Dietetic Products 2009). Additionally oat β-glucan has been suggested to have an important influence on human glycaemic control by lowering the postprandial blood glucose level (Jenkins *et al.* 2002; Lazaridou and Biliaderis 2007; Wood 2007). An immune stimulating effect of cereal β-glucan has been indicated (Volman *et al.* 2008).

Inulin-type fructans are natural components of several edible fruits and vegetables. The inulin commonly used in the food industry derives from chicory and consists of oligo- and polysaccharides which are composed of fructose units connected by β (2→1) links with terminal glucose moieties (Roberfroid 2007). Inulin-type fructans resist hydrolysis by human small intestinal digestive enzymes and reach the large intestine virtually intact where they are fermented by the intestinal microflora, stimulating selectively the growth and/or activity of intestinal bacteria associated with health and well-being (Gibson *et al.* 1995; Tuohy *et al.* 2007).

The many nutritional benefits of inulin and oat β-glucan have been shown in several previous publications (Gibson *et al.* 1995; Kaur *et al.* 2002; Liatis *et al.* 2009; Ripsin *et al.* 1992; Wood 2007; Zekovic *et al.* 2005). This work, however, focuses on the technological aspects of the addition of these substances to bread. Previous studies observed a decrease in loaf volume and height, and firmer crumb structure upon incorporation of β-glucan in wheat bread formulas (Brennan and Cleary 2007; Cavallero *et al.* 2002; Cleary *et al.* 2007). Lazaridou *et al.* (2007) included oat β-glucan into gluten free bread formulas and reported an increase in loaf volume and a lighter crust colour. The objective of this particular study was to determine the effect of inulin from chicory and β-glucan originating from oat on the rheological properties of a gluten free batter compared to wheat dough as well as a range of bread quality characteristics. The molecular weight distribution of the β-glucan before and after the addition to bread was determined. In addition, the influence of inulin and oat β-glucan on the ultra structural properties of bread was evaluated.

5.3 Materials and Methods

5.3.1 Materials

The ingredients used were potato starch (Emsland group, Germany), white rice flour (Doves Farm Foods Ltd, UK), dry yeast (Puratos, Belgium), margarine (Storck Unilever, Ireland), whey protein isolate (Isolac®, Carbery group, Ireland), sugar (Siucra, Ireland), salt (Glacia British Salt Limited, UK), Xanthan gum (SUMA Whole Foods, UK), HPMC (Dow Wolff Cellulosics, Germany), bakers flour from hard wheat (protein content 11.5 %; moisture 13.8 %) (Odlums, Ireland) and tap water. Beta-glucan powder extracted from oats was purchased from Bioatlantis (Ireland) and inulin Raftilose P95 from Beneo – Orafti (Belgium).

5.3.2 Rheology on gluten free batters and wheat dough

For the rheological measurements a Physica MCR 301 rheometer (Anton Paar Germany GesmbH, Germany) equipped with parallel plate geometry, consisting of a 50 mm diameter corrugated probe and plate, was used. A circulating water bath and a controlled pelletier system were utilised to set the temperature to 30 °C. Batter or dough samples, prepared as described for the bread making procedure (without the addition of yeast) were loaded onto the serrated plate. The probe was lowered to a gap width of 1 mm and the exposed edges of the samples were trimmed off. Samples rested for 5 min to allow equilibration and recovery. The edges of the samples were covered with mineral oil and the test was run in a humidified chamber to reduce water loss during testing. Storage modulus (G'), loss modulus (G''), complex modulus (G*) and phase angle (δ) were calculated using the manufacturers software (Rheo-plus, AntonPaar, Germany).

The linear viscoelastic range and the target strain were determined using amplitude sweeps applying strain (γ) increasing from 0.001 to 100 % at a constant frequency (ω) of 10 Hz. Frequency sweep tests were performed at frequencies between 0.1 and 50 Hz with a target strain of (0.01 %). All results are the average of two measurements.

5.3.3 Determination of the water addition level

The water level was determined as previously described by Nunes *et al.* (2009). Briefly, a single frequency test was performed at an angular frequency of 10 Hz and a strain of 0.01 %. Firstly, the complex modulus of the control formulations was determined. Then the complex moduli of the fiber containing samples were determined and the water level was altered until the complex modulus equalled that of the control formulation. Ten measuring points of G*, were recorded during each measurement and the calculated average value was used. Results of the single frequency test are the average of three measurements.

5.3.4 Bread production

Gluten free breads were prepared as described by Nunes *et al.* [2008]. The wheat dough was prepared, using a standard recipe (Ryan *et al.* 2008). Water and functional ingredients were incorporated at the levels shown in Table 5.1. Yeast and sugar were suspended in water (35 °C) and regenerated for a period of 10 min in a proofer (KOMA sunriser, Roermond, The Netherlands) set to 30 °C at a relative humidity (RH) of 85 %. This was added to the premixed dry ingredients. For the gluten free batter margarine was also added. Mixing was then carried out at low disk speed with a Kenwood chef classic equipped with a batter attachment (3.5 min for wheat dough and 1 min for the gluten free formulation). The bowl containing the gluten free batter was scraped down and a further mixing of 2 min at a higher disk speed was carried out. Bulk fermentation for the wheat dough was carried out for 15 min at 30 °C, 85 % RH. Then wheat doughs and gluten free batters were scaled to 90 g into 9 baking tins of 10 x 5.5 x 3.5 mm and placed in a proofer for 45 min and 25 min, respectively (30 °C, 85 % RH). The breads were baked for 25 min at 230 °C top and 240 °C bottom heat in a deck oven (MIWE condo, Arnstein, Germany), previously steamed with 0.3 L of water. Wheat and gluten free bread loaves were removed from the tins and cooled down at room temperature. The loaves were subsequently analysed or packaged in containers (polystyrol-ethylene vinyl alcohol-

polyethylene) under modified atmosphere (60 % N_2 and 40 % CO_2). Three batch replicas were prepared.

5.3.5 Bread analysis

Loaf specific volume were analysed upon cooling using rapeseed displacement method (AACC Method 10-05.01). Bake loss was determined by substracting loaf weight from dough weight. Moisture was determined using the AACC approved air-oven method (44-15A). Crumb texture and crust colour were determined after cooling at 2 and 5 days of storage. The two bread slices (20 mm thickness) taken from the centre of each loaf were used to evaluate the crumb texture. Texture profile analysis (TPA) was performed using a TA-XT2i texture analyser (Stable Micro Systems, Surrey, UK) equipped with a 25 kg load cell and a 20 mm aluminium cylindrical probe. The settings used were a test speed of 5 mm/s with a force of 0.98 N to compress the middle of the breadcrumb to 50 % of its original height. Crust colour was analysed with a Chroma Meter (Minolta CR-300, Japan) and expressed according to the CIE L*a*b* colour system. Three loaves per batch were analysed.

5.3.6 Beta-glucan degradation and molecular weight distribution

The level of β-glucan after baking was assessed with an enzyme test kit (Megazyme, Ireland) for mixed-linkage β-glucan. The molecular weight of β-glucan was estimated using a high performance size-exclusion chromatography system as described by Galle *et al* (2010). Beta-glucan was extracted from the bread sample with 1 N NaOH for 16 hrs on a shaker at room temperature, neutralized with 1 N HCl and precipitated by adding 2 volumes of 96 % ethanol and overnight storage at 4 °C (Tieking *et al*. 2003). Beta-glucan was collected by centrifugation; the pellet was redissolved in MES-TRIS blend buffer (pH 8.2) and treated with heat-stable α-amylase and protease (Megazyme, Ireland). Beta-glucan in solution were precipitated again with ethanol and freeze dried. The freeze dried samples were dissolved in Millipore H_2O [2 mg mL^{-1}] at 60 °C and analysed using a Superdex 200 Column (GE Healthcare, Canada). Water was used as a solvent at a flow rate of 0.4 mL min^{-1}. Beta glucan was detected with a RI detector.

The standards of known molecular weight used were inulin (10^4 Da) (Sigma, Canada) as well as high and low molecular weight dextran (5×10^6-4×10^7 and 10^5 Da) (Sigma, Canada).

5.3.7 Confocal Laser Scanning Microscopy

Methyl blue (Sigma, Ireland) 0.2 % in 150 mM KH_2PO_4 was used to selectively stain β-glucan. Bread samples were soaked in the dye solution over night and destained in 150 mM KH_2PO_4 for 15 min. Proteins and starch were stained for 1 min with 1 % solution of fluorescence isothiocyanate (FITC) (Sigma, Ireland) in N,N-Dimethylformamide (Sigma, Ireland) and rinsed with N,N-Dimethylformamide for approximately 30 min. An MRC-1024 confocal laser-scanning system (Biorad, Herts, UK) mounted on an upright microscope (Axioskop, Zeis, Germany) with 10x and 20x objectives was used. Fluorescence images of a number of optical sections were acquired by scanning the sample along the optical axis in 1.83 μm steps. For methyl blue and isothiocyanate, respectively, the 405 nm and 488 nm excitation line was used and signals were collected through a BA430-460 and a BA510IF emission filter.

5.3.8 Statistical analysis

Multiple samples were compared with PASW Statistics 18 (SPSS Inc., Chicago, Illinois). A One-way ANOVA and Tukey's Honesty significant differences post hoc test were used to describe means at a significance level of $p < 0.05$. For loaf specific volume, values are the average of nine measurements. All other values are the average of triplicate measurements.

5.4 Results and Discussion

5.4.1 Addition levels of dietary fibres

Oat β-glucan and inulin powders were incorporated in wheat dough and gluten free batters and their impact on a wide range of quality characteristics was evaluated. Beta-glucan has long been established as a functional ingredient, which can not only be used to reduce cholesterol levels (Davidson *et al*. 1991; Pomeroy *et al*. 2001), but also has been suggested to lower postprandial serum glucose levels (Wood 2007) and influence the human immune response (Jenkins *et al*. 2002; Volman *et al*. 2008). The physiological activity of oat β-glucans was shown

to be dosage dependent (Davidson *et al*. 1991; Ripsin *et al*. 1992). According to a recent study, the average bread consumption in Europe is 195 g /day of bread per capita (Gira Consultancy and Research 2006). In case of the oat β-glucan the addition level was chosen according to the EFSA health claim which recommends 3 g per day (EFSA Panel on Dietetic Products 2009) resulting in the addition of 5.6 % β-glucan based on the dry ingredients in the gluten free formulation and 2.6 % β-glucan based on wheat flour (Table 5.1). Inulin is a β-(1→2) linked linear fructan which has long been established as a prebiotic and is accepted in most countries as food ingredient that can be used without restrictions. In contrast to β-glucan, a "dose argument" cannot be generalized because the factors controlling the prebiotic effect are multiple and include for example the fecal flora composition of each individual (Roberfroid 2007). At high doses, increased flatulence and osmotic pressure can cause intestinal discomfort. If inulin is used to increase the dietary fibre content of food, addition levels ranging from 3-10 g per portion are recommended (Coussement 1999). Based on these considerations and the per capita consumption of bread 9 % of inulin based on flour was added to the gluten free bread formulation and 6.8 % based on flour to the wheat dough.

5.4.2 Batter Rheology

Amplitude sweep

To determine the limits of the linear viscoelastic range, an amplitude sweep was carried out and storage (G') and loss modulus (G'') were recorded. The G' value describes the deformation energy stored in the sample during the shear process. Upon removing the load, this energy is available and acts as driving force for reformation which partially or completely compensates the previous. The G'' value is a measure of the deformation energy used up during the shear process which is lost to the sample afterwards. As long as strain (γ) remains below the limiting value, the G' and G'' curves show a constantly high plateau value, indicating that the structure of the sample is stable under this low-deformation condition. At higher amplitudes, the limit of the linear viscoelastic range is exceeded and the structure of the sample is irreversibly changed

or completely destroyed (Metzger 2002). A target strain of 0.01 % was chosen for further tests. Higher breakpoints were observed for the wheat doughs than for the gluten free batters (data not shown). This is due to the unique properties of wheat gluten, forming a strong network during dough development. Other reasons for the rheological differences between gluten free and wheat doughs are changes in water level or starch properties.

Water level adjustment in batter

Water influences rheology of gluten free batters, gas holding capacity and sensory characteristics of the final bread, such as texture, visual appearance, flavour and staling behaviour. These quality parameters can be significantly changed by the addition of dietary fibres, due to the high water-binding capacity of these molecules. Therefore, the water level of the gluten free batter and the wheat dough had to be adjusted after the addition of inulin and β-glucan. For gluten free batters, the farinograph was found to be unsuitable. Therefore the level of water addition required was determined based on small deformation rheological measurements at constant strain (0.01 %) and frequency (10 Hz). The complex moduli G* of the controls were determined, and the amount of water added to the fibre containing batters and doughs was altered until the same G* was reached. The resulting water levels were utilized for the production of gluten free bread (Table 5.1). Skendi *et al.* (2009) and Izydorczyk *et al.* (2001) showed that the storage modulus G' of wheat dough increases significantly upon incorporation of cereal β-glucan. Lazaridou *et al.* (2007) showed the same effect of oat β-glucan on a rice flour based gluten free formulation. G' increases upon incorporation of oat β-glucan, therefore the batter or dough is more elastic and higher amounts of water are necessary in order to reach the same complex modulus as for the control. In agreement with previous publications, the addition of inulin led to a decrease in water level (O'Brien *et al.* 2003; Stephen *et al.* 2006; Wang *et al.* 2002). This effect is most likely related to the fact that dissolving sugar in water results in a volume increase. During fermentation, yeast invertase partially breaks down inulin into simple sugars which are soluble in water and hence the

solvent phase of the dough is increased (Delcour, personal communication). Another possible explanation for the reduction is the fact that inulin forms microcrystals when sheered in water, which interact forming particle gel leading to a smooth creamy texture (Kaur and Gupta 2002; Stephen *et al*. 2006). In contrast to other soluble fibres with high water binding capacities such as β-glucan, the macromolecules of inulin form junction zones and so enclose large amounts of water. The properties of inulin are based on water immobilisation during the formation of this particle gel rather than actual water-binding. While the addition of oat β-glucan increases the G', making the dough more elastic, the addition of inulin decreases G'. Therefore, the inulin containing dough is less elastic and less viscous and less water is needed to reach the same complex modulus.

Table 5.1 Level of dietary fibre addition calculated according to recommended levels from literature as well as water levels used as determined using a single frequency test (% based on flour weight)

Dietary fibre	Level of dietary fibre [%]	Water level [%]
Gluten free Control	0.0	90.0
Inulin	9.0	83.0
Oat beta-glucan	5.6	132.0
Wheat Control	0.0	62.0
Inulin	6.8	59.0
Oat beta-glucan	2.6	74.0

Frequency Sweep

Frequency sweeps on the batters after water level adjustment were performed in order to evaluate time dependent shear behaviour. Since during the water level adjustment, G* was set the same for all formulations, there were no significant differences regarding this value between controls and dietary fibre containing batters and doughs. Independently from the dietary fibre added, the elastic modulus G' was higher than the viscous modulus G'' resulting in tanδ<1, indicating that the batters had a solid, elastic-like behaviour (Table 5.2). This is in agreement with Lazaridou *et al.* (2007). Tanδ was significantly lower for batters containing oat-

β-glucan, indicating that the dietary fibre affects the ratio of the viscous to the elastic portion due to its water binding capacity. The addition of inulin did not significantly change the rheological properties of gluten free batters as indicated by constant tanδ (Peressini and Sensidoni 2009). Overall it can be said that complex modulus as well as damping factor are much higher in wheat dough compared to gluten free batter since the latter is more viscous.

Table 5.2 Damping factor (tanδ) as determined during frequency sweeps.

	Tanδ [1]*		
	1 Hz	7.07 Hz	50 Hz
Gluten free control	0.329 ± 0.02 a	0.284 ± 0.02 a	0.305 ± 0.01 a
Inulin	0.319 ± 0.02 a	0.292 ± 0.01 a	0.338 ± 0.02 a
Oat beta-glucan	0.260 ± 0.01 b	0.211 ± 0.01 b	0.239 ± 0.01 b
Wheat control	0.493 ± 0.05 a	0.393 ± 0.02 a	0.432 ± 0.01 a
Inulin	0.413 ± 0.01 ab	0.384 ± 0.01 a	0.435 ± 0.05 a
Oat beta-glucan	0.369 ± 0.01 b	0.326 ± 0.00 b	0.366 ± 0.04 b

*Values in one column followed by the same letter were not significantly different (p<0.05)

5.4.3. Bread analysis

The results for bake loss, loaf specific volume, moisture, crust colour and rate of staling are presented in Table 5.3. Loaf specific volume of gluten free breads was not influenced significantly by addition of dietary fibres. However, it increased by incorporation of inulin and somewhat decreased when β-glucan was added. The loaf specific volume of wheat breads was in general higher than that of gluten free breads due to the network forming properties of gluten. The addition of oat β-glucan reduced the specific volume, but was not affected by the addition of inulin. The baked loaves showed different values for crumb moisture (p<0.05), with values ranging from 44.14 % to 54.88 % for the inulin and oat β-glucan containing gluten free breads, respectively. The moisture content of the wheat breads was reduced in the inulin containing bread (43.16 %) and increased in the oat β-glucan containing bread (46.85 %) as compared to the control (44.53 %). The moisture content of the final breads showed a linear

correlation with the level of water incorporated into the batters. Regarding the bake loss, the addition of inulin did not have an influence. However, the addition of β-glucan increased the percentage bake loss of the gluten free loaf significantly. The bake loss of wheat breads did not change significantly. Crust colour of oat β-glucan containing gluten free breads were significantly lighter (higher L* values) compared to controls in accordance with previous studies (Lazaridou *et al*. 2007). The crust of wheat bread was not affected by the presence of β-glucan. Addition of inulin darkened the crust of wheat and gluten free loaves. The partial breakdown of the polysaccharide by yeast invertase (Nilsson *et al*. 1987; Verspreet *et al*. 2013) as well as the monosaccharides present in the inulin powder lead to a stronger Maillard reaction and therefore, to a darker product. This effect is desirable since the gluten free breads are considerably lighter than their wheat containing counterparts. Crumb hardness is correlated with perception of bread freshness. Crumb hardening, which is one of the most obvious manifestations of bread staling, is caused by starch retrogradation as well as differences in vapour pressure between crumb and crust resulting in moisture migration (Esteller and Lannes 2008). Figure 5.2a and 5.2b show crumb hardness of breads at day 0, 2 and 5 of storage. Incorporation of oat β-glucan softened the crumb of the gluten free formulation, as indicated by lower hardness values and a reduced rate of staling. The high water binding capacity of β-glucan and the higher initial water addition level possibly decreased water loss during storage (Sabanis *et al* 2009). On the contrary, in wheat bread oat-β-glucan addition resulted in higher crumb hardness values. Many authors have reported increased firmness as a result of β-glucan addition (Brennan and Cleary 2007; Cavallero *et al*. 2002). The authors proposed that this increase in hardness might be due to the fact that appreciable amounts of water are bound by the polysaccharide which is then no longer available for the development of the gluten network. As previously reported for wheat breads (Wang *et al*. 2002; O'Brien 2003; Peressini and Sensidoni 2009; Poinot *et al*. 2010), the

addition of inulin to wheat as well as gluten free breads resulted in higher crumb hardness as well as an increased rate of staling.

Table 5.3 Loaf characteristics of control gluten free and wheat bread as well as breads containing inulin or beta-glucan*

	Bake loss [%]	Loaf specific volume [L/g]	Moisture [%]	L*value crust colour	Staling rate	
					Day 0-2	Day 0-5
Gluten free control	17.52 ± 0.26 a	1.85 ± 0.04 a	48.71 ± 0.45 a	56.34 ± 0.35 a	16.81 ± 1.10 a	18.07 ± 1.20 a
Inulin	17.01 ± 0.20 a	1.99 ± 0.18 a	44.14 ± 0.92 b	52.37 ± 2.83 b	19.47 ± 1.83 a	34.02 ± 3.18 b
Oat beta-glucan	20.03 ± 0.42 b	1.79 ± 0.12 a	54.88 ± 0.06 c	65.07 ± 0.62 c	4.96 ± 0.77 b	15.48 ± 3.02 a
Wheat control	17.20 ± 0.47 a	2.37 ± 0.07 a	44.53 ± 1.08 a	56.91 ± 1.46 a	13.41 ± 0.80 a	17.02 ± 1.80 a
Inulin	17.18 ± 0.33 a	2.38 ± 0.19 a	43.16 ± 0.07 b	47.62 ± 3.90 b	14.47 ± 2.64 a	22.91 ± 2.60 a
Oat beta-glucan	16.67 ± 0.4 a	2.16 ± 0.13 a	46.85 ± 0.67 c	56.72 ± 0.35 a	13.45 ± 2.11 a	15.97 ± 5.57 a

*Values in one column followed by the same letter were not significantly different ($p < 0.05$)
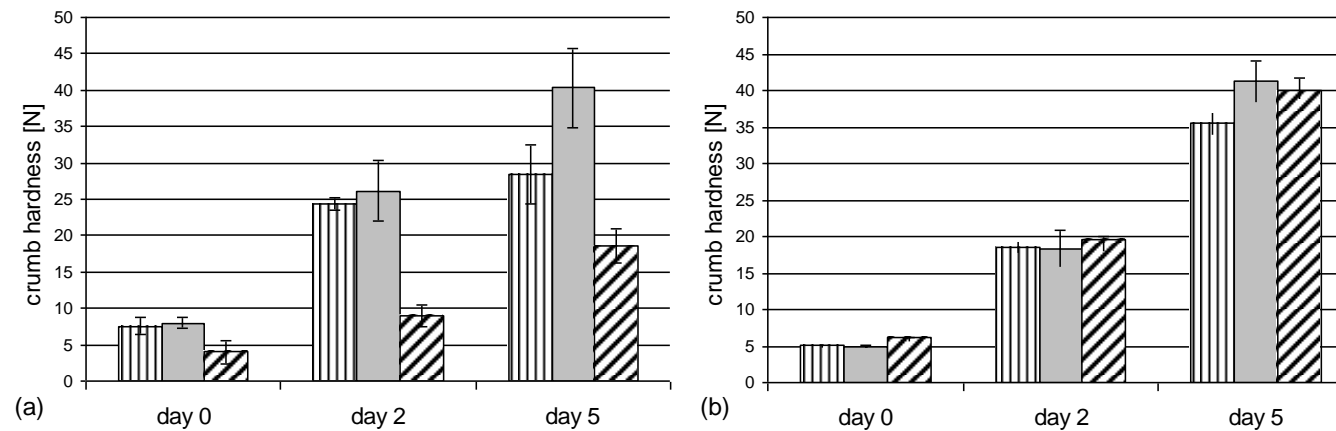
Figure 5.2a Crumb hardness of gluten free breads enriched with inulin or β-glucan over 5-days storage period: oat β-glucan (diagonal line bars); inulin (solid grey bars); control (vertical lines bar). Figure 5.2b Crumb hardness of wheat breads enriched with inulin or β-glucan over 5-days storage period: oat β-glucan (diagonal line bars); inulin (solid grey bars); control (vertical lines bar)

5.4.4. Beta-glucan degradation and molecular weight distribution

The physiological action of β-glucan depends on its molecular weight, solubility and concentration (Andersson *et al*. 2004). Shearing damage as a result of mechanical processing, enzymatic degradation through endogenous enzymes in flour or changes by excessive heat-treatments may occur during the bread making process, which can reduce the molecular weight of β-glucan and render it in such a way that its functional and physiological properties are impaired (Lazaridou and Biliaderis 2007). The amount of oat β-glucan in gluten free bread was 2.7 or 1.5 g 100 g^{-1} dry weight or fresh bread, respectively. This means that a portion (for bread defined as 50 g) contains 0.75 g β–glucan. The lowest suggested daily intake of β–glucan for achieving health effects is 3 g per day, i.e. 4 portions with 0.75 g of β–glucan. Beta-glucan content in wheat bread was 1.4 g or 0.63 g 100 g^{-1} dry weight or fresh bread, respectively. Therefore a portion of 50 g contains only 0.31 g. This reduction has to be kept in mind in respect to the 0.75 g per portion required to meet the health claim.

The results of the size-exclusion chromatography of the oat-β-glucan containing bread extracts show two peaks, indicating partial β-glucan breakdown (Figure 5.3a and b). Despite the partial breakdown, high molecular weight β-glucan was still detectable. Information about the molecular weight of the remaining β-glucan is important since it strongly influences viscosity (Colleoni-Sirghie 2003), which is in turn believed to be important for its health benefits particularly relating to its function in the gut (Wood 2007; Wood 2004). Beta-glucan was degraded to a wider extent in wheat compared to gluten free bread due to the presence of β-glucanase in wheat flour (Andersson *et al*. 2004; Trogh *et al*. 2004). Another possible explanation is the longer mixing and fermentation period of the wheat dough compared to the gluten free batter.
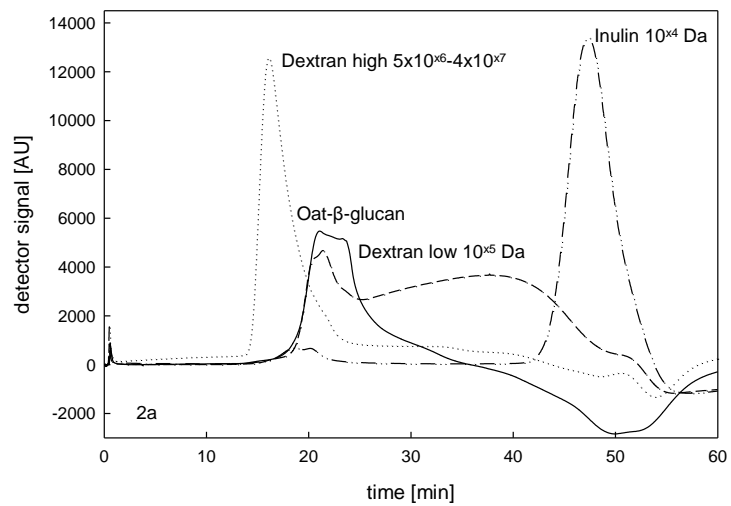
Figure 5.3a Raw material and standards: oat β-glucan (solid line), inulin (dash-dot line), dextran high (dotted line) and dextran low (dashed line)
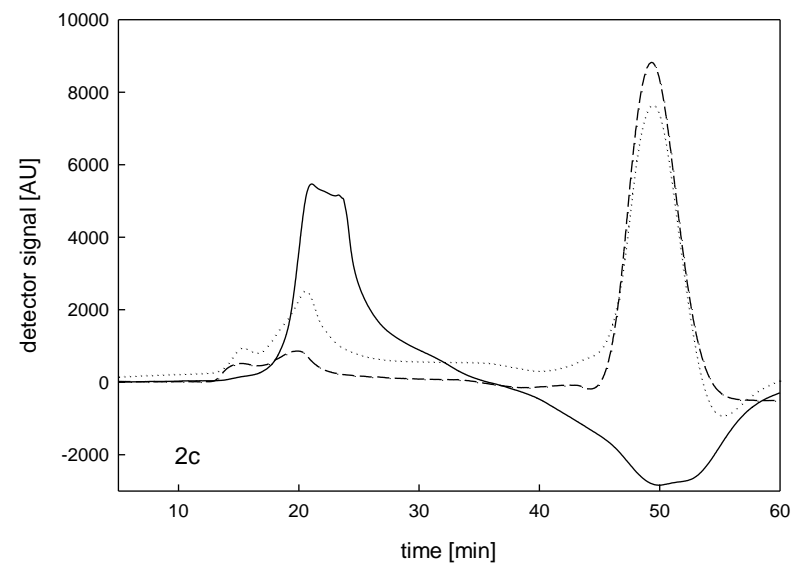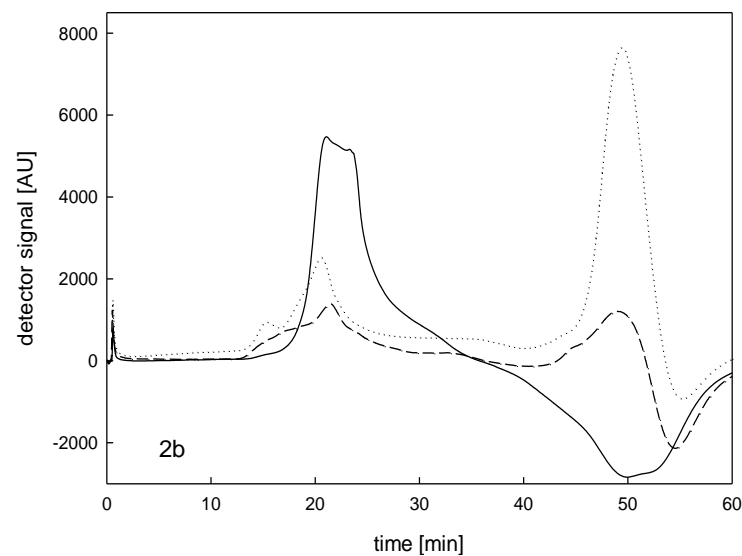
Figure 5.3b Raw material and extracts from gluten free bread: oat β-glucan (solid line), extract from oat β-glucan containing bread (dotted line) and extract from control bread (dashed line). Figure 5.3c Raw material and extracts from wheat bread: oat β-glucan (solid line), extract from oat β-glucan containing bread (dotted line) and extract from control bread (dashed line)

5.4.5. Bread Microstructure

Dough ingredients in combination with processing conditions determine the microstructure of the final product. Fluorescence microscopy was used to determine the effects of β-glucan and inulin addition to gluten free and wheat breads (Figures 5.4a-d). Methyl blue was used for the visualisation of β-glucan, and Fluorescein isothiocyanate for the selective staining of proteins (Autio 1997). In wheat bread the formation of a gluten network was observed (Figures 5a-b). In gluten free bread, proteins appear cloud like (Figures 5c-d). The lack of this gluten network explains the more viscous rheological behaviour of the gluten free batters. The weak signal for aniline blue in the control wheat breads is derived from β-glucan present in wheat grains (Stone and Clarke 1992). In enriched breads, aniline blue signal verified that β-glucan was still present in the bread and that it was distributed randomly.
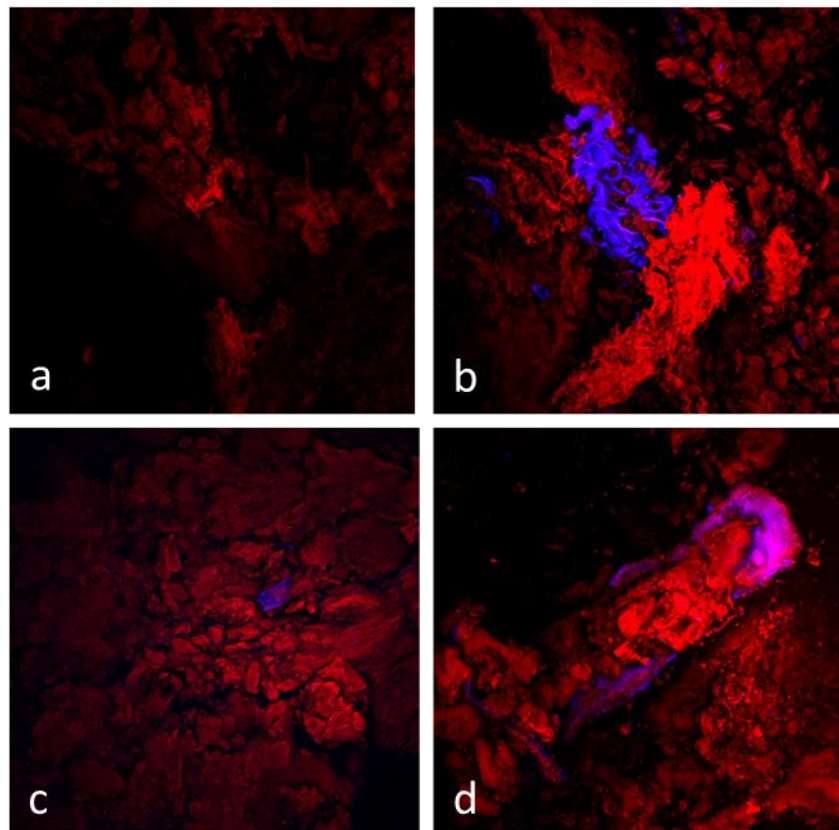
Figure 5.4: Microstructure of gluten free and wheat breads as observed with Confocal Laser Scanning Microscope. 5.4a and b control wheat bread and wheat bread containing oat β-glucan at 20x magnification; 5.4c and d gluten free control bread and gluten free bread containing oat β-glucan at 20x magnification

5.5 Conclusion

The incorporation of inulin and oat β-glucan has a positive effect on the nutritional value of gluten free as well as wheat bread. However, this study showed that several technological and textural properties are altered upon inclusion of these dietary fibres. In order to keep negative effects as limited as possible, it is crucial to adjust the water level of the dough upon addition of polysaccharides. Regarding the frequency sweeps performed it becomes clear that oat β-glucan affects the ratio of the viscous to the elastic portion (G*) due to its water binding capacity, whereas addition of inulin does not induce significant changes in the structure of the dough. Both substances influence bread quality characteristics. Addition of inulin leads to darkening of the crust in both wheat and gluten free bread. Gluten free bread shows a lighter crust colour upon addition of oat β-glucan. The addition of inulin to bread has unfavourable effects on crumb hardness and the rate of staling in both bread types. Oat-β-glucan has a remarkable effect softening the crumb and reducing the rate of staling of gluten free bread. Its incorporation into wheat bread increases crumb hardness values. Inulin, although interesting from a nutritional point of view, finds, due to its technological effects, little application in baking. It has to be kept in mind that β-glucan is partly degraded during bread production. This reduction in molecular weight is more pronounced in wheat than in gluten free bread. Considering both, extent of breakdown as well as technological effects of oat β-glucan, it can be concluded that this functional fibre is highly suitable to increase the nutritional value of gluten-free bread. Its potential as a fibre supplement in wheat bread however is limited.

5.6 Acknowledgements

5.7 References

Andersson A, Armo E, Grangeon E, Frederiksson H, Andersson R, Aman P. (2004) Molecular weight and structure units of (1-3, 1-4)-β-glucans in dough and bread made from hull-less barley milling fractions. Journal of Cereal Science, 40, 195-204

Autio K. (1997) Relationships between flour/dough microstructure and dough handling and baking properties. Trends in Food Science and Technology, 8, 181-185

Bell S, Goldman V, Bistrian B, Arnold A, Ostroff G, Forse R (1999) Effect of β-Glucan from Oats and Yeast on Serum Lipids. Critical Reviews in Food Science and Nutrition, 39, 189-202.

Brennan C, Cleary L (2005) The potential use of cereal (1→3,1→4)-β-glucans as functional food ingredients. Journal of Cereal Science, 42, 1-13.

Brennan C, Cleary L (2007) Utilisation Glucagel® in the β-glucan enrichment of breads: A physicochemical and nutritional evaluation. Food Research International, 40(2), 291-296

Cavallero A, Empilli S, Brighenti F, Stanca A (2002) High (1→3,1→4)-Glucan Barley Fractions in Bread Making and their Effects on Human Glycemic Response. Journal of Cereal Science, 36, 59-66

Cleary L, Andersson R, Brennan C (2007) The behaviour and susceptibility to degradation of high and low molecular weight barley β-glucan in wheat bread during baking and *in vitro* digestion. Food Chemistry, 102, 889-897

Colleoni-Sirghie M (2003) Rheological and molecular properties of water soluble (1,3) (1,4)-β-glucans from high-β-glucan and traditional oat lines. Carbohydrate Polymers, 52, 439-447

Coussement P.A (1999) Inulin and Oligofructose: Safe Intakes and Legal Status. Journal of Nutrition, 129(7), 1412S-1417S

Davidson M, Dugan L, Burns J, Bova J, Story K, Drennan K (1991) The Hypocholesterolemic Effects of β–glucan in Oatmeal and Oat Bran A Dose controlled Study. Journal of the American Medical Association, 265, 1833-1839

EFSA Panel On Dietetic Products (2009) Scientific Opinion on the substantiation of health claims related to beta-glucans and maintenance of normal blood cholesterol concentrations (ID 754, 755, 757, 801, 1465, 2934) and maintenance or achievement of a normal body weight (ID 820, 823). EFSA Journal, 7, 1254

Esteller M.S, Lannes S.C (2008) Production and Characterization of Sponge-Dough Bread Using Scalded Rye. Journal of Texture Studies, 39, 56-67

Galle S, Schwab C, Arendt E.K, Gänzle M (2010) Exopolysaccharide-forming Weissella strains as starter cultures for sorghum and wheat sourdoughs. Journal of Agriculture and Food Chemistry 58, 5834-5841

Gibson G.R, Beatty E.R, Wang X.I, Cummings J.H (1995) Selective Stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology, 108(4), 975-982

Gill S, Vasanthan T, Ooraikul B, Rossnagel B (2002) Wheat Bread Quality as Influenced by the Substitution of Waxy and Regular Barley Flours in Their Native and Extruded Forms. Journal of Cereal Science, 36, 219-237

GIRA Consultancy and Research (2006) Frozen BVP Markets – Western EuropeBread, Viennoiserie, Patisserie - Mini Market Report

Izydorczyk M, Hussain A, MacGregor A (2001) Effect of Barley and Barley Components on Rheological Properties of Wheat Dough. Journal of Cereal Science, 34, 251-260

Jenkins A.L, Jenkins D.J, Zdravkovic U, Würsch P, Vuksan V (2002) Depression of the glycemic index by high levels of beta-glucan fiber in two functional foods tested in type 2 diabetes. European Journal of Clinical Nutrition, 56, 622-8

Kaur N, Gupta A.K (2002) Applications of inulin and oligofructose in health and nutrition. Journal of Bioscience, 27, 703-714

Lazaridou A, Biliaderis C (2007) Molecular aspects of cereal β-glucan functionality: Physical properties, technological applications and physiological effects. Journal of Cereal Science, 46, 101-118

Lazaridou A, Duta D, Papageorgiou M, Belc N, Biliaderis C (2007) Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. Journal of Food Engineering, 79, 1033-1047

Liatis S, Tsapogas P, Chala E, Dimosthenopoulos C, Kyriakopoulos K, Kapantais E, Katsilambros N. (2009) The consumption of bread enriched with betaglucan reduces LDL-cholesterol and improves insulin resistance in patients with type 2 diabetes. Diabetes & Metabolism, 35, 115-120

Mezger T.G. (2002) The rheology Handbook: For users of rotational and oscillation rheometers. Vincentz Verlag, Hannover

Nilsson U., Öste R. and Jägerstad M. (1987) Cereal fructans: Hydrolysis by yeast invertase, in virto and during fermentation. Journal of Cereal Science, 77, 3325-3328

Nunes M.H, Moore M.M, Ryan L.A.M, Arendt E.K (2008) Impact of emulsifiers on the quality and rheological properties of gluten-free breads and batters. European Food Research and Technology, 228, 633-642

Nunes M.H, Ryan L.A.M, Arendt E.K (2009) Effect of low lactose dairy powder addition on the properties of gluten-free batters and bread quality. European Food Research and Technology, 229, 31-41

O'Brien C, Mueller A., Scannell A., Arendt E.K (2003) Evaluation of the effects of fat replacers on the quality of wheat bread. Journal of Food Engineering, 56, 265-267

Peressini D, Sensidoni A (2009) Effect of soluble dietary fibre addition on rheological and breadmaking properties of wheat doughs. Journal of Cereal Science, 49, 190-201

Poinot P, Arvisenet G, Grua-Priol J, Fillonneau C, Le-Bail A, Prost C. (2010) Influence of inulin on bread: Kinetics and physico-chemical indicators of the formation of volatile compounds during baking. Food Chemistry, 119, 1474-1484

Pomeroy S, Tupper R, Cehun-Aders M, Nestel P (2001) Oat B glucan lowers total and LDL-cholesterol. Australian Journal of Nutrition and Dietetics, 58, 51-55

Ripsin C, Keenan J, Jacobs D, Elmer P, Welch R, Van Horn L, Liu K, Turnbull W.H, Thye FW, Kestin M, Hegsted M, Davidson D.M, Davidson M.H, Dugan L.D, Denmark-Wahnefried W, Beling S. (1992) Oat Products and Lipid Lowering - A Metaanalysis. Journal of the American Medical Association, 267, 3317-3325

Roberfroid M.B (2007) Inulin-type fructans: functional food ingredients. Journal of Nutrition, 137, 2493-2502

Ryan L.A.M, Dal Bello F, Arendt E.K (2008) The use of sourdough fermented by antifungal LAB to reduce the amount of calcium propionate in bread. International Journal of Food Microbiology, 125, 274-278

Sabanis D, Lebesi D. and Tzia C. (2009) Effect of dietary fibre enrichment on selected properties of gluten-free bread. LWT - Food Science and Technology, 42, 1380-1389

Skendi A, Papageorgiou M. and Biliaderis C. (2009) Effect of barley beta-glucan molecular size and level on wheat dough rheological properties. Journal of Food Engineering, 91, 594-601

Stephen A.M, Phillips G.O, Williams P.A (2006) Food Polysaccharides and their application. Taylor & Francis, New York

Stone B.A, Clarke A.E (1992) Chemistry and Biology of (1->3)-B-Glucans. La Trobe University Press, Victoria, Australia

Symons L. and Brennan C. (2004) The Influence of (1→3) (1→4)-B-D-Glucan-rich Fractions from Barley on the Physicochemical Properties and *In Vitro* Reducing Sugar Release of White Wheat Breads. Food Chemistry and Toxicology, 69, 463-467

Tieking M, Korakli M, Ehrmann M.A, Ga M.G, Vogel R.F. (2003) In Situ Production of Exopolysaccharides during Sourdough Fermentation by Cereal and Intestinal Isolates of Lactic Acid Bacteria. Applied Environmental Microbiology, 69, 945-952

Tiwari U, Cummins E (2009) Factors influencing β-glucan levels and molecular weight in cereal based products. Cereal Chemistry, 86, 290-301

Trogh I, Courtin C.M, Andersson A.A, Aman P, Sorensen J.F, Delcour J.A (2004) The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley flour breads with increased arabinoxylan and (1➔3,1➔4)-β-D-glucan levels. Journal of Cereal Science, 40, 257-267

Tuohy K.M, Kolida S, Lustenberger A.M, Gibson G.R. (2007) The prebiotic effects of biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides – a human volunteer study. British Journal of Nutrition, 86, 341-348

Volman J, Ramakers J. and Plat J. (2008) Dietary modulation of immune function by beta-glucans. Physiology and Behaviour, 94, 276-284

Wang J, Rosell C, Beneditodebarber C. (2002) Effect of the addition of different fibres on wheat dough performance and bread quality. Food Chemistry, 79, 221-226

Wood P. (2004) Relationships between solution properties of cereal β-glucans and physiological effects — a review. Trends in Food Science and Technology, 15, 313-320

Wood P. (2007) Cereal β-glucans in diet and health. Journal of Cereal Science, 46, 230-238

Zekovic D, Kwiatkowski S, Vrvic M, Jakovljevic D, Moran C. (2005) Natural and modified (1-->3)-beta-D-glucans in health promotion and disease alleviation. Critical Reviews in Biotechnology, 25, 205-30

Chapter 6: Influence of hydroxypropylmethylcellulose (HPMC), xanthan gum and their combination on loaf specific volume, crumb hardness and crumb grain characteristics of gluten free breads based on rice, maize, teff and buckwheat

Anna-Sophie Hager, Elke K. Arendt (2013)

6.1 Abstract

Currently, the only effective treatment for coeliac disease is the complete avoidance of gluten, a protein found in wheat, rye and barley. The production of high-quality leavened baked goods made from ingredients other than wheat flour represents a major technological challenge, due to the absence of the visco-elastic gluten compound. To tackle these problems, hydrocolloids such as xanthan gum and hydroxypropylmethylcellulose (HPMC) are often incorporated into gluten free formulations. This study used systematic baking trials based on a central composite design to investigate the influence of these two gums and their combination on gluten free model systems. It was found that the effect of the hydrocolloids on the gluten free model systems of this study varied according to the raw materials used. HPMC had a positive linear effect on volume of teff and maize breads and a negative linear effect on this parameter in rice breads, while the volume of buckwheat bread did not change. Xanthan addition had a negative linear effect on loaf volume of all breads. HPMC addition reduced crumb hardness of teff, buckwheat, maize and rice bread. Xanthan increased the crumb hardness of teff and buckwheat breads, while rice bread crumb remained uninfluenced. Crumb hardness values of maize breads were reduced by xanthan addition. Also crumb grain charactersitics such as area of cells and wall thickness was influenced by the hydrocolloids. Optimisation trials were carried out in order to determine optimal water and hydrocolloid addition levels.

6.2 Introduction

Coeliac patients suffer from an immune mediated disease, triggered by the ingestion of gluten, a protein found in wheat, rye and barley. The only effective treatment is the complete avoidance of this protein, i.e. the adherence to a gluten free diet. Increasing numbers of diagnosed cases and growing awareness makes the availability of gluten free foods an important socioeconomic issue. The production of high-quality leavened baked goods made from ingredients other than wheat flour represents a major technological challenge. The absence of the visco-elastic gluten compound results in reduced gas retention and structure formation. Hence, breads based solely on gluten free flours are usually characterised by significantly lower volumes and a firmer crumb when compared to wheat counterparts (Hager *et al*. 2012). To tackle these problems, hydrocolloids such as xanthan gum and hydroxypropylmethylcellulose (HPMC) are often incorporated into gluten free formulations.

Xanthan gum is an extracellular heteropolysaccharide of high molecular weight secreted by the microorganism *Xanthomonas campestris*. It consists of repeating units of D-glucose, linked to form the β-1,4-D-glucan cellulosic backbone. Side chains (β-D-mannose-(1,4)-β-D glucuronic acid-(1,2)-α-D-mannose) are attached to alternate glucose residues. The terminal mannose moiety may carry pyruvate residues linked to the C4 and C6 position. The internal mannose is acetylated at C6. Acetyl- and pyruvate substituents are linked in variable amounts to the side chains, depending on fermentation conditions and strain used (Born *et al*. 2002). Regarding its secondary structure, the molecule may be in ordered or disordered conformation, depending on the matrix. Xanthan is present in its native ordered form below the melting point, a temperature which depends on the ionic strength of the surrounding media. It is a rigid double helix structure stabilised by non-covalent bonds (i.e. hydrogen bonding, electrostatic interaction and steric effects). The temperature induced transformation from its ordered to disordered conformation is attributed to a complete or partial separation of the double strands (Born *et al*. 2002). Xanthan is soluble in cold water and solutions exhibit highly

pseudoplastic flow. In solution the molecules are able to form intermolecular bonds that result in the formation of a weakly bound complex network.

HPMC is a cellulose ether obtained by chemically linking hypdroxypropyl and methyl groups to the β-1,4-D-glucan cellulosic backbone. This chemical modification leads to a water-soluble polymer with high surface activity and unique properties regarding its hydration characteristic in solution as well as during temperature changes (Sarkar and Walker 1995). In the solution state at lower temperatures HPMC has great water-holding capacity: molecules are hydrated and there is little polymer-polymer interaction other than simple entanglement. Upon heating it forms a gel while simultaneously releasing water. During gelation, HPMC is believed to form stronger hydrophobic bonds with other HPMC chains, resulting in stronger gel networks at higher temperatures. The varying ratios of hydroxypropyl- and methyl- substitution influences the solubility and the thermal gelation temperature.

When comparing the available publications on the effect of HPMC and xanthan on bread quality it becomes apparent that the observed effects are diverging. Therefore, this study used systematic baking trials based on a central composite design to investigate the influence of these two gums and their combination on gluten free model systems. Additionally, the water level was also varied, as it is well known that hydrocolloids can bind large amounts of water. As opposed to the complex recipes utilised in previous publications the basic formulations of this study contained only flour, water, salt, sugar and yeast. Rice, maize, teff and buckwheat flours were chosen because these raw materials represent commonly used gluten free ingredients. Response surface methodology was used as it does not only allow the evaluation of the relative effect of predictor variables (e.g. hydrocolloid and water level) on response variables (e.g. loaf volume, crumb hardness, area of cells and wall thickness) but also allows the determination of optimum ingredient levels.

6.3 Materials and Methods

6.3.1 Materials

The suppliers for the ingredients used were Doves Farm Foods Ltd, UK, for white rice flour

(12.8 % moisture) and buckwheat flour (12.6% moisture); Trouw, The Netherlands, for teff

flour (9.5% moisture); Smiths Flour Mills, UK, for maize flour (14.0% moisture). Dry yeast was

obtained from Puratos, Belgium; sugar from Siucra, Ireland, and salt from Glacia British Salt

Limited, UK. Xanthan (Keltrol F) was sourced from CP Kelko (Atlanta, Georgia, U.S.A.);

according to product specifications, the viscosity of a 1% xanthan solution at 20°C is 1470 mPas

(measured with a Brookfield LVF viscometer). HPMC (Metolose NE-4000) with 27-30%

methoxyl substitution and 4-7.5% hydroxypropoxyl substitution was sourced from Harke,

Germany. Viscosity of a 2% aqueous solution at 20°C was specified as lying between 3000-5600

mPas.

6.3.2 Characterisation of flour raw materials

Crude fat was determined following AACC method 30-10, protein and moisture contents of

flours were determined according to the AACC methods 46-12 and 44-15A, respectively (AACC

International 1995). Protein content was calculated with a protein factor of 6.25. Ash content

was determined according to Matissek (2006). Dietary fibre, phytate, total and damaged starch

levels as well as amylose/amylopectin ratio were determined using enzyme kits (K-TDFR, K-

PHYT, K-TSTA, K-SDAM, K-AMYL) supplied by Megazyme, Ireland. Polyphenol content was

determined according to Alvarez-Jubete *et al.* (2010). The gelatinisation temperatures were

determined by differential scanning calorimetry (DSC, Mettler Toledo DSC821^{e}). Flour samples

of 3-5 mg were weighed directly into aluminium pans and 10 mg of water were added. An

empty container was used as reference. Samples were heated from 25°C to 105°C at a rate of

5°C/min and onset temperatures (T_O), peak temperature (T_P) as well as end temperature (T_E)

were recorded. Results are the average of triplicate measurements.

6.3.3 Experimental design

Response surface methodology was used to evaluate the effect of the independent variables (level of water, HPMC and/or xanthan) on the dependent variables (loaf volume, crumb hardness, area of cells and wall thickness). Hereupon, optimum ingredient levels could be determined. A circumscribed, two-dimensional central composite design was developed featuring variations in the addition levels of water (ranging from 85-95% based on buckwheat flour, 95-105% based on teff flour, 120-130% based on rice flour and 90-100% based on maize flour). The upper and lower limits of these levels were selected based on preliminary trial-and-error baking tests. Crumb structure of resulting breads was evaluated and also loaf specific volumes were considered (Hager *et al*. 2012). Addition levels of HPMC and/or Xanthan ranged from 0-2% based of flour, according to values found in literature. A total of 13 trials was carried out, comprising four for the factorial, six for the axial and three as central points. The response of each of the investigated parameters was analysed by fitting quadratic models to the data with least square regression in order to identify significant ($p < 0.05$) effects of the variations in ingredient levels on the responses. Three dimensional graphs for the models were used to visualise overall trends. Significance of the lack-of-fit error term, R^2 value, coefficient of variation, and model significance were used to judge adequacy of model fit (F-test has to show significant effects ($P < 0.05$) and lack-of-fit insignificant ($P > 0.05$)). The multiple regression coefficient R^2 represents the power of fit and is a measure of how well the regression model fits the raw data. It ranges from 0-1, where 1 is the perfect model. Where contradiction between these three requirements existed, the best overall solution was chosen. For optimisation of hydrocolloid and water level, a multiple response method called desirability was applied. The following responses were used: loaf specific volume (maximise), crumb hardness (minimise), area of cells (maximise) and wall thickness (minimise).

6.3.4 Baking tests

Gluten free breads were prepared using 2 % salt, 2 % sugar and 3 % yeast, based on flour weight. Yeast and sugar were suspended in the water (35 °C) and regenerated for a period of 10 min in a proofer (KOMA sunriser, Roermond, The Netherlands) set to 30 °C at a relative humidity (RH) of 85 %. This suspension was added to the premixed dry ingredients. Mixing was then carried out with a batter attachment for 1 min at low disk speed with a Kenwood chef classic. The bowl was scraped down and a further mixing at a higher disk speed was carried out (1.5 min for the gluten free formulation). Batters were scaled to 400 g into 10 baking tins of 15 x 9.5 x 7 cm and placed in a proofer for 30 min (30 °C, 85 % RH). The breads were baked for 45 min at 190 °C top and bottom heat in a deck oven (MIWE condo, Arnstein, Germany), previously steamed with 0.3 L of water. Bread loaves were removed from the tins, cooled to room temperature and subsequently analysed. Loaf specific volume was measured upon cooling using a Volscan Profiler (Stable Micro Systems, UK). Texture profile analysis (TPA) was performed using a TA-XT2i texture analyser (Stable Micro Systems, Surrey, UK) equipped with a 25 kg load cell and a 35 mm aluminium cylindrical probe. Three bread slices (25 mm thickness) taken from the centre of each loaf were used to evaluate the physical crumb texture. The settings used were a test speed of 5 mm/s with a trigger force of 0.98 N to compress the middle of the breadcrumb to 50 % of its original height. Wait time between first and second compression cycle was 5 sec. Crumb grain was described by the following parameters: area of cells (the total area of cells as a percentage of the total slice area) and wall thickness (the average thickness of cell walls) using a C-cell bread imaging system (Calibre Control International Ltd., UK).

6.3.5 Statistical analysis

Design Expert Version 7 (Stat-Ease, U.S.A.) was used for experimental design and to generate surface response plots that permitted evaluation of effects of independent variables on the selected dependent variables and to optimise ingredient levels. With the results of

compositional analysis, analysis of Variance (One-way ANOVA) followed by Fisher LSD post hoc test, was performed to determine significant differences between samples made from different flours. Statistica 7.1 (StatSoft, U.S.A.) was used for this purpose.

6.4 Results

6.4.1 Characterisation of flour raw materials

Raw material characterization was carried out and results are shown in Table 6.1. Ingredients differ significantly in protein, fat, and mineral content, with rice and maize being significantly lower (p<0.05) than buckwheat and teff. Hence, the former two show higher total starch levels. Fiber content is highest in teff flour, followed by maize and buckwheat flour. Teff and buckwheat flours contain significant amounts of phytate and polyphenols. Also the temperature range of starch gelatinization is shown in Table 6.1. Onset temperature is lowest for buckwheat flour, while gelatinization commences at higher temperatures for maize, teff and rice flour. Endpoint temperatures are similar for rice, buckwheat and maize flour, but slightly higher for teff.

Table 6.1 Compositional data of flour raw materials[$]

	Rice	Buckwheat	Maize	Teff
Moisture [g/100g]	12.8 ± 0.2^{b}	12.6 ± 0.1^{b}	14.0 ± 0.1^{a}	9.5 ± 0.1^{c}
Protein [g/100g]	7.3 ± 0.0^{b}	12.2 ± 0.4^{a}	5.5 ± 0.2^{c}	12.8 ± 0.5^{a}
Fat [g/100g]	0.9 ± 0.1^{c}	4.2 ± 0.7^{a}	2.5 ± 0.5^{b}	4.4 ± 0.3^{a}
Ash [g/100g]	0.5 ± 0.0^{c}	1.7 ± 0.0^{b}	0.4 ± 0.0^{d}	2.2 ± 0.1^{a}
Total starch [g/100g]	77.5 ± 0.4^{a}	61.4 ± 2.2^{b}	71.5 ± 0.4^{a}	57.8 ± 5.9^{b}
Amylose [% of total starch]	21.4 ± 0.9^{ab}	16.0 ± 0.6^{c}	22.9 ± 0.8^{a}	19.7 ± 1.0^{b}
Damaged Starch [g/100g]	15.2 ± 1.5^{a}	2.6 ± 0.3^{c}	4.5 ± 0.3^{b}	2.1 ± 0.2^{c}
Total dietary fibre [g/100g]	0.4 ± 0.2^{c}	2.2 ± 0.1^{b}	2.6 ± 0.5^{b}	4.5 ± 0.6^{a}
Soluble dietary fibre [g/100g]	0.1 ± 0.1^{c}	0.5 ± 0.2^{bc}	0.6 ± 0.1^{ab}	0.9 ± 0.2^{a}
Phytate [g/100g]	0.2 ± 0.0^{c}	0.6 ± 0.1^{b}	0.1 ± 0.0^{c}	1.5 ± 0.2^{a}
Polyphenols [mg/100g]	14.2 ± 2.5^{d}	465.5 ± 22.4^{a}	97.9 ± 0.6^{c}	175.7 ± 1.5^{b}
T at onset of gelatinisation [°C]	61^{c}	58^{c}	63^{b}	66^{a}
T at peak of gelatinisation [°C]	67^{c}	65^{c}	69^{b}	71^{a}
T at end of gelatinisation [°C]	72^{bc}	72^{c}	74^{ab}	77^{a}

[$]Values in one row followed by the same letter are not significantly different (p<0.05)

6.4.2 Influence of hydrocolloids on loaf specific volume

Loaf specific volume is one of the most important visual characteristic of breads, strongly influencing consumer's choice. Hence, it is a key parameter looked at when evaluating bread quality.

Analysis of variance (ANOVA) confirmed that a significant effect was found of HPMC and water level on specific volume of teff, buckwheat, maize and rice bread (P <0.05). Increase of water addition level had a positive linear effect in all formulation except maize. Even though also the volume of maize breads could be improved by increased water addition, the observed effect was not linear over the entire range but volumes decreased again upon a water addition of 95 % (based on flour). The influence of HPMC was depending on the flour matrix. HPMC had a positive linear effect on volume of teff breads and a negative linear effect on this parameter in rice breads, while the volume of buckwheat bread did not change. For maize breads, volume was increased by addition of HPMC.

ANOVA demonstrated that the models for effects of xanthan and water level on specific volume were adequate for teff, buckwheat and maize formulations (P<0.05). However, regarding rice breads, the model was less suitable as the lack of fit was significant. Nevertheless, a surface plot was generated, because it offered a reasonable initial solution for describing the quality response of this parameter. Xanthan addition had a negative linear effect on loaf volume of all breads. Again, increased water levels resulted in higher specific volumes.

ANOVA showed that the predictive models developed for the influence of the combination of the two hydrocolloids on teff, maize and rice bread were considered adequate because they possessed a non-significant lack of fit and had satisfactory levels of R^2, CV and model significance. The developed model for the influence of the combination of the two hydrocolloids on buckwheat bread was less predictive because lack of fit was significant at the 5% level. Nevertheless explanatory analysis of data was performed in order to verify the

tendency of this parameter. Upon addition of both hydrocolloids in combination to the teff

formulation, an increase in xanthan resulted in decreased volumes, while HPMC did not show

an effect. Also in maize bread the combination resulted in an overall decreased volume, even if

addition of HPMC increases this parameter slightly. In buckwheat and rice bread, HPMC and

xanthan decreased specific volume, the effect of the latter being more pronounced. Response

surface plots are a helpful tool for a better understanding of the link between each factor and

the response. Three dimensional response surface plots, showing the effect of xanthan and

HPMC   on   loaf   specific   volume   are   presented   in   Figure   6.1.
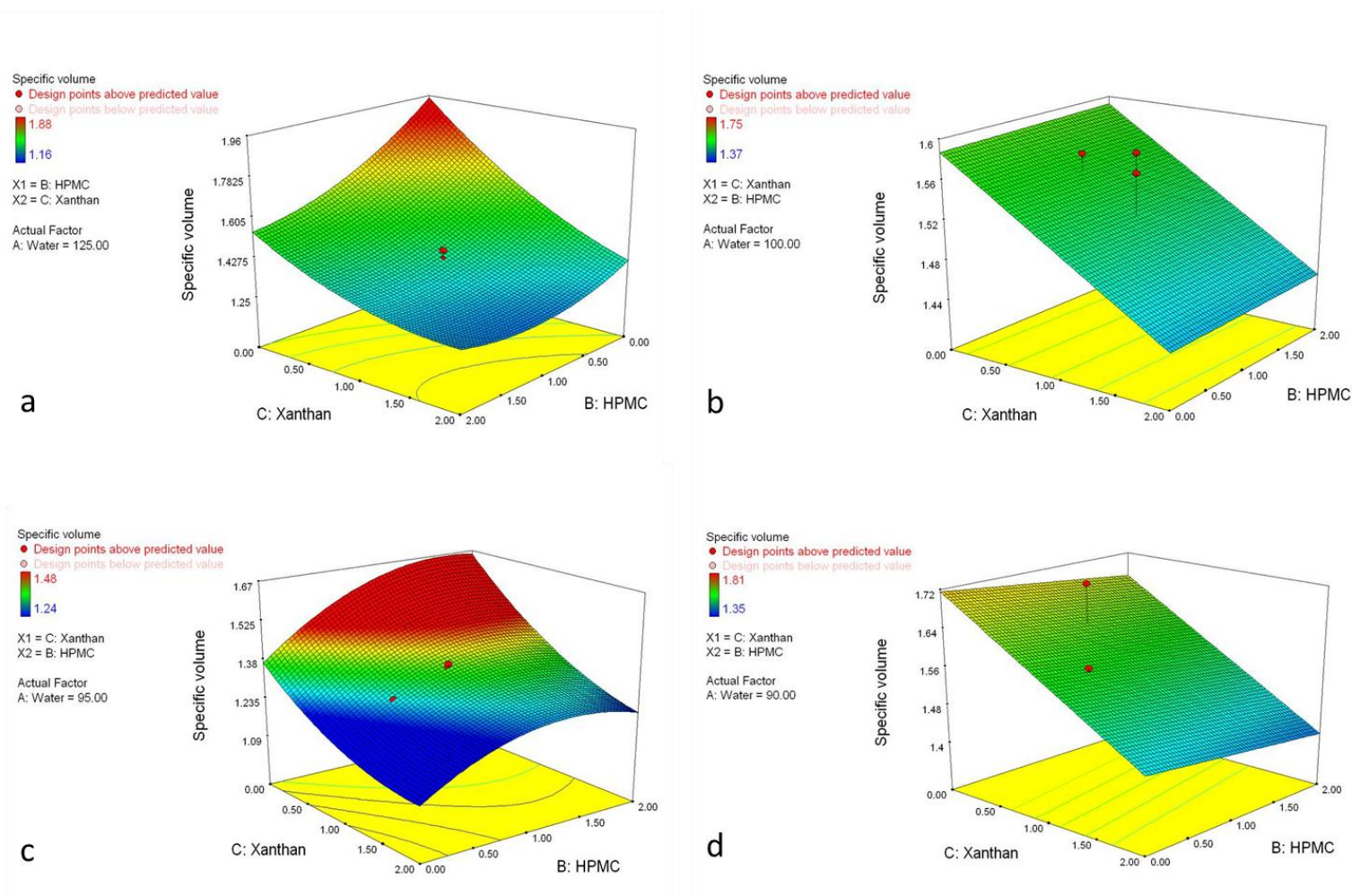
Figure 6.1 Influence of HPMC and Xanthan on specific volume of (a) rice, (b) teff, (c) maize and (d) buckwheat bread

6.4.3 Influence of hydrocolloids on crumb hardness

Another important quality characteristic of bread is texture, with consumers desiring soft and flexible crumbs, i.e. low hardness values. ANOVA demonstrated that the models for effects of HPMC and water level as well as models for the effect of xanthan and water level on crumb hardness were adequate for all formulations (P<0.05). The increase of water levels had a negative linear effect, decreasing crumb hardness. HPMC addition decreased this parameter in teff, buckwheat, maize and rice bread.

Xanthan increased the crumb hardness of teff and buckwheat breads, while rice bread crumb remained uninfluenced. Crumb hardness values of maize breads were reduced by xanthan addition.

ANOVA showed that the predictive models developed for the influence of the combination of the two hydrocolloids on teff, maize and buckwheat bread was considered adequate because they possessed a non-significant lack of fit and had satisfactory levels of R^2, CV and model significance. The developed model for the influence of the combination of the two hydrocolloids on rice bread was less predictive because lack of fit was significant at the 5% level. However, a surface plot was generated also for this model, because it offered a reasonable initial solution for describing the quality response of this parameter. The resulting response surface plots (Figure 6.2) show that at constant water level HPMC and xanthan addition increase hardness of rice and buckwheat breads. Xanthan addition increases, while HPMC addition decreases crumb hardness of teff crumb and hardness of maize breads was reduced by both hydrocolloids.
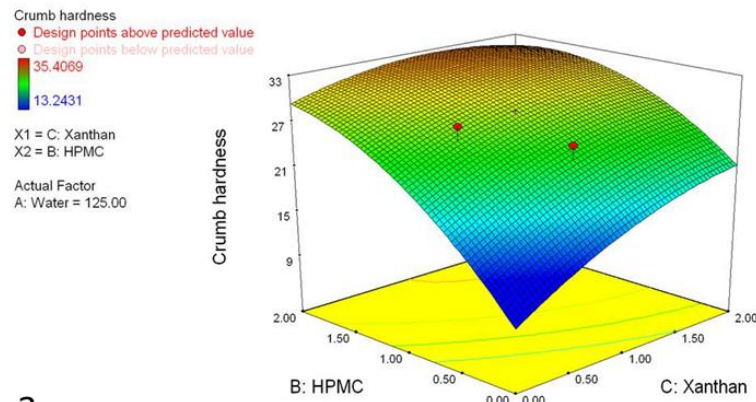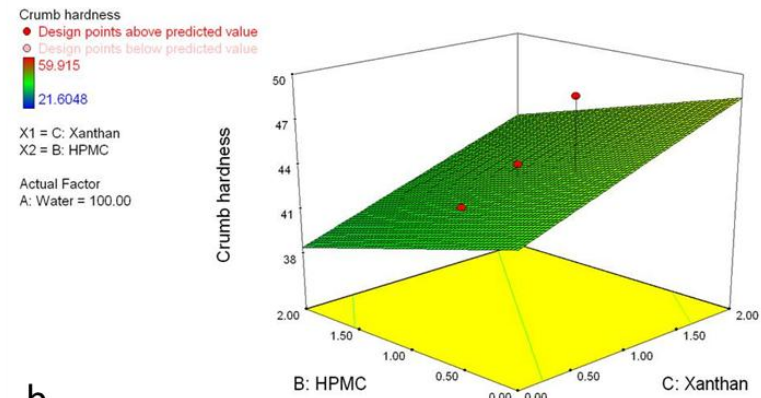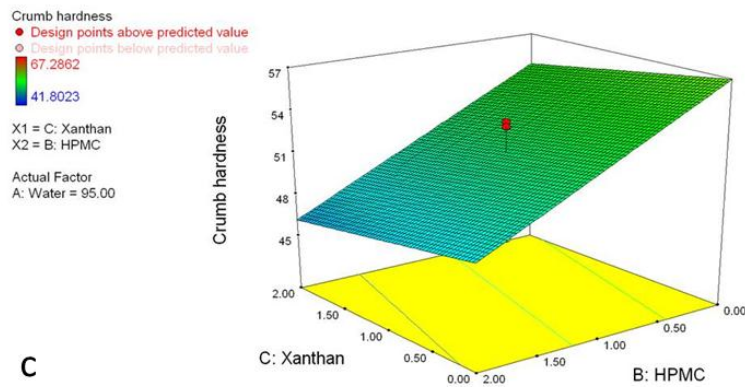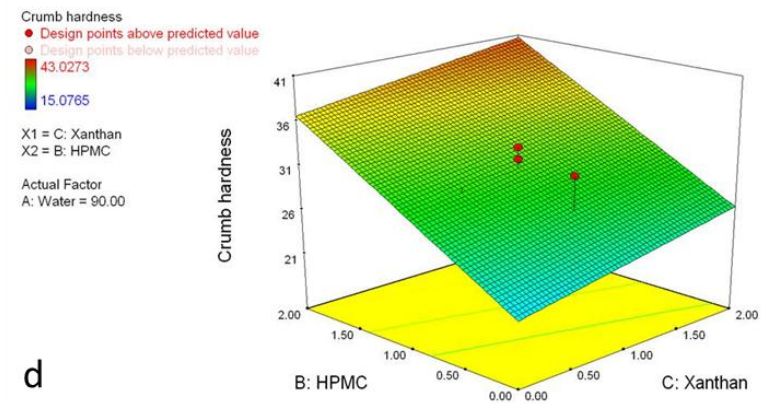
Figure 6.2 Influence of HPMC and Xanthan on crumb hardness of (a) rice, (b) teff, (c) maize and (d) buckwheat bread

6.4.4 Influence of hydrocolloids on crumb grain

The area of cells as a percentage of total slice area was used to quantitatively describe crumb grain properties. Higher values indicate a more open texture. However, not the holes themselves are the most significant contributor to mechanical strength of the baked product, but the surrounding matrix referred to as "cell walls" (Cauvain *et al*. 1999). The mouth feel of bread is known to be strongly influenced by these cell characteristics: finer, thin-walled uniform cells yield a softer and more elastic texture, than coarse, thick-walled cell structures (Scalon and Zghal 2001). ANOVA showed that the predictive models developed for the influence of HPMC and water level on area of cells and wall thickness of teff bread as well as on area of cells of maize bread was considered inadequate because they possessed a significant lack of fit and had unsatisfactory levels of R^2, CV and model significance. Hence, no trends can be reported for these combinations. Models for the influence of HPMC and water level on crumb grain characteristics of all other formulations were adequate ($P<0.05$). While water level had a negative effect on area of cells of buckwheat bread, HPMC increased the ratio. In rice bread, both independent variables decreased area of cells. Wall thickness of rice bread was reduced by water and HPMC addition. Addition of HPMC to the buckwheat formulation resulted in an initial decrease of wall thickness. At higher levels (HPMC > 1.5%) wall thickness increased again. Increased water and hydrocolloid levels initially increased wall thickness of maize bread; after a certain level the wall thickness decreased again. Lowest wall thickness of maize bread crumb is obtained at the lowest levels of HPMC and water addition.

ANOVA demonstrated that the models for effects of xanthan and water level on crumb grain were adequate for all formulations ($P<0.05$). A negative effect of xanthan and water level on area of cells was observed in all formulations. Water addition showed a positive effect on wall thickness, increasing this value in all formulations, while xanthan addition showed the opposite effect, reducing wall thickness of all breads.

Also all models for effects of the hydrocolloid combination and water level on crumb grain were adequate, as demonstrated by ANOVA (P<0.05). Resulting response surface plots are shown in Figure 6.3 and Figure 6.4. Upon xanthan addition decreased areas of cells can be observed. However, in the case of rice and buckwheat bread, this parameter increases again at xanthan levels of around 2%. The influence of HPMC on area of cells was less pronounced, with no effect on buckwheat bread. A slight decrease of area of cells was observed in teff bread and an increase in rice and maize breads. While xanthan shows a decreasing effect on wall thickness of rice, teff and maize bread, HPMC only decreases wall thickness of rice and maize breads but did not show an effect on teff or buckwheat breads.
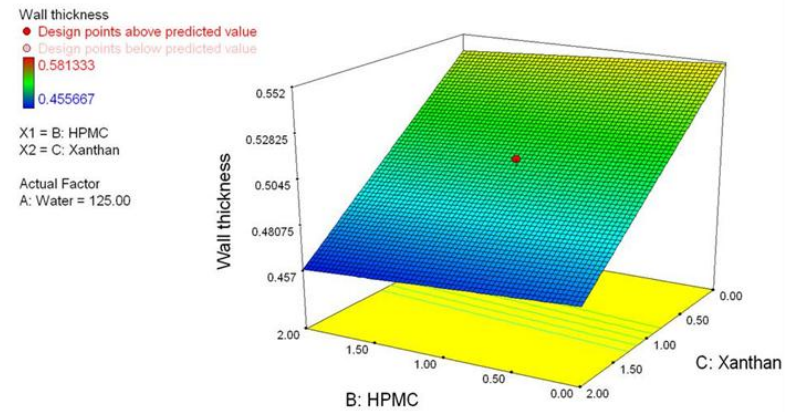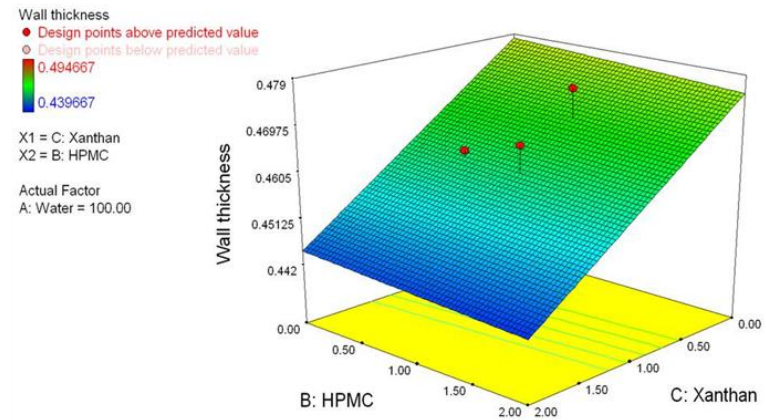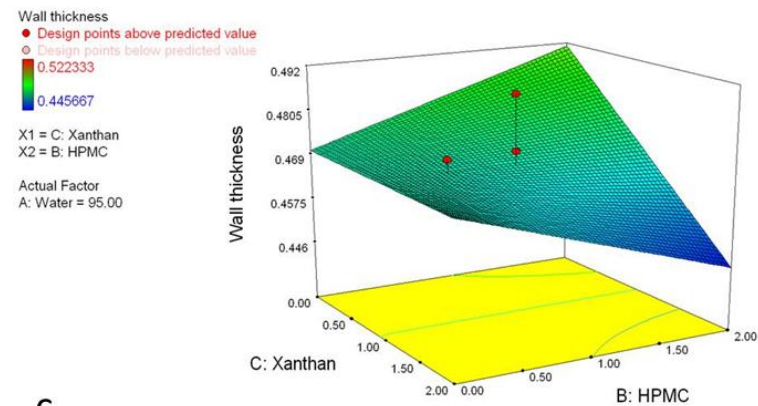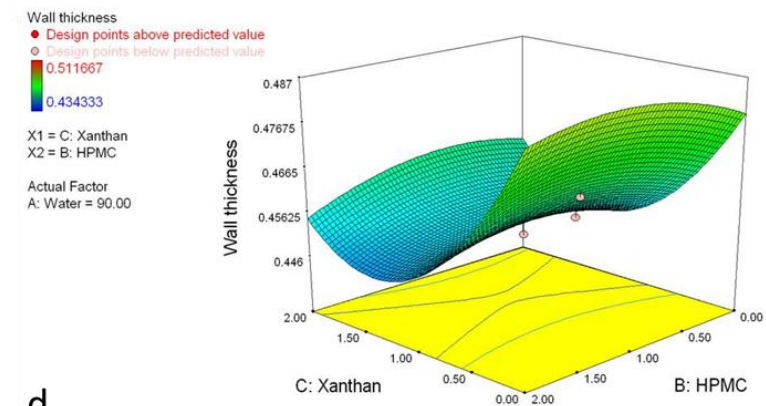
Figure 6.3 Influence of HPMC and Xanthan on wall thickness of (a) rice, (b) teff, (c) maize and (d) buckwheat bread
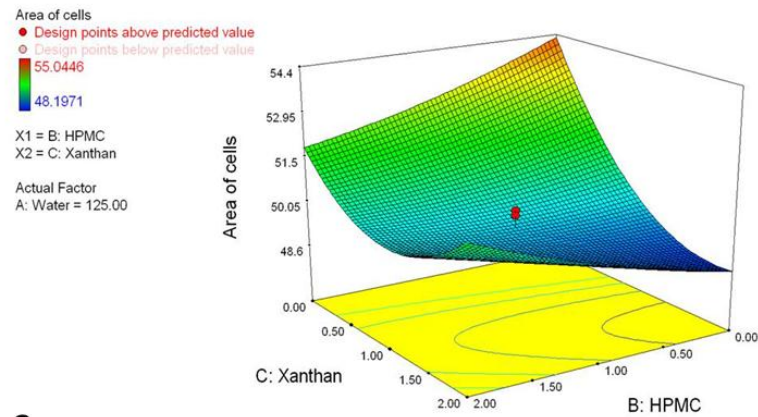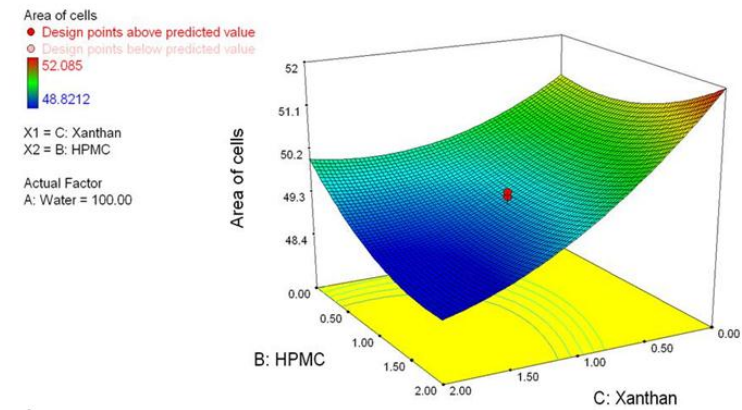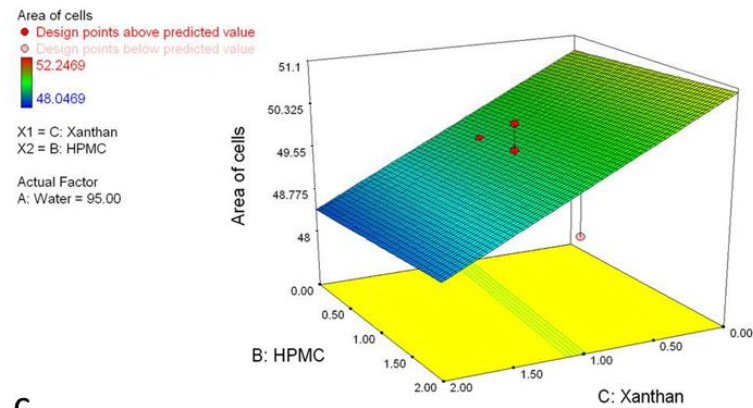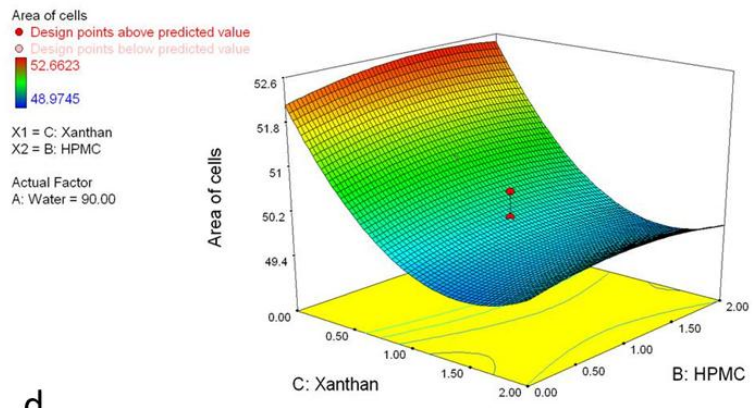
Figure 6.4 Influence of HPMC and Xanthan on area of cells of (a) rice, (b) teff, (c) maize and (d) buckwheat bread

6.4.5 Optimisation

The influence of hydrocolloid and water levels on the individual responses as illustrated by response surface plots was described above. The next step was the identification of optimal ingredient levels. The optimal solution arises from a compromise between all responses and the criteria of optimisation have to be chosen, i.e. each independent variable has to be either maximised or minimised. Although the final judgement of what represents a good quality bread is highly personal, there are certain widely accepted criteria such as a high volume and a soft crumb (Cauvain 2012). Hence, loaf specific volume was maximised while crumb hardness was minimised. As gluten free products often show a too dense crumb, area of cells was maximised. Thin-walled cells are said to yield a softer more elastic texture and therefore wall-thickness was minimised (Scalon and Zghal 2001).

In preliminary trials, control breads were produced. Instead of applying response surface methodology, optimal water levels for these breads were determined by empirical trial-and-error testing by measuring loaf specific volume and visually evaluating crumb structure. Based on these preliminary baking trials the following levels were determined as optimal: 120 % of rice flour, 95 % of teff flour, 90 % of maize flour, 85 % of buckwheat flour. These empirically optimized breads without hydrocolloids (Table 6.2) were then compared to the breads resulting from the optimized formulations using RSM.

Table 6.2 Properties of control breads produced without hydrocolloid addition

	Teff	Buckwheat	Rice	Maize
Water [%]	95	85	120	90
Specific volume [ml/g]	1.60 ± 0.03	1.69 ± 0.06	1.80 ± 0.05	1.33 ± 0.10
Crumb hardness [N]	43.1 ± 5.2	42.9 ± 4.0	18.8 ± 1.9	66.7 ± 3.6
Area of cells [%]	47.75 ± 0.41	49.92 ± 0.09	53.85 ± 0.11	47.76 ± 0.22
Wall thickness [mm]	0.45 ± 0.01	0.45 ± 0.00	0.54 ± 0.01	0.43 ± 0.00

The ingredient levels obtained as a result of the optimization step as well as the calculated desirabilities for these formulations are shown in Table 6.3-6.5. Water level adjustment and HPMC addition was able to improve loaf quality of buckwheat (1.50% HPMC), teff (2.00%

HPMC) and maize bread (0.63% HPMC). The increase of water addition in rice bread alone was sufficient in ameliorating bread properties, HPMC addition did not result in further improvement (Table 6.3). Relatively lower levels of xanthan were suggested by the software: 0.45 % for teff, 0.52 % for buckwheat, 0.43 % rice and 0.30 % for maize bread formulations (Table 6.4). Regarding the combination of both hydrocolloids, the following addition levels were suggested: 2.00% HPMC and 0.04% xanthan for teff breads, 0.14 % xanthan without HPMC addition for buckwheat breads and 1.77% HPMC without xanthan addition for maize bread. Regarding rice bread, again hydrocolloid addition could not further improve loaf quality and hence HPMC and xanthan levels of 0.00% were suggested by the software (Table 6.5).

Breads were baked using the ingredient levels resulting from the optimization process and subsequently analyzed in order to compare responses predicted by the software to the measured values (Table 6.3-6.5). For buckwheat breads, volume measurements of xanthan containing breads corresponded well with the predictions but higher volumes than expected were measured upon addition of HPMC or the combination of both hydrocolloids. The predictions for loaf volumes of rice breads not containing HPMC correspond well (Table 6.3). However, the measured loaf volume upon xanthan incorporation was higher than predicted (Table 6.4), while values were overestimated for the formulations without hydrocolloid addition (Tables 6.3 and 6.5). In teff and maize breads lower volumes were predicted by the software. For optimization of addition level of HPMC as well as the combination, predictions for crumb hardness were generally in good agreement with measurements, with the exception of maize breads where this value was overestimated. Regarding optimization of xanthan addition levels, crumb hardness was generally overestimated. For crumb grain characteristics however, the measured responses compared favorably to the predicted values.

Table 6.3 Predicted and measured values for the responses (independent variables) at optimum HPMC and water levels

	Teff		Buckwheat		Rice		Maize	
HPMC [%]	2.00		1.50		0.00		0.63	
Water [%]	105		95		130		100	
Desirability	0.697		0.747		0.653		0.543	
	Predicted	Measured	Predicted	Measured	Predicted	Measured	Predicted	Measured
Specific volume [ml/g]	1.86	1.99	1.85	1.96	1.97	1.94	1.44	1.62
Crumb hardness [N]	18.0	16.9	13.4	13.0	11.0	15.5	43.9	32.2
Area of cells [%]	53.35	52.99	54.19	54.69	53.89	53.00	50.97	53.60
Wall thickness [mm]	0.54	0.51	0.51	0.51	0.57	0.58	0.48	0.54

Table 6.4 Predicted and measured values for the responses (independent variables) at optimum Xanthan and water levels

	Teff		Buckwheat		Rice		Maize	
Xanthan [%]	0.45		0.52		0.43		0.30	
Water [%]	105		93		130		97	
Desirability	0.668		0.614		0.585		0.557	
	Predicted	Measured	Predicted	Measured	Predicted	Measured	Predicted	Measured
Specific volume [ml/g]	1.56	1.82	1.76	1.77	1.53	1.93	1.34	1.47
Crumb hardness [N]	32.6	26.2	25.4	20.8	20.0	7.0	58.4	44.2
Area of cells [%]	51.78	51.35	51.67	52.72	52.21	54.72	49.95	50.64
Wall thickness [mm]	0.49	0.49	0.47	0.49	0.55	0.60	0.44	0.49

Table 6.5 Predicted and measured values for the responses (independent variables) at optimum Xanthan and HPMC concentrations and water levels

	Teff		Buckwheat		Rice		Maize	
Xanthan [%]	0.04		0.14		0.00		0.00	
HPMC [%]	2.00		0.00		0.00		1.77	
Water [%]	105		95		121		100	
Desirability	0.612		0.745		0.723		0.864	
	Predicted	Measured	Predicted	Measured	Predicted	Measured	Predicted	Measured
Specific volume [ml/g]	1.67	1.93	1.77	1.85	1.88	1.78	1.48	1.63
Crumb hardness [N]	29.0	24.1	15.9	17.7	14.3	14.4	44.1	31.5
Area of cells [%]	52.0	51.6	51.99	54.15	54.83	53.62	51.02	51.04
Wall thickness [mm]	0.48	0.50	0.48	0.51	0.54	0.58	0.46	0.53

6.5 Discussion

HPMC and Xanthan have been extensively studied in gluten free systems, but results are contradictory. Hydrocolloids are frequently used in gluten free baking to mimic the visco-elastic properties of gluten, thereby increasing gas retention during proofing and baking and hence increasing loaf specific volume. Table 6.6 suggests, that xanthan gum increases volume only at low levels up to 0.5% (Peressini and Sensidoni 2009; Sciarini *et al*. 2010). Higher addition levels of xanthan do not significantly affect this parameter or result in decreased volumes (Crockett *et al*. 2011; Lazaridou *et al*. 2007; Sabanis and Tzia 2011a). Comparing the results of previous studies it becomes apparent that HPMC is much more suitable for increasing loaf specific volume. A volume increase upon HPMC addition at various levels was reported in several publications (Haque and Morris, 1994; Mezaize *et al*. 2009; Sabanis and Tzia, 2011a,b). However, the study of Crockett *et al*. (2011) clearly shows that the grade of the hydrocolloid is crucial in determining its effect. While these authors observed a volume increase after addition of 3 and 5% low methoxy HPMC, the addition of high methoxy HPMC did not have a significant influence.

Even though the effect of the hydrocolloids on the gluten free model systems of this study varied according to the raw materials used, some observed trends are reflective of the physico-chemical properties of the gums. In bread making, there are two principally different phases: before and after starch gelatinisation (Schober 2009). The former includes mixing, resting, fermentation and early stages of baking and it is at this stage where hydrocolloids have to take effect. HPMC is believed to maintain homogeneity of a batter due to its affinity to both the aqueous and non-aqueous phases. The hydrocolloid has surface active properties, with the methoxyl and hydroxypropyl showing affinity to the non-polar or lipid phase of a multi-phase system such as bread dough. Therefore it forms an interfacial film at the boundaries of gas cells and possibly provides stability for the cells during expansion (Bell 1990). Also the increase of batter viscosity upon gelation of HPMC results in improved gas retention. According to the

producer's homepage, gelation temperature of HPMC NE-4000 is 75°C. This is higher than the gelatinisation temperatures of the utilised flours (Table 6.1). On the contrary, xanthan addition already results in a viscosity increase of the batter at room temperature and xanthan is believed to keep rheological properties stable upon heating. It was hypothesised previously that the increased viscosity of gluten free batters upon xanthan addition helps keeping gas bubbles from rising and prevents their coalescence, keeping the system homogeneous until starch gelatinization (Lazaridou *et al*. 2007; Schober, 2009). The results of this study contradict this theory somewhat as xanthan addition reduced loaf volume as well as area of cells. The altered rheological properties of the batters, i.e. increased viscosity, seemed to have a positive impact on cell walls, reducing their thickness in all produced breads. In view of the fact that xanthan works well in wheat starch based breads, a possible explanation for the lack of success in breads of this study might be the different gelatinisation temperatures. When baking breads with teff, maize, buckwheat and rice, compared to wheat bread higher temperatures are required for the formation of a starch gel resulting in a longer time period in which bubbles expand due to heat (Schober 2009).

Hydrocolloids are typically added to baked goods for increased water-binding, producing moister and softer crumbs. Several authors however observed an increased crumb hardness upon xanthan addition (Crockett *et al*. 2011; Lazaridou *et al*. 2007; Peressini and Sensidoni, 2009; Sabanis and Tzia, 2011a). Again, the utilisation of HPMC was more successful in improving loaf quality, with reduced crumb hardness being reported in most publications. However, two studies using response surface methodology to simultaneously alter HPMC and water level showed a positive linear effect of hydrocolloid addition, i.e. increased crumb hardness (McCarthy *et al*. 2005; Sabanis and Tzia, 2011b). As mentioned for loaf volume, the HPMC grade plays a role also in determining crumb hardness. (Crockett *et al*. 2011) showed that high methoxy HPMC (2, 3 and 5% addition levels) and low methoxy HPMC (2%) significantly reduces crumb hardness. Addition of 3 % low methoxy HPMC did not influence

this parameter, while addition of 5% low methoxy HPMC increased hardness significantly. HPMC addition decreased crumb hardness of all breads of this study. The hydrocolloid forms a thermo-reversible gel which strengthens when heated and reverts back to weak entanglement after cooling. This temperature dependence stabilizes the gelatinizing crumb structure during baking but reduces crumb hardness of the final bread (Crockett *et al*. 2011).

By changing the rheological properties of gluten free batters, hydrocolloids are believed to influence crumb structure. Reports on the utilisation of image analysis to evaluate the influence of HPMC and Xanthan on specific crumb grain characteristics are scarce. (Mezaize *et al*. 2009) observed that HPMC addition (2.3%) increased the number of cells per cm^2 and reduced porosity (total cell area divided by slice area). Also McCarthy *et al*. (2005) found that up to a certain limit, the number of cells per cm^2 increased as HPMC and water increased. Incorporation of xanthan (0.6%) did not influence these parameters. Also Turabi *et al*. (2010) did not observe a significant effect of xanthan (1%) on the ratio of pore to slice area or roundness of cells. However, these authors detected an increased number of small cells. Sciarini *et al*. (2010) observed no significant difference in total cell area upon incorporation of 0.5% xanthan gum, but a reduced number of cells per mm^2 and an increase in the average cell size, indicating a more open structure of xanthan containing breads compared to the control.

It was hypothesised previously, that the combination of hydrocolloids results in a synergistic effect (Arendt and Dal Bello 2008). However during this study HPMC and xanthan combined did not produce an effect greater than the sum of their individual effects.

The bread quality parameters measured for breads baked with optimum conditions were compared to control breads without hydrocolloid addition. Using response surface methodology and the described optimization process, volumes of teff, buckwheat and maize breads were increased by the described hydrocolloid and water level combinations. As expected, the incorporation of hydrocolloids allowed for an increase in water level in all formulations. This resulted in reduced crumb hardness values compared to control breads.

Area of cells of teff, buckwheat and maize bread crumbs was increased following the optimisation process. No real improvement of this parameter was observed for rice breads. Wall thickness was not improved by the optimisation process. All breads showed higher values than the controls.

Results clearly showed that HPMC and xanthan are powerful ingredients that influence bread properties even at very low addition levels. However, the application of these hydrocolloids has to be well thought through as their effects depend strongly on the surrounding matrix. The present results confirm the observation made when comparing available publications: the suitability of xanthan and HPMC for bread improvement depends on the formulation used. This is due to the fact that xanthan and HPMC are influenced by pH and ionic strength of the surrounding media as well as heat and shearing (Abdel-Aal 2009). Furthermore, as shown above, cereal flours diverge strongly in their chemical composition and certain components may interact to different extents with the hydrocolloids. Another explanation for the contradictions between studies might be the use of different bread pans, as gluten free batters are very soft and mechanical support from bottom and sidewalls of tins is necessary. This means that in larger containers a certain amount of batter has to support relatively more weight than in a small pan (Schober 2009). Besides heat transfer during baking depends on tin dimensions and material as well as ovens used.

In conclusion it can be said that HPMC and xanthan have the potential to improve bread properties, but might also deteriorate loaf quality. Hydrocolloids are expensive ingredients that should be used at as low levels as possible. Manufacturers of gluten free bread are advised to carefully evaluate the effect of these chemicals in the respective formulation.

Table 6.6 Influence of HPMC and Xanthan on gluten free bread and cake

Recipe base	Hydrocolloid	Level	Effect on Volume	Effect on crumb hardness	Reference
Sorghum flour, corn starch	Xanthan gum (Quest International, The Netherlands)	0.3-1.2	Decrease	Increase	(Schober *et al*. 2005)
Rice and buckwheat flour	Xanthan gum (Chimab Food Ingredients, Italy)	0.5%*	Increase	Increase	(Peressini and Sensidoni 2009)
		1%*	No sign. effect	Increase	
		1.5%*	Decrease	Increase	
Rice flour, corn starch	Xanthan gum (Saporiti A.A., Argentina)	0.5%	Increase	Decrease	(Sciarini *et al*. 2010)
Rice flour ,corn starch, sodium caseinate	Xanthan (KELTROL, CP Kelco, Denmark)	1%*	No sign. effect	Increase	(Lazaridou *et al*. 2007)
		2%*	Decrease	Increase	
Rice flour, Sugar, Shortening, Egg (=cake)	Xanthan gum (Fluka, France)	0.3%	-	No sign. effect	(Sumnu *et al*. 2010) (Turabi *et al*. 2010)
		1%	-	Decrease	
Rice flour, Corn flour, Sugar, Egg, Milk (=cake)	Xanthan gum (Farmaquimica Industrial Ltd, Brasil)	0.2%	No sign. effect	Decrease	(Preichardt *et al*. 2011)
		0.3%	Increase	Decrease	
		0.4%	Increase	Decrease	
Rice flour and cassava starch	Xanthan Grindsted 200 (Danisco, U.S.A.)	2	No sign. effect	No sign. effect	(Crockett *et al*. 2011)
		3	Decrease	No sign. effect	
		5	Decrease	Increase	
	HPMC Methocel K4M (Dow Chemicals, U.S.A.)	2	Increase	Decrease	
		3	No sign. effect	No sign. effect	
		5	No sign. effect	Increase	
	HPMC Methocel E15	2	Increase	Decrease	

	(Dow Chemicals, U.S.A.)	3	Increase	Decrease	
		5	Increase	Decrease	
Rice flour, Corn starch, corn flour, potato starch	Xanthan gum (Cargill, France)	0.6%	No sign. effect	Decrease	(Mezaize *et al*. 2009)
	HPMC (Dow Europe GmbH, Germany)	2.3%	Increase	Decrease	
Corn starch, Rice flour	Xanthan gum (Luxara 7571/200, UK)	1%*	No sign. effect	Increase	(Sabanis and Tzia 2011a)
		1.5%*	No sign. effect	Increase	
		2%*	No sign. effect	Increase	
	HPMC (Dow Wolff Cellulosics, Europe)	1%*	Increase	Decrease	
		1.5%*	Increase	Decrease	
		2%*	Increase	Decrease	
Maize starch, rice flour	HPMC Methocell (Dow Chemical Company)	0.5 – 2.5%*	Increase up to medium level, hereupon decrease	Increase	(Sabanis and Tzia 2011b)
Rice flour, potato starch, skim milk powder	HPMC (Food Ingredient Technology Ltd., UK)	0.5 – 2.5%*	Decrease	Increase	(McCarthy *et al*. 2005)
	HPMC		Increase up to medium level, hereupon decrease		(Haque and Morris 1994)
Rice flour	HPMC 90 HG 4000 (Dow Chemical Company, Michigan)	1.5%	Increase	-	(Nishita *et al*. 1976)
		3.0%	Increase		
		4.5%	Increase		
		7.0%	Increase		

*Based on flour base

6.6 Acknowledgements

6.7 References

AACC International (1995) Method 30-10 Crude Fat. In: Approved Methods of Analysis, 9th Ed. AACC International, St. Paul, MN, U.S.A.

AACC International (1995) Method 46-12 Crude Protein—Kjeldahl Method, Boric Acid Modification. In: Approved Methods of Analysis, 9th Ed. AACC International, St. Paul, MN, U.S.A.

AACC International (1995) Method 44-15A Moisture—Air-Oven Methods. In: Approved Methods of Analysis, 9th Ed. AACC International, St. Paul, MN, U.S.A.

Abdel-Aal E.M. (2009) Functionality of starches and hydrocolloids in gluten-free foods. In: Gallagher, E. (Ed.) Gluten-free Food Science and Technology. Oxford: Blackwell publishing Ltd.

Alvarez-Jubete L, Wijngaard H, Arendt E.K. and Gallagher E. (2010) Polyphenol composition and *in vitro* antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. Food Chemistry, 119(2), 770-778

Arendt E.K. and Dal Bello F. (2008) Gluten-Free Cereal Products and Beverages. London: Academic Press

Bell D.A. (1990) Methylcellulose as a structure enhancer in bread baking. Cereal Foods World, 35(10), 1001-1006

Born K, Langendorff V. and Boulenguer P. (2002) Xanthan. In: Steinbüchel, A., Vandamme, E. J. & De Baets, S. (Eds.) Biopolymers. Weinheim: Weley-VCH

Cauvain S.P. (2012). Breadmaking Improving quality. Cambridge: Woodhead Publishing

Cauvain S.P, Whitworth M.B, and Alava J.M. (1999) The Evolution of Bubble Structure in Bread Doughs and Its Effect on Bread Structure. In: Campbell, G.M. (Ed.) Bubbles in Food. St. Paul, Minnesota: American Association of Cereal Chemists

Crockett R, Ie P. and Vodovotz Y. (2011) How Do Xanthan and Hydroxypropyl Methylcellulose Individually Affect the Physicochemical Properties in a Model Gluten-Free Dough? Journal of Food Science, 76(3), E274-E282

Hager A.S, Wolter A, Czerny M, Bez J, Zannini E, Arendt E.K. and Czerny M. (2012) Investigation of product quality, sensory profile and ultra-structure of breads made from a range of commercial gluten free flours compared to their wheat counterparts. European Food Research and Technology, 235(2), 333-344

Haque A. and Morris E.R. (1994) Combined use of Ispaghula and HPMC to replace or augment gluten in breadmaking. Food Research International, 27(4), 379-393

Lazaridou A, Duta D, Papageorgiou M, Belc N. and Biliaderis C.G. (2007) Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. Journal of Food Engineering, 79, 1033-1047

Matissek R. and Steiner G. (2006) Bestimmung des Gluehrueckstandes durch direkte Veraschung (Aschegehalt). In: Lebensmittelanalytik –Grundzuege, Methoden, Anwendungen. Berlin: Springer-Verlag

McCarthy D.F, Gallagher E, Gormley T.R, Schober T.J. and Arendt E.K. (2005) Application of Response Surface Methodology in the Development of Gluten-Free Bread. Cereal Chemistry, 82, 609-615

Mezaize S, Chevallier S, Le Bail A. and de Lamballerie M. (2009) Optimization of gluten-free formulations for French-style breads. Journal of food science, 74(3), E140-E146

Nishita K.D, Roberts R.L, Bean M.M. and Kennedy B.M. (1976) Development of a yeast-leavened rice-bread formula. Cereal Chemistry, 53(5), 626-635

Peressini D. and Sensidoni A. (2009) Effect of soluble dietary fibre addition on rheological and breadmaking properties of wheat doughs. Journal of Cereal Science, 49, 190-201

Preichardt L.D, Vendruscolo C.T, Gularte M.A. and Moreira A.D. (2011) The role of xanthan gum in the quality of gluten free cakes: improved bakery products for coeliac patients. International Journal of Food Science and Technology, 46(12), 2591-2597

Sabanis, D. and Tzia, C. (2011a) Effect of hydrocolloids on selected properties of gluten-free dough and bread. Food Science and Technology International, 17(4), 279-291

Sabanis, D. and Tzia C. (2011b) Selected Structural Characteristics of HPMC-Containing Gluten Free Bread: A Response Surface Methodology Study for Optimizing Quality. International Journal of Food Properties, 14(2), 417-431

Sarkar, N. and Walker L.C. (1995) Hydration Dehydration properties of methylcellulose and hydroxypropylmethylcellulose. Carbohydrate Polymers, 27(3), 177-185

Scalon, M.G. and Zghal M.C. (2001) Bread properties and crumb structure. Food Research International, 34, 841-864

Schober T.J. (2009) Manufacture of gluten-free speciality breads and confectionary products. In: Gallagher, E. (Ed.) Gluten-free Food Science and Technology. Oxford: Blackwell publishing Ltd.

Sciarini L.S, Ribotta P.D, Leon A.E, Perez G.T. (2010) Effect of hydrocolloids on gluten-free batter properties and bread quality. International Journal of Food Science and Technology 45(11), 2306-2313

Schober T.J, Messerschmidt M, Bean S.R, Park S.H. and Arendt E.K. (2005) Gluten-Free Bread from Sorghum: Quality Differences Among Hybrids. Cereal Chemistry, 82, 394-404

Sumnu G, Koksel F, Sahin S, Basmanm A. and Meda V. (2010) The effects of xanthan and guar gums on staling of gluten-free rice cakes baked in different ovens. International Journal of Food Science and Technology 45(1), 87-93

Turabi E., Sumnu G. and Sahin S. (2010) Quantitative analysis of macro and micro-structure of gluten-free rice cakes containing different types of gums baked in different ovens. Food Hydrocolloids, 24, 755-762

Chapter 7: Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours

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7.1 Summary

Coeliac patients suffer from an immune mediated disease, triggered by the ingestion of a protein composite (gluten) found in wheat, rye and barley. Consequently, there is a need for products such as bread or pasta, made from alternative cereal grains or pseudocereals. A fair proportion of the gluten free products currently on the market are nutritionally inadequate. Hence, it was the aim of this study to investigate the nutrient composition of seven commonly used commercial gluten free flours (oat, rice, sorghum, maize, teff, buckwheat and quinoa) and compare them to wheat and wholemeal wheat flour. In addition to the levels of all major compounds, also mineral composition, fatty acid profile, phytate, polyphenols and folate content were determined. Furthermore, properties of carbohydrates were studied in greater detail, looking at total and damaged starch levels; total, soluble and insoluble dietary fibre content as well as amylose/amylopectin ratio. Proteins were further investigated by means of capillary electrophoreses. Additionally, the ultra-structure of these materials was explored using scanning electron microscopy. The results show that maize and rice flour are poor regarding their nutritional value (low protein, fibre, folate contents). In contrast, teff as well as the pseudocereals quinoa and buckwheat show a favourable fatty acid composition and are high in protein and folate. In particular, quinoa and teff are characterised by high fibre content and are high in calcium, magnesium and iron. Therefore these flours represent nutrient dense raw materials for the production of gluten free foods.

7.2 Introduction

Growing interest exists in the utilisation of alternative grains for the production of cereal based foods due to their high nutritional value and the dietary needs of a significant part of the human population (e.g. coeliac disease patients). The use of wheat flour (*Triticum aestivum*) for human consumption has a long tradition and it is the dominant crop in temperate countries. Wheat contributes essential amino acids, minerals, beneficial phytochemicals and dietary fibre to the human diet, and these are particularly enriched in wholemeal flour. The success of wheat relies mainly on the gluten protein fraction, which is responsible for the formation of a viscoelastic dough that can then be processed into bread, pasta and other food products (Shewry 2009). This protein fraction cannot be tolerated by patients suffering from coeliac disease. Yet, there are a number of cereals available, which do not contain gluten and are therefore safe to use even by coeliac patients. Probably the most commonly used gluten free flour in industry as well as for research purposes is rice flour (*Oryza sativa*). Rice flour is a cheap nutrient source. It consists of about 80 % starch and its proteins are not considered coeliac toxic. Sorghum (*Sorghum bicolor*) and maize (*Zea mays*) are two closely related species. The latter is grown worldwide and ranks third only to wheat and rice in world's grain consumption. Even though maize supplies many micro- and macronutrients necessary for human metabolism, the amounts of some essential nutrients are inadequate (Nuss and Tanumihardjo 2010). Sorghum has been neglected over the past decades and currently doesn't play an important role in commercialized food systems. Limited research efforts in grain processing and product technologies have been made to assess the potential of this crop for food uses (Rai *et al*. 2008). Although the proximate composition and nutritional value of sorghum is similar to that of maize, its proteins are less digestible (Wrigley *et al*. 2004). Teff (*Eragrostis tef*), which can be considered a minor crop when compared to the above mentioned, originates from Ethiopia where it is used for the production of several types of flat bread (Tatham *et al*. 1996). It is a small-seeded annual grass and falls into the group of millet.

Quinoa (*Chenopodium quinoa*) is a typical crop of the Andean region. It has been recognized as an extremely nutrious grain, due to the good quality and high quantity of its protein and essential fatty acids (Wrigley *et al*. 2004). Buckwheat (*Fagopyrum esculentum*) is also interesting from a nutritional point of view, since it contains protein of high value, dietary fibre, essential vitamins and minerals (Wijngaard and Arendt 2006). Quinoa and buckwheat are not true cereal grains since they are dicotyledonous (as opposed to monocotyledonous). Due to the fact that they produce starch-rich seeds like cereals they are called pseudocereals. Oat (*Avena sativa*) was included into the study although its status in the gluten free diet is controversial. Most but not all people with intolerance to gluten can include oats in their diet without adverse effect on their health (Commission of the European Communities 2009).

Much information is available on the chemical composition of these cereal grains, but data on the composition of gluten free flours is scarce. Due to the fact that processes such as dehulling and milling significantly change the nutrient profile, the characterisation of resulting flours is interesting. The aim of this fundamental study was to characterise the chemical composition of commercial gluten free flours made from teff, sorghum, maize, quinoa, buckwheat, oat and rice and to compare their nutritional properties to that of wheat and wholemeal wheat flour. Investigating the ultra-structure of flours gives valuable information on the nature of starch granules, which in turn significantly influences technological properties. In the course of this study, scanning electron microscopy was used to evaluate and compare the different flours. This publication is the first of its kind to directly compare a wide range of chemical and ultra-structural properties of seven gluten free and two wheat flours. The information gained is crucial for the formulation of nutritionally valuable gluten free products such as bread and pasta.

7.3 Materials and Methods

7.3.1 Materials

The suppliers for the ingredients used were Doves Farm Foods Ltd, UK for white rice flour (12.8 % moisture) and buckwheat flour (12.6 % moisture); Odlums, Ireland for wholemeal wheat (13.1 % moisture) and baker's flour (12.7 % moisture); Trouw, The Netherlands for teff flour (9.5 % moisture); Smiths Flour Mills, UK for maize flour (14.0 % moisture); Ziegler Naturprodukte, Germany for quinoa flour (12.3 % moisture); E. Flahavan & Son Ltd, Ireland for oat flour (10.4 % moisture) and Twin Valley Mills, Nebraska for sorghum flour (11.1 % moisture).

7.3.2 Compositional analysis

Crude fat, protein and moisture content of flours were determined according to the AACC methods 30-10, 46-12 and 44-15A, respectively. Protein content was calculated with a protein factor of 6.25, except for wheat flours where 5.7 was used. Ash content was determined according to Matissek (2006). Dietary fibre, phytate, total and damaged starch levels as well as amylose/amylopectin ratio were determined using enzyme kits (K-TDFR, K-PHYT, K-TSTA, K-SDAM, K-AMYL) supplied by Megazyme, Ireland. Polyphenol content was determined according to Alvarez-Jubete *et al*. (2010). The fatty acid profile was determined using gas chromatography following the trimethylsulfoniumhydroxide (TMSH) derivatisation method described by the DGF (Deutsche Gesellschaft für Fettwissenschaften) (method number: DGF C-VI 11e). Minerals were analyzed by inductively coupled plasma mass spectrometry (ICP-AES) following the method EN ISO 11885 E22. The chloride concentration of flours was determined according to the Analysenkommision (1996). Calorie contents were calculated using the following specific energy factors: 8.37 for fat, 4.12 for carbohydrates and 3.91 for protein (Schakel *et al*. 2009). Carbohydrate contents were calculated by difference (100 – moisture – fat – protein – ash). Folate levels were determined according to AOAC 944.12 (AOAC International 2006). Folate was extracted from the sample in an autoclave using a buffer

solution, followed by an enzymatic digestion with human plasma and pancreas deconjugase and finally by a second autoclave treatment. After dilution with basal medium containing all required growth nutrients except folic acid the growth response of Lactobacillus rhamnosus (ATCC 8043) to extracted folate was measured turbidimetrically and was compared to calibration solutions with known concentrations.

7.3.3 Capillary electrophoreses of extracted proteins

Proteins were extracted for 5 min in an ultrasonic waterbath with an extraction buffer containing 2 M Urea, 15 % glycerol, 0.1 M Tris, pH 8.8 and 0.1 M Dithiothreitol. Thereupon the samples were subjected to capillary electrophoresis, using a lab-on-a-chip technique (Agilent Technologies, Palo Alto, CA). For each protein extract, an aliquot of 4 µL sample was mixed with 2 µL Agilent sample buffer and loaded, under reducing conditions on a 230 kDa Protein chip in an Agilent Bioanalyzer. Protein peaks with an average concentration lower than 20 ng/µL were not considered, since their significance is low to the detection limit of the method.

7.3.4 Scanning electron microscopy

Oven-dried flour samples were attached onto double-sided carbon tape fixed to an aluminium specimen stub and were preliminary gold-coated in a SEM coating system (BIORAD Polaron Division) with a layer of 25 nm in thickness. Hereupon samples were examined under high vacuum in a field emission scanning electron microscope (JEOL, JSM-5510 SEM) with a working distance of 8 mm. Secondary electron images were acquired at an accelerating voltage of 5 kV.

7.3.5 Statistical analysis

SigmaPlot (Systat, Chicago, Illinois) was used to carry out statistical analysis on the test results. Normality test (Shapiro-Wilk) was followed by an all pair wise multiple comparison procedure (Fisher LSD Method) to evaluate significant differences. Analysis was performed in triplicates.

7.4 Results

7.4.1 Carbohydrates

Cereals usually comprise of about 50-80 % carbohydrate on a dry weight basis. Starch is the

main cereal polysaccharide and a major food reserve providing a bulk nutrient and energy

source in the human diet (Dewettinck *et al*. 2008). It is stored in granular form of variable size

and shapes characteristic of the species (Figure 7.1). Granules consist of starch molecules

which are arranged radially forming a series of concentric layers that alternate as amorphous

and semi-crystalline regions. Wheat starch differs to that of other botanical sources in that it

contains two, possibly three, distinct populations of granules differing in shape, dimension,

composition, and properties (Maningat and Seib 2010). Apart from large lenticular starch

granules (A-granules), smaller spherical granules (B-granules) can be observed. The crystalline

polymorphic form influences the extent of starch digestibility, with A-type granules being more

susceptible to amylolysis than B-type (Srichuwong *et al*. 2005). On the contrary to wheat

starch, granules found in the other cereals have a simple size distribution, being of similar

shape and diameter. Maize shows only spherical granules with a diameter of approximately 10

µm (Figure 7.1e). The starch granules in rice flour are polyhedral and very small (<5 µm). The

individual granules are organised together forming compound granules. Figure 7.1f shows

indentations where granules have been broken off during milling. Also oat starch is a

compound starch, comprised by bigger granules than in rice (up to 10 µm). The micrographs of

sorghum flour show polygonal starch granules of approximately 10 µm. These are surrounded

by smaller spherical bodies of only a few micrometers, likely to be protein bodies (Delcour and

Hoseney 2010). Teff granules are polygonal in shape and between 2 and 7 µm in diameter.

They are packed together and protein seems to attach outside of the compound starch

granule. Also buckwheat starch has granular shape (up to about 5 µm in diameter) and is

organised in bigger compounds. Quinoa has significantly smaller starch granules than all other

flours (<2 µm). They are polygonal and present both singly and in aggregates. The scanning

electron micrographs show that although in several cereal species starch is organised compound-like, on milling individual starch granules are released.
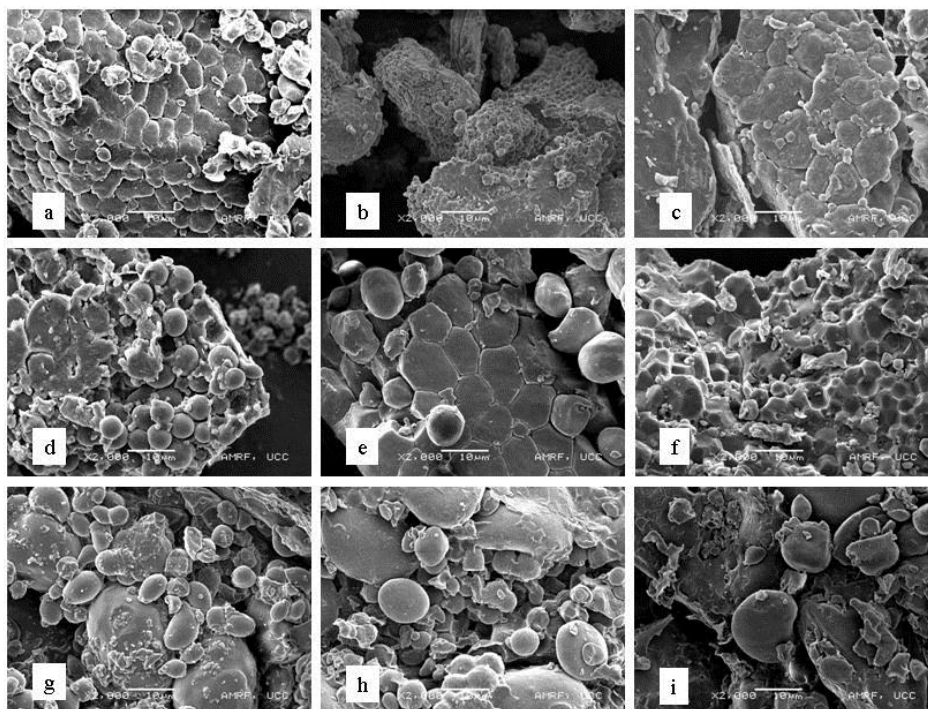
Figure 7.1 Scanning electron micrographs of the gluten free and wheat flours: (a) buckwheat; (b) quinoa; (c) teff; (d) sorghum; (e) maize; (f) rice; (g) oat; (h) wholemeal wheat; (i) wheat

Table 7.1 shows the total starch contents of the studied flours, which ranged from 49 g/100 g (quinoa) to 78 g/100 g (rice). Wholemeal wheat flour contains 57 g starch per 100 g flour. Wheat flour, due to a greater proportion of endosperm material, shows a higher starch level (68 g/100 g). From a chemical point of view, starch is comprised of two polymers of D-glucose: amylose and amylopectin. Amylose is linear (only lightly branched) and completely amorphous. Amylopectin is a highly branched polymer and provides partial crystallinity to the starch granule. The ratio of amylose to amylopectin is of technological relevance especially in connection to bread staling (Kulp and Ponte, 1981), but is also an important nutritional feature of starch (Akerberg *et al*. 1998). The percentage amylose of total starch is in the same range for maize flour (23 %), the wheat flours and rice flour (21 %), oat and teff flour (20 %) (Table 7.1). Sorghum and buckwheat flour had a lower percentage of amylose (18 and 16 %,

respectively). Quinoa starch showed a significantly lower amylose content of only 5 % of total starch. It was previously shown that higher amylose/amylopectin ratios result in increased levels of resistant starch upon heating (Akerberg *et al*. 1998, Granfeldt *et al*. 1993).

The milling of grains causes physical damage to a proportion of the starch granules (Table 7.1). Their altered properties are of technological significance, as damaged starch granules increase water absorption and are also more susceptible to enzyme hydrolysis. In this study, the highest amount of damaged starch was found in rice flour (15.24 g/100 g), followed by white wheat flour (7.85 g/100 g). Buckwheat and teff flour contained the lowest amounts (2.63 g/100 g and 2.08 g/100 g respectively). These variations are not only due to difference in biological origin of the flours, but result also from the different milling procedures applied and equipment used.

Apart from starch, cereals also contain significant amounts of carbohydrates which are resistant to digestion in the human small intestine and are completely or partly fermented in the large intestine (i.e. non-starch polysaccharides or dietary fibre). However, compared to their grain counterparts, flours are significantly lower in fibre because the milling process removes the bran and germ to a certain extent. Due to differences in chemical composition of the grains and applied milling procedures, the fibre content of the flours in this study varied widely (Table 7.1). Endosperm-derived white wheat flour contains only 3.4 g/100 g dietary fibre, while wholemeal wheat flour, where the bran fraction is reintroduced into the milled white flour, contains 11.4 g/100 g. Quinoa flour contained the highest amount of dietary fibre among the gluten free flours screened (7.1 g/100 g). This high level is due to the fact that quinoa flour is made by milling the whole seed. Sorghum and teff flours have a similar fibre content (both 4.5 g/100 g) and oat contains 4.1 g/100 g. The fibre content of the maize kernel is naturally lower than that of other cereals and therefore the fibre content of the resulting flour is as low as 2.6 g/100 g. Buckwheat is dehulled and milled into flour, containing mainly starchy endosperm and therefore fibre content in buckwheat flour is lower than in most other

flours (2.2 g/100 g). During production of white rice flour, hull and bran are removed from paddy rice. Therefore the resulting product contains only negligible amounts of fibre (0.4 g/100 g in this study). Dietary fibre is commonly fractionated into soluble and insoluble fibre; the latter being associated with intestinal regulation (increased stool weight and frequency and reduced intestinal transit time). Viscous soluble dietary fibre is linked to reduction of serum cholesterol levels and an attenuation of postprandial glycaemic response. The soluble fibre contents of the gluten free and wheat flours screened are shown in Table 7.1. White wheat flour, quinoa and maize as well as buckwheat are characterized by a high proportion of soluble fibre (39 %, 25 %, 24 % and 22 % of total dietary fibre).

7.4.2 Proteins

Amino acids, peptides and proteins are important constituents of food. Besides their nutritional significance, they contribute to flavour and texture of food. Wheat flour commonly used for bread making has a protein content of approximately 11 % (11.5 % in this study). In comparison teff and buckwheat flour showed higher protein contents (12.8 % and 12.2 %). With 13.5 %, quinoa flour showed the highest protein content. This pseudocereal is higher in protein and fat and lower in carbohydrates due to the proportional size of the embryo within the grain (up to 30 % of the grains gross weight, compared with 1 % for most cereals) (Wrigley *et al.* 2004). Sorghum and maize flour had the lowest protein contents of 4.7 % and 5.5 %. This value was also relatively low for rice and oat flour (7.3 % and 6.9 %). The wide variation in protein content is not only due to genetic factors, but also environmental effects. Protein is synthesized during the fruiting period, whereas starch synthesis starts later. If growing conditions in the late fruiting period are good, starch yield will be high but protein content will be relatively low (Lasztity 1996). Capillary electrophoresis was used to further investigate the proteins present in the flours of this study. Electropherograms were obtained for each extract and a representative gel is shown in Figure 7.2. Protein peaks below 7 kDa or peaks with an

average concentration lower than 20 ng/ll were not considered, since their significance is close to the detection limit of the instrument.
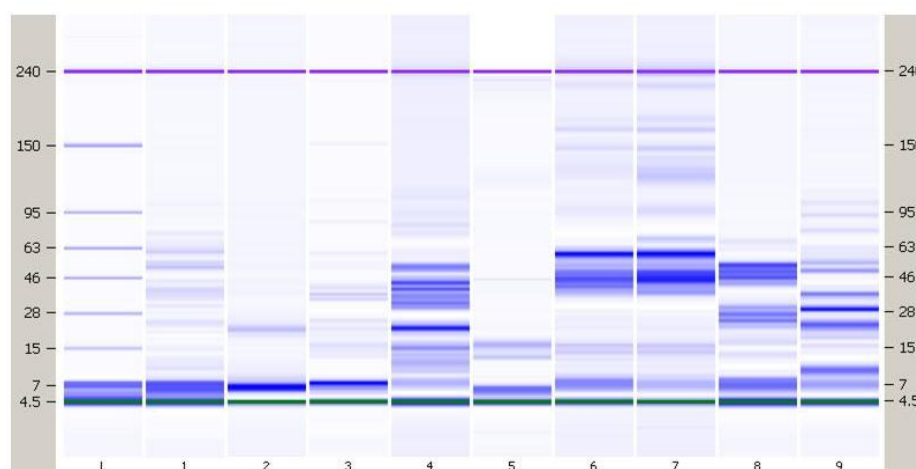
Figure 7.2 Gel view representative of banding patterns: (L) Ladder; (1) teff; (2) sorghum; (3) rice; (4) buckwheat; (5) maize; (6) wholewheat; (7) wheat; (8) oat; (9) quinoa

When comparing results of the current study to literature it has to be kept in mind, that the protein banding pattern is characteristic of species but also variety. However, in the course of this study commercial flours were evaluated which are often composed of a mixture of varieties. Extracts of wheat and wholemeal wheat flour produce several bands between 14 and 223 kDa. Protein peaks were found at 14, 16, 40, 45, 53 59, 97, 139, 147 and 170 kDa. The peptides triggering coeliac disease are contained in the prolamin fraction of wheat protein. Molecular weights of prolamins vary greatly from approximately 10 kDa to 100 kDa (Shewry and Halford 2002). Even though also panicoideae such as maize, sorghum and teff, contain significant amounts of prolamins, this group of storage proteins has separate evolutionary origins of those in triticeae (Shewry and Halford 2002). The 33-mer gluten peptide, responsible for the immune reaction in genetically susceptible persons, is absent in these grains and therefore they are not coeliac-toxic. The major group in maize prolamins are α-zeins, which result in bands at 19 kDa and 22 kDa on the electropherogram. As also shown by Moroni *et al*. (2010) for maize flour only three protein components in the 9-25 kDA range can be detected. The low molecular weight storage protein of sorghum, named kafirin, produced major bands

at 22 kDa (corresponding to α-kafirin) and at 19 kDa (corresponding to β-kafirin) (Lasztity 1996). In teff extracts major bands were present at 25, 40 and 61 kDa, possibly corresponding to the prolamin fraction (Tatham *et al*. 1996). Bands could also be observed at 15, 32, 37, 53 and 77 kDa. As previously observed, the protein fractions of teff are less complex than those of wheat, in terms of their apparent molecular size differences, and resemble more the pattern found in maize (Shewry and Tatham 1990). The major storage proteins in wheat, maize, sorghum and teff are prolamins. This is not the case for other plants such as oats and pseudocereals, where globulins are the major storage proteins; or rice, where glutelins are most abundant (Gorinstein *et al*. 2002). Oat prolamins (avenins) are similar to wheat gluten. However, due to different composition and amino acid sequence, oat might not belong to the grains harmful to coeliac disease patients (Vader *et al*. 2002). This has been supported by clinical observations (Janatuinen *et al*. 2002). The electropherogram of the extracted oat flour proteins showed bands at 14 and 17 kDa and several bands between 23 and 28 kDa as well as between 44 and 54 kDa and at 70 kDa. These findings compare well with those of Hüttner *et al*. (2010). Capillary electrophoreses of quinoa proteins resulted in major bands at 23 kDa and at 30 and 38 kDa. These bands are likely to represent the principal protein of quinoa, the chenopodina. Electropherograms of buckwheat protein shows bands at 14, 15, 22 and 53 kDa as well as between 32-44 kDa, corresponding to the albumin and globulin fractions. This banding pattern was also observed by Vallons *et al*. (2011). The extracted rice proteins result in a protein peak at 16 kDa and a minor one at 22 kDa, corresponding to the low molecular weight storage proteins (prolamins). The electropherogram shows proteins of molecular weights between 35 and 40 kDa, representing the α-glutelin subunits and between 19 and 25 kDa, representing β-glutelin subunits (Van der Borght *et al*. 2006).

7.4.3 Lipids

Although lipids comprise only about 1.5-7.0 % of cereal grains, they are of nutritive and physiological importance due to their role as energy supply and source of essential fatty acids.

Furthermore, they play a role in food quality as they may cause off-flavours in stored flours. Fat content was significantly different in the analysed samples, ranging from 0.9 % for rice flour up to 8.6 % for quinoa flour (Table 7.2). Oat, teff, buckwheat, wholewheat and sorghum flour had relatively higher fat contents (6.7 %, 4.4 %, 4.2 %, 3.6 % and 3.5 % respectively), as compared to wheat flour (1.8 %). The amount of fat in the pseudocereal quinoa is higher than in any other grain. However, the fat is characterised by a high content of nutritionally valuable unsaturated fatty acids, with linoleic acid accounting for 53 % of total fatty acids. The results were in agreement with literature (Schoenlechner *et al*. 2008). In the flours of this study, palmitic acid (C16:0) was the most abundant saturated fatty acid, being especially high in wheat, oat and rice (19.7%, 20.6% and 22.4%, respectively). Cereal lipids include a range of essential fatty acids such as linoleic and α-linolenic acid. In teff, sorghum and maize, quinoa and wholemeal wheat flour, linoleic acid is the most abundant fatty acid (Table 7.2). Oats contain a considerable amount of oleic acid (41.9 % w/w). Also in wheat, buckwheat and rice flour, oleic acid makes the highest proportion of the fatty acids (31.1 %, 36.5 % and 40.0 %, respectively). These findings are in accordance with Dewettinck *et al*. (2008). Also in buckwheat flour unsaturated fatty acids (oleic and linoleic) prevail (36.4 % and 33.0 %, respectively). The major fatty acids in sorghum flour are linoleic acid (49.3 %), oleic (30.4 %) and palmitic (13.5 %), making up over 90 % of the total fatty acids. These values compare well with literature (Wrigley *et al*. 2004). Sorghum oil is very similar to maize oil in quality and fatty acid content (Table 7.2). Wholewheat, buckwheat and quinoa flour show a high amount of α-linolenic acid (5.1 % w/w, 4.6 % w/w and 3.8 % w/w respectively). Buckwheat was found to be high in eicosenoic acid (3.3%). Comparing omega-6/omega-3 it can be seen that the pseudocereals quinoa and buckwheat have the most favourable ratio (11/1 and 9/1, respectively). Also wheat and rice flours have similar ratios (12/1 for wholemeal wheat, 14/1 for wheat and 15/1 for rice flour). The other flours have much higher ratios up to 37/1 for oat flour.

Table 7.1 Chemical composition of gluten free and wheat flours (values based on fresh weight of samples)*

	Wheat	Wholewheat	Rice	Oat	Quinoa	Buckwheat	Sorghum	Maize	Teff
Protein [g/100g]	11.54 ± 1.07^{c}	9.89 ± 0.17^{d}	7.33 ± 0.03^{e}	6.91 ± 0.08^{e}	13.48 ± 0.04^{a}	12.19 ± 0.38^{bc}	4.68 ± 0.04^{f}	5.50 ± 0.19^{f}	12.84 ± 0.51^{ab}
Total starch [g/100g]	68.06 ± 2.34^{b}	57.24 ± 0.26^{c}	77.52 ± 0.42^{a}	69.38 ± 1.66^{c}	48.88 ± 2.07^{d}	61.35 ± 2.15^{c}	73.20 ± 1.52^{a}	71.52 ± 0.42^{a}	57.77 ± 5.94^{c}
Amylose [% of total starch]	21.10 ± 1.29^{ab}	21.10 ± 2.08^{abc}	21.38 ± 0.90^{ab}	20.42 ± 2.43^{bc}	4.62 ± 0.83^{e}	15.95 ± 0.61^{d}	18.18 ± 0.55^{cd}	22.91 ± 0.82^{a}	19.72 ± 099^{bc}
Damaged Starch [g/100g]	7.85 ± 0.41^{b}	4.06 ± 0.68^{c}	15.24 ± 1.53^{a}	4.91 ± 0.06^{c}	4.71 ± 0.70^{c}	2.63 ± 0.25^{d}	4.66 ± 1.03^{c}	4.52 ± 0.30^{c}	2.08 ± 0.22^{d}
Total dietary fibre [g/100g]	3.44 ± 0.01^{cd}	11.42 ± 1.27^{a}	0.43 ± 0.15^{f}	4.05 ± 0.40^{c}	7.14 ± 0.23^{b}	2.18 ± 0.11^{e}	4.51 ± 0.01^{c}	2.62 ± 0.45^{de}	4.54 ± 0.57^{c}
Soluble dietary fibre [g/100g]	1.34 ± 0.11^{a}	1.60 ± 0.40^{a}	0.14 ± 0.06^{d}	0.36 ± 0.02^{cd}	1.77 ± 0.14^{a}	0.48 ± 0.17^{cd}	0.72 ± 0.04^{bc}	0.64 ± 0.14^{bd}	0.85 ± 0.17^{b}
Phytate [g/100g]	0.16 ± 0.03^{e}	0.77 ± 0.01^{b}	0.21 ± 0.01^{e}	0.27 ± 0.01^{de}	1.34 ± 0.00^{a}	0.64 ± 0.06^{bc}	0.49 ± 0.02^{cd}	0.09 ± 0.03^{e}	1.52 ± 0.21^{a}
Polyphenols [mg/100g]	13.04 ± 0.23^{d}	82.20 ± 0.42^{c}	14.16 ± 2.45^{d}	22.16 ± 0.16^{d}	78.24 ± 0.46^{c}	465.47 ± 22.41^{a}	103.30 ± 6.06^{c}	97.85 ± 0.64^{c}	175.65 ± 1.48^{b}
Calories [kcal/100g]	361	366	359	393	385	368	376	362	380

*Values followed by the same letter in the same row are not significantly different ($p<0.05$)

Table 7.2 Fat content [% (w/w) based on fresh weight] and fatty acid profile of flour samples [% (w/w) of total lipids]*

	Wheat	Wholewheat	Rice	Oat	Quinoa	Buckwheat	Sorghum	Maize	Teff
Fat	1.81 ± 0.05[d]	3.63 ± 0.104[c]	0.90 ± 0.06[e]	6.74 ± 0.80[b]	8.59 ± 0.25[a]	4.21 ± 0.74[c]	3.50 ± 0.31[c]	2.48 ± 0.46[d]	4.39 ± 0.26[c]
Myristic 14:0	1.48 ± 0.014[a]	0.10 ± 0.000[f]	0.44 ± 0.002[b]	0.24 ± 0.001[c]	0.12 ± 0.000[e]	0.11 ± 0.000[e]	0	0	0.22 ± 0.003[d]
Palmitic 16:0	19.74 ± 0.076[c]	16.97 ± 0.011[d]	22.43 ± 0.014[a]	20.62 ± 0.001[b]	9.77 ± 0.004[i]	15.78 ± 0.03[e]	13.52 ± 0.21[f]	12.62 ± 0.01[g]	10.86 ± 0.042[h]
Stearic 18:0	10.41 ± 0.094[a]	0.75 ± 0.000[g]	2.45 ± 0.012[c]	1.71 ± 0.007[e]	0.63 ± 0.004[h]	2.08 ± 0.001[d]	1.28 ± 0.00[f]	2.07 ± 0.00[d]	4.14 ± 0.031[b]
Oleic 18:1, 9c	31.14 ± 0.006[d]	12.73 ± 0.007[i]	40.01 ± 0.019[b]	41.85 ± 0.004[a]	23.93 ± 0.004[h]	36.53 ± 0.012[c]	30.40 ± 0.05[e]	26.08 ± 0.01[g]	29.47 ± 0.320[f]
Linoleic 18:2 9, 12	23.74 ± 0.034[i]	60.79 ± 0.020[a]	29.38 ± 0.003[g]	26.56 ± 0.011[h]	52.68 ± 0.012[c]	33.01 ± 0.010[f]	49.31 ± 0.13[e]	54.73 ± 0.01[b]	49.99 ± 0.183[d]
α-Linolenic 18:3 9, 12, 15	1.74 ± 0.004[h]	5.04 ± 0.002[a]	1.91 ± 0.009[g]	0.71 ± 0.014[i]	4.60 ± 0.001[b]	3.78 ± 0.005[c]	2.22 ± 0.01[e]	2.08 ± 0.00[f]	2.29 ± 0.072[d]
Eicosenoic 20:1 11	1.61 ± 0.016[b]	0.72 ± 0.001[f]	0.53 ± 0.007[g]	1.06 ± 0.001[d]	1.56 ± 0.001[c]	3.27 ± 0.007[a]	0.32 ± 0.01[h]	0.26 ± 0.00[i]	0.78 ± 0.017[e]
Saturated fatty acids	38.94 ± 0.038[a]	18.97 ± 0.004[e]	26.35 ± 0.035[b]	23.42 ± 0.001[c]	11.56 ± 0.005[h]	21.43 ± 0.021[d]	15.19 ± 0.21[g]	15.21 ± 0.00[g]	16.14 ± 0.075f
Unsaturated fatty acids	60.06 ± 0.044[g]	80.72 ± 0.001[d]	73.25 ± 0.038[e]	71.78 ± 0.005[f]	85.44 ± 0.003[a]	60.06 ± 0.044[g]	83.81 ± 0.21[c]	84.29 ± 0.00[b]	83.66 ± 0.075[c]
ω6/ω3	14/1	12/1	15/1	37/1	11/1	9/1	22/1	26/1	21/1

*Values followed by the same letter in the same row are not significantly different ($p < 0.05$)

7.4.4 Folate and Minerals

Folate, an essential component in the human diet, is involved as a cofactor in metabolic reactions (e.g. the biosynthesis of nucleotides, the building blocks of DNA and RNA) and plays a critical role in the prevention of neural tube defects. Determination of folate content of the different flours showed large variations between the samples. Wheat and wholemeal wheat flour as well as rice and oat flour contained low levels: 18, 34, 33, and 30 µg/100 g, respectively. Sorghum (77 µg/100 g), maize (37 µg/100 g) and teff (96 µg/100 g) contain notably higher levels. The pseudocereals quinoa and buckwheat contain the highest amounts of folate among the flours screened: 180 µg/100 g and 132 µg/100 g, respectively. Results for wheat and quinoa flour compare well with those of (Schoenlechner *et al*. 2010), but folate content of buckwheat flour was lower in the study of these authors (25 µg/100 g dwb).

Minerals are important for various physiological functions in the human body. Per day, more than 100 mg of the major minerals (Na, Mg, K, Ca, P, and Cl) and less than 100 mg of trace elements (Fe, Cu, Zn) are required (Insel, 2004). Table 7.3 shows the ash content of the flours as well as the mineral composition. In this study ash content ranged from 0.4 g/100 g (maize flour) to 2.4 g/100 g (quinoa flour). Phosporus contents were high with concentrations up to 441.6 mg/100 g (quinoa flour). Only white wheat flour had lower phosphorus content (10 % of total ash). The majority of the phosphorus in cereals occurs as phytic acid, an inositol hexaphosphoric acid. Potassium and sodium, two elements of concern with regard to health care, were also detected in the flours screened. Potassium contents were high, ranging from 97.4 mg/100 g (rice flour) to 553.8 mg/100 g (quinoa flour). However, cereal flours are not considered a high or even moderate source of sodium. Contents in this study were between 0.5 mg/100 g for sorghum and maize flour and about 3.7 mg/100 g for wheat and quinoa flour, contributing to less than 1% of the dietary reference amount (Table 7.4). Ash content of quinoa flour is significantly higher than in the other samples and its composition is superior to most cereals, with potassium, phosphorous, magnesium and calcium prevailing (553.8, 441.6,

229.9 and 49.8 mg/100 g). Additionally quinoa flour is high in iron and zinc (5.4 and 3.3 mg/100 g). Teff flour can be considered a good source of calcium (154.3 mg/100 g). The high amount of calcium in white wheat flour of this study (179.8 mg/100 g) compared to literature (17 mg/100 g, Wrigley *et al.* 2004) is due to the fact that calcium carbonate is added to this product (personal communication with producer). Magnesium levels are relatively high in buckwheat and teff flour (173.6 mg/100g and 169.0 mg/100g). Wheat is known to be a source of iron with contents ranging from 1 mg/100g to 5 mg/100g (1.3 mg/100g flour in this study) (Dewettinck *et al.* 2008). This study however showed that flours made from buckwheat, quinoa or teff are even higher in their iron content (2.9 mg/100g, 5.4 mg/100g and 8.5 mg/100g, respectively). It is well known from literature that wheat is a good source of zinc (1-5 mg/100g) and copper (0.1-1 mg/100g) (Dewettinck *et al.* 2008). Wholemeal wheat flour of this study contained 1.8 mg/100g of zinc, while this value was lower for refined wheat flour (0.8 mg/100g). However, also the other cereal flours analyzed, apart from maize flour, contained comparable or even higher levels of zinc and copper (Table 7.3). Buckwheat is a richer mineral source (except for calcium) than many cereals such as rice, sorghum and maize, with high levels of magnesium (173.6 mg/100g), zinc (1.88 mg/100g), potassium (402.3 mg/100g) and copper (0.51 mg/100g).

Table 7.3 Ash content [% (w/w) based on fresh weight] and mineral composition of flours [mg/kg]*

	Wheat	Wholewheat	Rice	Oat	Quinoa	Buckwheat	Sorghum	Maize	Teff
Ash [% w/w]	0.92 ± 0.02[f]	1.32 ± 0.01[d]	0.51 ± 0.01[h]	0.82 ± 0.01[g]	2.43 ± 0.03[a]	1.65 ± 0.01[c]	0.97 ± 0.01[e]	0.37 ± 0.03[i]	2.15 ± 0.05[b]
Calcium [mg/kg]	1797.7 ± 10.5[a]	307.7 ± 4.5[d]	50.7 ± 0.5[h]	224.3 ± 2.1[e]	497.3 ± 1.2[c]	148.2 ± 1.7[f]	97.6 ± 1.4[g]	33.2 ± 1.1[i]	1543.0 ± 2.0[b]
Magnesium [mg/kg]	244.0 ± 1.0[i]	782.7 ± 2.5[e]	338.0 ± 4.0[g]	392.7 ± 4.5[f]	2299.0 ± 17.0[a]	1736.0 ± 13.0[b]	849.7 ± 8.5[d]	315.7 ± 1.5[h]	1689.7 ± 14.5[c]
Sodium [mg/kg]	38.1 ± 3.7[b]	19.9 ± 0.2[c]	15.1 ± 0.3[d]	16.7 ± 3.5[cd]	37.0 ± 0.2[b]	10.8 ± 2.6[e]	4.9 ± 0.1[f]	4.8 ± 0.3[f]	59.8 ± 2.1[a]
Potassium [mg/kg]	1520.3 ± 8.5[f]	3997.7 ± 29.5[b]	973.7 ± 4.5[h]	1743.7 ± 16.5[e]	5537.7 ± 23.5[a]	4022.7 ± 24.5[b]	2581.0 ± 5.0[d]	1487.0 ± 7.0[g]	3827.7 ± 4.5[c]
Iron [mg/kg]	13.4 ± 0.1[f]	26.9 ± 0.2[d]	6.0 ± 0.3[h]	16.4 ± 0.1[e]	53.5 ± 0.4[b]	28.5 ± 0.0[c]	26.7 ± 0.2[d]	9.1 ± 0.1[g]	85.3 ± 2.0[a]
Copper [mg/kg]	1.51 ± 0.0[h]	4.0 ± 0.0[d]	2.2 ± 0.0[f]	2.7 ± 0.1[e]	7.7 ± 0.1[b]	5.1 ± 0.0[c]	1.8 ± 0.0[g]	0.9 ± 0.0[i]	9.3 ± 0.1[a]
Manganese [mg/kg]	8.3 ± 0.1[g]	23.4 ± 0.1[c]	7.3 ± 0.02[h]	27.7 ± 0.4[b]	13.5 ± 0.0[d]	11.8 ± 0.0[e]	11.3 ± 0.1[f]	1.5 ± 0.0[i]	34.5 ± 0.4[a]
Zinc [mg/kg]	7.59 ± 0.00[h]	17.5 ± 0.4[e]	17.8 ± 0.2[d]	11.3 ± 0.1[f]	32.8 ± 0.1[b]	18.8 ± 0.0[c]	10.0 ± 0.0[g]	6.6 ± 0.1[i]	41.5 ± 0.1[a]
Chloride [mg/kg]	825.6 ± 42.6[b]	998.0 ± 23.7[a]	350.6 ± 31.5[g]	670.0 ± 48.5[c]	433.8 ± 0.3[e]	144.0 ± 31.0[h]	402.8 ± 25.36[f]	484.2 ± 23.1[d]	481.0 ± 39.1[d]
Phosphorus [mg/kg]	908.7 ± 3.5[h]	2040.7 ± 3.5[d]	954.7 ± 16.5[g]	1476 ± 25[f]	4415.7 ± 57.5[a]	2787.0 ± 14.0[c]	1631.0 ± 13.0[e]	813.7 ± 8.5[i]	3617.0 ± 11.0[b]

*Values followed by the same letter in the same row are not significantly different (p<0.05)

7.4.5 Phytate and polyphenols

Cereal grains being an important source of minerals also contain phytic acid. Phytate is considered to be an anti-nutritional factor as it has a high chelating activity, which may decrease the bioavailability of certain elements such as calcium, magnesium, iron and zinc. Phytate also adversely affects the absorption of other nutrients such as amino acids, proteins and starch. In this study, teff and quinoa flour contained high amounts of phytate (1.52 g/100g and 1.34 g/100g), followed by wholewheat, buckwheat and sorghum (0.77 g/100g, 0.64 g/100g and 0.49 g/100g). White wheat flour, rice, oat and maize flour showed low phytate concentrations (Table 7.1). These results compare well with literature (Garcia-Estepa *et al.* 1999), apart from maize where a much lower level of phytate was detected in this study.

Polyphenols are a heterogeneous group of molecules (large multiples of phenol structural units) produced as secondary plant metabolites which affect nutritional and sensory properties. The total phenol content (gallic acid equivalents) is shown in Table 7.1. Among the different flours this value was significantly higher in buckwheat (465.47 mg/100g) and teff (175.65 mg/100g) and decreased in the following order buckwheat > teff > sorghum > maize > wholewheat > quinoa. Wheat, rice and oat flour showed significantly lower values.

7.4.6 Energy content

Looking at the calculated calorie content of the different flours (Table 7.1), oat had the highest value of 393 kcal/100g, followed by quinoa, teff and sorghum (385, 380 and 376 kcal/100g). Rice flour showed the lowest calorie content of only 359 kcal/100g. The other flours had calorie contents in the range of 368 kcal/100g (buckwheat) to 361 kcal/100g (wheat).

7.5 Discussion

According to the Codex Alimentarius (2008) gluten free products that substitute important basic foods (e.g. flour, bread, pasta), should supply approximately the same amount of vitamins and minerals as the original food they replace. The energy and nutrient content of gluten free products require attention as the substitution of food with gluten free alternatives may result in inadequate intakes of important nutrients. As expected in a malabsorptive condition like coeliac disease, nutritional deficiencies occurr frequently. Weight-loss, osteoporosis and iron-deficiency anaemia are common. Also deficiencies of several minerals, such as calcium, magnesium, zinc, copper and selenium have been reported. These result from malabsorption, increased requirement and/or a lower intake due to the gluten free diet (Kennedy and Feighery 2000). In the course of this study, seven commercially available gluten free flours have been analysed and their nutritional value compared to that of wheat flours.

Table 7.4 summarises the Dietary Reference Intakes of selected nutrients set by the United States Department of Agriculture (National Nutrient Database for Standard Reference) and shows the percentage contribution of 100g of each of the flours. One of the most noticeable differences in contribution between the different breads was the protein content of gluten free flours. By consuming 100g of wheat flour, 21% (for men) and 25% (for women) of the daily required amount of protein is reached. Most gluten free flours contribute less to the protein content than wheat flours do. Intakes would be significantly higher if quinoa, buckwheat or teff were used for the production of gluten free breads instead of rice, maize or sorghum. With respect to proteins, it is important to not only consider the amount but also the quality. For instance protein found in oats is known to be superior to that of wheat, due to higher lysine contents, a limiting amino acid in cereals (Lasztity 1996). Protein quality of maize and sorghum is meagre; the flours contain low amounts of total protein and the protein efficiency ratio is low (Nuss *et al*. 2010). Pseudocereals are known for their high lysine contents and quinoa and

buckwheat are also superior regarding protein digestibility, net protein utilisation and protein efficiency ratio (Saturni 2010).

Low bone mineral density (osteopenia, osteomalacia and osteoporosis) in children and adults with coeliac disease has been described. Osteopenia is reversible in time with a gluten free diet (Kennedy and Feighery 2000). However, the study of Pazianas *et al*. (2005) provided evidence, that even after prolonged gluten withdrawal, calcium absorption remains impaired. Therefore a higher calcium intake by coeliac patients is necessary. From this point of view teff flour is interesting for the production of gluten free products. It contains over 30 times more calcium than rice and over 47 times more than maize flour. Hundred gram contribute to 15% of the daily recommended intake. The calcium content of quinoa is also higher than in other gluten free raw materials. The mechanism of bone loss in coeliac disease is considered multifactorial and has also been attributed to trace element and magnesium deficiencies (Sategna-Guidetti *et al*. 2000). Ohlund *et al*. (2010) showed a too low magnesium intake in children on a gluten free diet. Incorporation of quinoa, buckwheat and teff flour would have a positive influence on magnesium levels. Already 100 g of these flours supply the human body with over 40% of the daily required amount of magnesium (Table 7.4). Adoption of a gluten free diet may also reduce the intake of iron (Mariani *et al*. 1998). Thompson (2000) could show that gluten free products often contain lower amounts of iron than their gluten-containing counterparts. Sorghum, rice and maize flour are deficient in iron. On the contrary, teff, quinoa and buckwheat flour show a high iron level. Their incorporation into gluten free products would therefore be beneficial. The consumption of 100g quinoa flour contributes 67% of the male and 30 % of the female daily recommended allowance. Teff flour is outstanding, with 100g providing 107% and 47% (male and female, respectively) of the daily required iron (Table 7.4). However, it has to be kept in mind that despite quinoa and teff showing a favourable mineral composition; their high phytic acid levels are considered an antinutritional factor, since this compound binds minerals such as calcium, iron, magnesium, manganese and zinc.

This is disadvantageous especially for coeliac patients who often suffer from micronutrient deficiencies. Phytic acid can be enzymatically degraded by phytase (myo-inositol hexakisphosphate phosphohydrolase), improving the nutritional value of the product. It was previously shown that cereals show endogenous phytase activity, which is especially high in wheat and buckwheat (Egli *et al*. 2002). Under conventional processing conditions such as pasta or bread making, optimal conditions for the degradation of phytate are rarely reached. However, sourdough fermentation provides optimum pH conditions for phytase (Zannini *et al*. 2011).

Dietary fibre is another highly important nutrient, which has been repeatedly shown not to be consumed in sufficient amounts among coeliac disease patients as well as the general population (Hager *et al*. 2011). Compared to refined wheat flour, oat, quinoa, sorghum and teff flour show higher fibre contents. However, none of the flours screened contain fibre amounts comparable to wholemeal wheat flour. The most commonly used raw materials for gluten free products, show very low fibre levels. Hence, a large number of gluten free products are fibre enriched. The consumption of 100g rice flour provides only 1-2% of the dietary reference intake (Table 7.4).

Fatty acids of specific chain length and saturation are required by humans for structural and metabolic needs. Linoleic and α-linolenic acid are two essential fatty acids, which can not be synthesized by the body but have to be taken up as part of the daily diet. All studied cereals are a good source of linoleic acid. Quinoa is relatively higher in α-linolenic acid, contributing to 26% and 37% (male and female) of the dietary reference intake. Ohlund *et al*. (2010) could show that children on a gluten free diet frequently have a too high intake of saturated fatty acids and a too low intake of polyunsaturated fatty acids. This trend can also be observed in the general population. The characterization of the fatty acid profiles show that in all cereal flours the majority of the lipids are unsaturated.

As antioxidants, polyphenols may protect cell constituents against oxidative damage and may limit the risk of various degenerative diseases associated with oxidative stress. Therefore much interest in the polyphenol composition of foods has been raised over the past decade (Alvarez-Jubete *et al*. 2010). Compared to all other cereals screened, buckwheat flour showed the highest polyphenols content, followed by teff flour.

In view of evidence linking low folate intake with neural tube defects in the foetus, health authorities recommend that women, who could become pregnant, should increase their dietary folic acid intake. In several countries, including the US and Canada, fortification of wheat flour is mandatory. However, no recommendations or regulations exist on the fortification of gluten free cereal products, even though people suffering from coeliac disease show an inflamed small intestine, leading to malabsorption of folate (Kennedy and Feighery 2000; Murray 1999). Generally, levels in gluten free products are much lower than those in their gluten containing counterparts (Thompson 2000; Yazynina *et al*. 2008). Therefore the use of nutrient-dense ingredients is important to improve the nutritional quality of gluten free bread. The results of this study show significant variations in folate levels among the gluten free raw materials screened. Folate levels in rice and oat flours are similarly low as in wheat flour. Maize, sorghum and teff flour contain higher amounts. The highest folate contents were detected in the flours of the pseudocereals quinoa and buckwheat. Consumption of 100 g of these flours contributes to 45% and 33% of the recommended daily folate intake.

Several studies could show that overweight and obesity are problems often connected with a strict gluten free diet (Castelluzzo *et al*. 2011; Mariani *et al*. 1998). Moreover, once coeliac disease has been diagnosed, regeneration of the intestinal mucosa and normalisation of absorptive processes often result in weight gain (Castelluzzo *et al*. 2011). This can be partly related to the fact that gluten free bread products often contain higher amounts of calories than wheat breads of the same category (Hager *et al*. 2011). Therefore the use of raw

materials with low energy content such as buckwheat, teff and quinoa flour are recommended. Oat and rice flour provide the most calories. This is due to the high starch level of rice and the high fat content of oat flour.

Table 7.4 Dietary Reference Intakes (DRIs) for the female and male adult general population and the contribution of 100 g flour to the DRIs. Recommended Dietary Allowances (RDAs) are presented in ordinary type, Adequate Intakes (AIs) are followed by an asterisk (*)

Nutrient	Gender	DRIs[a]		Contributions of 100 g flour to DRIs (%)								
				Wheat	Wholewheat	Rice	Oat	Quinoa	Buckwheat	Sorghum	Maize	Teff
Protein	Male	56	g/d	21	18	13	12	24	22	8	10	23
	Female	46	g/d	25	22	16	15	29	27	10	12	28
Linoleic Acid	Male	17*	g/d	3	13	2	11	26	8	10	8	13
	Female	12*	g/d	4	18	2	15	37	12	14	11	18
α-Linolenic Acid	Male	1.6*	g/d	2	11	1	3	24	10	5	3	6
	Female	1.1*	g/d	3	17	2	4	36	15	7	5	9
Dietary Fibre	Male	38*	g/d	9	30	1	11	19	6	12	7	12
	Female	25*	g/d	14	46	2	16	29	9	18	10	18
Folate	Male/ Female	400	µg/d	5	9	8	7	45	33	19	9	24
Calcium	Male/ Female	1000	mg/d	18	3	1	2	5	1	1	0	15
Phosphorus	Male/ Female	700	mg/d	13	29	14	21	63	40	23	12	52
Sodium	Male/ Female	1.5*	g/d	<1	<1	<1	<1	<1	<1	<1	<1	<1
Potassium	Male/ Female	4.7*	g/d	3	9	2	4	12	9	5	3	8
Chloride	Male/ Female	2.3*	g/d	4	4	2	3	2	1	2	2	2
Copper	Male/ Female	900	µg/d	17	44	24	30	86	57	20	10	103

Magnesium	Male	420	mg/d	6	19	8	9	55	41	20	8	40
	Female	320	mg/d	8	24	11	12	72	54	27	10	53
Zinc	Male	11	mg/d	7	16	16	10	30	17	9	6	38
	Female	8	mg/d	10	22	22	14	41	24	13	8	52
Iron	Male	8	mg/d	17	34	8	21	67	36	3	11	107
	Female	18	mg/d	7	15	3	9	30	16	2	5	47

[a] Source: USDA Dietary Reference Intakes

7.6 Conclusion

Looking at gluten free products currently on the market, undoubtedly the most commonly used ingredient is rice flour. However, considering its nutritional value, it is inferior to many other gluten free flours. Although being an economical ingredient, rice flour lacks important nutrients. This study shows that inclusion of alternative grains provide cereal products of higher nutritional value. Even though most gluten free products are based on rice or maize flour, other flours especially teff or the pseudocereals quinoa and buckwheat present a higher nutritional value. Several publications showed that many gluten free foods lack dietary fibre, micronutrients and protein. As an alternative to enrichment, a more natural way of achieving nutritionally more balanced products is the use of carefully selected raw materials. The use of nutrient-dense flours is a way to improve the nutritional quality of gluten free products. Regarding its nutritional value, quinoa flour is outstanding and can therefore be used alone or in combination with other cereal flours to improve quality of gluten free products. However, it has to be kept in mind that this study only focuses on the chemical characterisation of the different flours. The use of the pseudocereals quinoa and buckwheat, although nutritionally superior to cereal grains, may be limited due to technological or sensory properties. These were subject to further research and are evaluated in a later publication (Hager *et al*. 2012).

7.7 Acknowledgements

7.8 References

AACC International (1995) Approved Methods of Analysis, 9th Ed. Method 30-10 Crude Fat. In: Flour, Bread, and Baked Cereal Products Not Containing Fruit. AACC International, St. Paul, MN, U.S.A.

AACC International (1995) Method 46-12 Crude Protein—Kjeldahl Method, Boric Acid Modification. In: Approved Methods of Analysis, 9th Ed. AACC International, St. Paul, MN, U.S.A.

AACC International (1995) Method 44-15A Moisture—Air-Oven Methods. In: Approved Methods of Analysis, 9th Ed. AACC International, St. Paul, MN, U.S.A.

Akerberg A, Liljeber H. and Björk I. (1998) Effects of Amylose/Amylopectin Ratio and Baking Conditions on Resistant Starch Formation and Glycaemic Indices. Journal of Cereal Science, 28, 71-80

Alvarez-Jubete L, Wijngaard H, Arendt E.K, Gallagher E. (2010). Polyphenol composition and *in vitro* antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. Food Chemistry, 119(2), 770-778

Analysenkommision (1996). MEBAK Brautechnische Analysenmethoden III. Selbstverlag der Mebak, Freising-Weihenstephan.

AOAC International (2006) Microbiological Methods. http://www.aoac.org/omarev1/944_12.pdf ; accessed June 7th, 2013

Castelluzzo M.A, Massoud M, Valitutti F, Tambucci R, Rea F, Di Iorio L, Polito A, Mino G, Cotugno N, Paone F.M. (2011) Coexisting Celiac Disease and Overweight/Obesity in an Italian Pediatric Population. Gastroenterology, 140(5), S685-S685

Commission of the European Communities (2009) Commission Regulation (EC) No 41/2009 concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten.

Delcour J.A. and Hoseney R.C. (2010) Principles of Cereal Science and Technology, AACC International Press, St.Paul, Minnesota

Deutsche Gesellschaft für Fettwissenschaften (1981) Deutsche Einheitsmethoden zur Untersuchung von Fetten, Fettprodukten, Tensiden und verwandten Stoffen. Method DGF C-VI 11e. e.V. Munster; Wissenschaftliche Verlagsgesellschaft mbH: Stuttgart, Germany

Dewettinck K, Vanbockstaele F, Kuhne B, Vandewalle D, Courtens T, Gellynck X. (2008) Nutritional value of bread: Influence of processing, food interaction and consumer perception. Journal of Cereal Science, 48, 243-257

Egli I, Davidsson L, Juillerat M.A, Barclay D, Hurrell R.F, (2002) The influence of Soaking and Germination on the Phytase activity and Phytic Acid Content of Grains and Seeds Potentially Useful for Complementary Feeding. Journal of Food Science, 67(9), 3484-3488

Dubois M. (1949) Incidences du réglage de la mouture sur les propriétés des farines. Bulletin ENSMIC, 113, 170-187

Garcia-Estepa R. M, Guerra-Hernandez E, Garcia-Villanova B. (1999) Phytic acid content in milled cereal products and breads. Food Research International, 32(3), 217-221

Gorinstein S, Pawelzik E, Delgado-Licon E, Haruenkit R, Weisz M, Trakhtenberg S. (2002) Characterisation of pseudocereal and cereal proteins by protein and amino acid analyses. Journal of the Science of Food and Agriculture, 82(8), 886-891

Granfeldt Y, Drews A, Björck I. (1995) Arepas made from high amylose corn flour produce favorably low glucose and insulin responses in healthy humans. Journal of Nutrition 125, 459-465

Hager A.-S, Axel C, Arendt E.K. (2011) Status of Carbohydrates and Dietary Fiber in Gluten-free Diets. Cereal Foods World, 56(3), 109-114

Hager A.-S., Wolter A, Czerny M, Bez J, Zannini E, Arendt E.K, Czerny M. (2012) Investigation of product quality, sensory profile and ultrastructure of breads made from a range of commercial gluten-free flours compared to their wheat counterparts. European Food Research and Technology, 235(2), 333-344

Hüttner E.K, Dal Bello F, Arendt E.K. (2010) Rheological properties and bread making performance of commercial wholegrain oat flours. Journal of Cereal Science, 52, 65-71

Insel P, Turner R.E. and Ross D. (2004) Water and major minerals. In: Nutrition. Jones and Bartlett, Sudbury, Massachusetts

International Organization for Standardization (1998) Method EN ISO 11885 E22, Geneva, Switzerland

Janatuinen E.K, Kemppainen T.A, Julkunen R.J.K, Kosma V.M, Maki M, Heikkinen M, Uusitupa M.I.J. (2002) No harm from five year ingestion of oats in coeliac disease. Gut, 50(3), 332-335

Kennedy N. P. and Feighery C. (2000) Clinical features of coeliac disease today. Biomedicine & Pharmacotherapy, 54(7), 373-380

Kulp K., Ponte J.G. (1981) Staling of white pan bread – fundamental causes. Critical Reviews in Food Science and Nutrition, 15(1), 1-48

Lasztity R. (1996) The Chemistry of Cereal Proteins. CRC Press, Boca Raton, Florida

Maningat C.C. and Seib A.P. (2010) Understanding the Physicochemical and Functional Properties of Wheat Starch in Various Foods. Cereal Chemistry, 87(4), 305-314

Mariani P., Viti M.G., Montouri M., La Vecchia A., Cipolletta E., Calvani L., Bonamico M. (1998) The Gluten-Free Diet: A Nutritional Risk Factor for Adolescents with Celiac Disease? Journal of Pediatric Gastroenterology and Nutrition, 27, 519-523

Matissek R. and Steiner G. (2006) Bestimmung des Gluehrueckstandes durch direkte Veraschung (Aschegehalt). In: Lebensmittelanalytik –Grundzuege, Methoden, Anwendungen. Springer-Verlag, Berlin, Germany

Moroni A.V, Iametti S, Bonomi F, Arendt E.K, Dal Bello F, (2010) Solubility of proteins from non-gluten cereals: A comparative study on combinations of solubilising agents. Food Chemistry, 121(4), 1225-1230

Murray J.A. (1999) The widening spectrum of coeliac disease. American Journal of Clinical Nutrition, 69, 354-365

Nuss E.T. and Tanumihardjo S.A. (2010) Maize: A Paramount Staple Crop in the Context of Global Nutrition. Comprehensive Reviews in Food Science and Food Safety, 9(4), 417-436

Ohlund K, Olsson C, Hernell O, Ohlund I., (2010) Dietary shortcomings in children on a gluten-free diet. Journal of Human Nutrition and Dietetics, 23(3), 294-300

Pazianas M, Pazianas M, Butcher G.P, Subhani J.M, Finch P.J, Ang L, Collins C, Heaney R.P, Zaidi M, Maxwell J.D, (2005) Calcium absorption and bone mineral density in celiacs after long term treatment with gluten-free diet and adequate calcium intake. Osteoporosis International, 16(1), 56-63

Rai K.N, Gowda C.L.L, Reddy B.V.S, Seghal S. (2008) Adaptation and potential uses of sorghum and pearl millet in alternative and health foods. Comprehensive Reviews in Food Science and Food Safety, 7(4), 340-352

Sategna-Guidetti C, Grosso S.B, Sategna-Guidetti C, Grosso S.B, Grosso S, Mengozzi G, Aimo G, Zaccaria T, Di Stefano M, Isaia G.C. (2000) The effects of 1-year gluten withdrawal on bone mass, bone metabolism and nutritional status in newly-diagnosed adult coeliac disease patients. Alimentary Pharmacology & Therapeutics, 14(1), 35-43

Saturni L, Ferretti G, Bacchetti T, (2010) The Gluten-Free Diet: Safety and Nutritional Quality. Nutrients, 2, 16-34

Schakel S.F, Jasthi B, Van Heel N, Harnack L. (2009) Adjusting a nutrient database to improve calculation of percent calories from macronutrients. Journal of Food Composition and Analysis 22, S32-S36

Schoenlechner R, Wendner M, Siebenhandl-Ehn S, Berghofer E. (2010) Pseudocereals as alternative sources for high folate content in staple foods. Journal of Cereal Science 52(3), 475-479

Schoenlechner R, Siebenhandl-Ehn S, Berghofer E. (2008) Pseudocereals. In: Arendt E.K., Dal Bello F. (Eds.), Gluten-free Cereal Products and Beverages. Academic Press, Burlington, Massachusetts, pp. 149-176

Shewry P.R. (2009) Wheat. Journal of Experimental Botany, 60, 1537-1553

Shewry P.R. and Halford N.G. (2002) Cereal seed storage proteins: structures, properties and role in grain utilization. Journal of Experimental Botany, 53(370), 947-958

Shewry P.R. and Tatham A.S. (1990) The prolamin storage proteins of cereal seeds – structure and evolution. Biochemical Journal, 267(1), 1-12

Srichuwong S, Sunarti T.C, Mishima T, Isono N, Hisamatsu M. (2005) Starches from different botanical sources I: Contribution of amylopectin fine structure to thermal properties and enzyme digestibility. Carbohydrate Polymers, 60, 529-538

Tatham A.S, Fido R.J, Moore C.M, Kasarda D.D, Kuzmicky D.D, Keen J.N, Shewry P.R. (1996) Characterisation of the major prolamins of tef (Eragrostis tef) and finger millet (Eleusine coracana). Journal of Cereal Science, 24(1), 65-71

Thompson T. (2000) Folate, Iron, and Dietary Fiber Contents of the Gluten-free Diet. Journal of the American Dietetic Association, 100(11), 1389-1396

Vader L.W, De Ru A, van der Wal Y, Kooy Y.M.C, Benckhuijsen W, Mearin M.L, Drijfhout J.W, van Veelen P, Koning F. (2002) Specificity of tissue transglutaminase explains cereal toxicity in celiac disease. Journal of Experimental Medicine, 195(5), 643-649

Vallons K.J.R, Ryan L.A.M, Arendt E.K. (2011) Promoting structure formation by high pressure in gluten-free flours. Lwt-Food Science and Technology, 44(7), 1672-1680

Van der Borght A, Vandeputte G.E, Derycke V, Brijs K, Daenen G, Delcour J.A. (2006) Extractability and chromatographic separation of rice endosperm proteins. Journal of Cereal Science, 44(1), 68-74

Wijngaard H.H. and Arendt E. K. (2006) Buckwheat. Cereal Chemistry, 83(4), 391-401

Wrigley C, Corke H, Walker C.E. (2004) Encyclopedia of grain science, Elsevier Academic Press, Waltham, Massachusetts

Yazynina E, Johansson M, Jagerstad M, Jastrebova J. (2008) Low folate content in gluten-free cereal products and their main ingredients. Food Chemistry, 111(1), 236-242

Zannini E, Pontonio E, Waters D.M, Arendt E.K. (2011) Applications of microbial fermentations for production of gluten-free products and perspectives. Applied Microbiology and Biotechnology, 93 (2), 473-485

Chapter 8: Investigation of product quality, sensory profile and ultra-structure of breads made from a range of commercial gluten free flours compared to their wheat counterparts

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8.1 Summary

Bread is a major staple food consumed daily in all parts of the world. A significant part of the human population cannot tolerate gluten, a storage protein found in wheat, rye and barley and therefore products made from alternative cereals are required. During this study the bread making potential of seven gluten free flours, wheat and wholemeal wheat flour was compared. Fermentation potential of the different flours was determined, showing that dough development height of gluten free and wholemeal wheat samples was lower than for wheat and oat flour. Apart from standard bread quality parameters such as loaf specific volume and physical crumb texture, also water activity and shelf life have been determined. The shelf life of gluten free breads was reduced compared to wheat bread. Aroma profiles were evaluated by a trained panel. Wheat, oat and wholemeal wheat breads were liked moderately, while the remaining samples had lower liking scores. Crumb grain characteristics were investigated using image analysis and microstructure was observed by scanning electron microscopy. Overall only breads produced from oat flour were of similar quality to wheat bread and that the utilisation of buckwheat, rice, maize, quinoa, sorghum and teff flours resulted in breads of inferior quality.

8.2 Introduction

Bread is one of the major staple foods and is consumed daily in all parts of the world. Although a wide range of different types exist, the term "bread" usually refers to yeast leavened wheat products. Wheat (*Triticum aestivum*) is outstanding among cereals, because of its gluten

protein fraction. This protein is responsible for the unique visco-elastic properties of wheat dough and hence for the exceptional bread making potential. However, this protein composite found in wheat, rye and barley triggers gluten sensitive enteropathy, i.e. coeliac disease. Consequently, there is a need for bread products made from alternative raw materials. Flours milled from rice (*Oryza sativa*), maize (*Zea mays*), teff (*Eragrostis tef*), sorghum (*Sorghum bicolor*) as well as the pseudocereals quinoa (*Chenopodium quinoa*) and buckwheat (*Fagopyrum esculentum*) can be included into a gluten free diet. The inclusion of oat (*Avena sativa*) in products for coeliac disease patients is controversial. Studies showed that ingestion of moderate amounts of oat is well tolerated by a vast majority (Thompson 2003). Hence, wholegrain oat flour has been incorporated into this study. An extensive review of the available literature showed that these flours have previously been used in baking. However, they were added to the recipe as a component in complex gluten free formulations or to replace a certain proportion of wheat flour. During this study, breads were baked using 100% of the respective gluten free flour. Besides, this is the first publication directly comparing the bread making potential of such a high number of flours.

Rice flour is an economical ingredient widely used in gluten free baking. Its suitability for bread products is due to its white colour, bland taste and easy digestibility. Despite these advantages, rice proteins have poor functional properties. Therefore, many rice based gluten free formulations contain hydrocolloids (Gurjal *et al*. 2003; Nunes *et al*. 2009; Rosell and Marco 2008). Maize is a major cereal grain grown worldwide, ranking second only to wheat in total production area and second only to rice in total amount produced (Moore *et al*. 2004). Limitations in the use of conventional maize flour for bread production are partly due to the distinctive yellow colour and flavour. However, Brites *et al*. (2010) successfully applied *broa* bread making technology (Portuguese ethnic bread) for the production of gluten free maize bread. Using different strains of lactic acid bacteria Sanni *et al*. (1998) produced sour maize bread of varying quality. These authors reported a hard crumb but a shelf life of six days.

Organoleptic qualities were within the acceptable limits expected for sourdough bread. More suitable for the production of bread is white maize. The grain is biologically and genetically similar, but carotin oil pigments responsible for yellow colouring are absent. Flander *et al*. (1998) incorporated wholemeal oat flour into wheat breads and hence improved nutritional quality due to increased fibre and β-glucan content. Even though its status in a gluten free diet is controversial, several publications exist on the use of oat flour for the production of gluten free bread. Huettner *et al*. (2010) investigated the bread making performance of several commercial wholegrain oat flours, concluding that flours with coarse particle size, limited starch damage and low protein content are to be favoured. The same authors showed a positive effect of high pressure treatment on baking performance of oat flour. Comparing the different cereals and pseudocereals, it can be stated that the main efforts of scientists have been concentrated on wheat, rice, maize and oat whereas the investigation of alternative grains such as sorghum, buckwheat, quinoa and teff are less developed. Even though recently an increasing interest in the exploration of these grains has been evident, publications on their use for the production of bread are scarce. Rosell *et al*. (2009) studied the effect of the addition of flours from the highly nutritious crop quinoa to wheat bread formulation. Replacement of wheat flour up to 50% still resulted in breads with acceptable sensory quality, however colour was compromised. Alvarez-Jubete *et al*. (2010) investigated the potential of quinoa and buckwheat as healthy high-quality ingredients in gluten free bread. These authors found that the addition of the pseudocereals resulted in higher loaf volume and softer crumb compared to the control. Despite its name, buckwheat is not taxonomically related to wheat and hence can be considered as gluten free. In Southeast Asia it is traditionally used to make unleavened breads called *chapatti*. Moore *et al*. (2004) produced buckwheat sourdough breads, while Mezaize *et al*. (2009) used buckwheat flour in a composite formulation to produce French style gluten free breads. Teff, a small seeded tropical grain, originates from Ethiopia and is traditionally used for the production of *injera* (fermented flatbread) (Mezaize *et*

al. 2009). Mohammed *et al.* (2009) supplemented wheat bread up to a level of 20% with teff flour, noticing a significant decrease of organoleptic overall acceptability. Using a simple recipe based on 100 % teff flour Renzetti *et al.* (2008) produced gluten free breads of relatively low specific volume and crumb hardness. Several researchers have reported on the production of gluten free sorghum breads and much of this work is reviewed by Schober and Bean (2008) Vallons *et al.* (2010) attempted an improvement of sorghum bread by using high-pressure treated sorghum, while Schober *et al.* (2007) used sourdough fermentation. Hugo *et al.* (2003) showed that fermentation of sorghum flour has potential also to increase the utilisation of sorghum flour in composite wheat breads.

While a recent publication looks into the nutritional value of this wide range of flours (Hager *et al.* 2012), the aim of this study was to evaluate their potential for the production of bread and to evaluate several quality criteria of the end products. A basic bread recipe was used, consisting simply of flour, water, sugar, salt and yeast. Using a Rheofermentometer, the fermentation potential of the different flours was compared. Apart from standard bread quality parameters such as loaf specific volume and physical crumb texture, also water activity and shelf life of the final products have been determined and aroma profiles were evaluated by a trained panel. As bread is an aerated product and its texture depends strongly on the size and distribution of the gas cells within, crumb grain characteristics were investigated additionally using image analysis and microstructure was observed by means of scanning electron microscopy. All flours used in this study are commercially available. Hence the research findings can be adapted to industrial bread production. This publication aims at supporting technologists in the development and improvement of gluten free breads by providing basic knowledge of the function of flours from different botanical sources.

8.3 Materials and Methods

8.3.1 Materials

The suppliers for the ingredients used were Doves Farm Foods Ltd, UK, for white rice flour

(12.8 % moisture, 0.5 % ash) and buckwheat flour (12.6 % moisture, 1.7 % ash); Odlums,

Ireland for wholemeal wheat (13.1 % moisture, 1.3 % ash) and bakers' flour (12.7 % moisture,

0.9 % ash); Trouw, The Netherlands, for wholegrain white teff flour (9.5 % moisture, 2.1 %

ash); Smiths Flour Mills, UK, for maize flour (14.0 % moisture, 0.4 % ash); Ziegler

Naturprodukte, Germany, for quinoa flour (12.3 % moisture, 2.4 % ash); E. Flahavan & Son Ltd,

Ireland, for wholegrain oat flour (10.4 % moisture, 0.8 % ash) and Twin Valley Mills, Nebraska,

USA, for sorghum flour (11.1 % moisture, 1.0 % ash). Dry yeast was obtained from Puratos,

Belgium; sugar from Siucra, Ireland, and salt from Glacia British Salt Limited, UK. Extensive

compositional analysis was carried out on all flours and results are presented in a previous

study [20].

8.3.2 Rheofermentometer analysis

Gaseous release and dough development of gluten free batters and wheat doughs were

measured using a Rheofermentometer (Chopin, France). Three hundred grams of each dough

were prepared in the same manner as described below for baking trials. The tests were

performed at 30 °C over a period of 90 min. For wheat samples a cylindrical weight of 1500 g

was applied onto the fermentation chamber. The leavening process is described in terms of

Hm (maximum height of dough development curve), T1 (time at maximum of dough

development curve), (Hm-h)/Hm (dough height at the end of the test, calculated as percentage

of the maximum), and Vt (total volume of carbon dioxide released by the dough). Results are

presented as the average of two measurements.

8.3.3 Bread making procedure

The optimal water addition level for wheat as well as wholemeal wheat flour was determined

using a farinograph (AACC method 54-21). However, this method is not applicable for gluten

free systems. Therefore, empirical trial-and-error testing was conducted and the resulting crumb structure was evaluated visually. In addition, loaf specific volume was considered. The following levels were determined as optimal: 120 % of rice flour, 95 % of oat, quinoa, sorghum and teff flour, 90 % of maize flour, 85 % of buckwheat flour, 67 % of wholemeal and 63 % of wheat flour. The gluten free flours needed higher amounts of water to form an acceptable crumb than the wheat flours and therefore resulted in cake-like batters rather than workable doughs.

Gluten free and wheat breads were prepared using 2 % salt, 2 % sugar and 3 % yeast, based on flour weight. Yeast and sugar were suspended in the water (35 °C) and regenerated for a period of 10 min in a proofer (KOMA sunriser, Roermond, The Netherlands) set to 30 °C at a relative humidity (RH) of 85 %. This suspension was added to the premixed dry ingredients. Mixing was then carried out with a batter attachment for 1 min (gluten free batter) or with a dough hook for 30 sec (wheat dough) at low disk speed with a Kenwood chef classic. The bowl was scraped down and a further mixing at a higher disk speed was carried out (7 min for wheat dough and 1.5 min for the gluten free formulation). Bulk fermentation for the wheat dough was carried out for 15 min at 30 °C, 85 % RH. Wheat doughs and gluten free batters were scaled to 400 g into 10 baking tins of 15 x 9.5 x 7 cm and placed in a proofer for 30 min and 75 min, respectively (30 °C, 85 % RH). The breads were baked for 45 min at 190 °C top and bottom heat in a deck oven (MIWE condo, Arnstein, Germany), previously steamed with 0.3 L of water. Bread loaves were removed from the tins, cooled down at room temperature and subsequently analysed or stored in plastic bags at ambient temperature. Three batch replicas were prepared.

8.3.4 Loaf characteristics

Loaf specific volume were analysed upon cooling using a Volscan Profiler (Stable Micro Systems, UK). Bake loss was determined by substracting loaf weight from dough weight. Moisture was determined using the AACC approved air-oven method (44-15A). Crumb texture

was determined at 2 and 5 days of storage. The three bread slices (25 mm thickness) taken from the centre of each loaf were used to evaluate the physical crumb texture. Texture profile analysis (TPA) was performed using a TA-XT2i texture analyser (Stable Micro Systems, Surrey, UK) equipped with a 25 kg load cell and a 35 mm aluminium cylindrical probe. A test speed of 5 mm/s was used to compress the middle of the breadcrumb to 50 % of its original height. The following values calculated by the TPA software were chosen to describe crumb texture: Hardness (peak force of first compression cycle), springiness (how well the product physically springs back after it has been compressed in the first cycle, calculated as the distance of the detected height of the product on the second compression cycle divided by the original compression distance), chewiness (area of second compression cycle divided by the area of first compression cycle multiplied by springiness). Three loaves per batch were analysed on day 0, day 2 and day 5 of storage. Rate of staling was calculated using the following equation

$$(\quad rate\ of\ staling\ =\ \frac{crumb\ hardness\ day\ 5\ -\ crumb\ hardness\ day\ 0}{5\ days} \quad).$$ Water activity

measurements of the bread crumb were determined using an AquaLab 4TE water activity meter (Decagon Devices Inc., Pullman, Washington, USA).

8.3.5 Crumb grain

The structure of bread slices was characterised using a C-cell Bread Imaging system (Calibre Control International Ltd., UK). To describe the crumb grain the following parameters were chosen: Slice area (total area of a product slice), number of cells (number of discrete cells detected within the slice), area of cells (the total area of cells as a percentage of the total slice area), cell elongation (the degree of overall elongation of the cell structure in a particular direction) and wall thickness (the average thickness of cell walls).

8.3.6 Shelf life

The shelf life of the breads was determined using the method described by Dal Bello *et al*. (2007). Each loaf was sliced transversely in a sterile manner to obtain uniform slices of 25 mm

thickness. Each slice was exposed to the air for 5 min on each side and then packed in a plastic bag and heat sealed, during which procedure a small slot was left open and a tip of a transfer pipette was inserted to ensure comparable aerobic conditions in each bag. Bags were incubated at room temperature and examined for mould growth during a 12-day storage period. A series of 9 slices was inoculated. Mould growth was quantified as being the number of slice surfaces, i.e. both front and back of the slice, showing aerial mycelia.

8.3.7 Energy content

For the breads produced in this study, calories have been calculated according to Schakel *et al*. (2009) using the compositional data of the flours shown previously (Hager *et al*. 2012).

8.3.8 Sensory evaluation

All sensory analyses were performed using a trained panel consisting of 22 members (5 male, 17 female, aged 23 – 43 years). The panellists were trained, in weekly sessions, to orthonasally recognize about 120 selected odorants at different odorant concentrations according to their odour qualities. Training courses were done at least six months prior to participation in the actual sensory experiments. At least ten assessors participated in each sensory session. Sensory analyses were performed in a sensory panel room at 21 ± 1 °C over three different sessions. A flavor language was developed, based on reference aroma solutions at defined concentrations, defining the specific smell of a compound corresponding to a certain aroma attribute.

For aroma profile analysis bread loaves were cut in slices (thickness about 2 cm) and the crusts were removed. The samples were presented to the sensory panel, which had to sniff the crumbs and describe the odour qualities they perceived during sniffing the crumbs. The panel finally agreed on characteristic odour attributes in a group discussion. Crumb samples were presented again to the panel in a second session and the intensities of the predefined odour attributes were evaluated on a scale from 0 (not detectable) over 1 (weak intensity), 2

(medium intensity), to 3 (high intensity). The results of each attribute were calculated as arithmetic mean. The assessors were trained immediately prior to analysis with aqueous odorant solutions in defined concentrations (factor 100 above the odour threshold) [22-24]. The odorant solutions reflected the evaluated characteristic odour attributes of the flours: buttery (butane-2,3-dione; 120 µg/L), cooked potato-like (3-(methylthio-)propanal; 140 µg/L), malty (3-methylbutanal; 120 µg/L), mouldy (geosmin; 2.1 µg/L), oat flakes-like ((E,E,Z)-nona-2,4,6-trienal; 2,6 µg/L), pea-like (3-isobutyl-2-methoxypyrazine; 3.9 µg/L), popcorn-like, roasty (2-acetyl-1-pyrroline; 12 µg/L), vinegar-like (acetic acid; 18,000,000 µg/L), vomit-like, cheesy (butanoic acid; 120,000 µg/L). The odorant references were purchased from Sigma-Aldrich (Taufkirchen, Germany) Acros (Geel, Belgium) and AromaLab (Freising, Germany). The attribute yeast dough-like was evaluated based on the experience of the assessors. In order to evaluate aroma liking, bread crumb slices were prepared as described above and presented to the panel. The assessors had to evaluate the liking of the samples on a nine-point-scale from 1 (dislike very much) over 5 (neither like nor dislike) to 9 (like very much). The results were calculated as the arithmetic mean.

8.3.9 Microstructure

Scanning electron microscopy was chosen to investigate the microstructure of dough and bread. Samples were freeze-dried for approximately 20 h, grinded shortly with mortar and pestle and then attached onto double-sided carbon tape fixed to an aluminium specimen stub and were preliminary gold-coated in an SEM coating system (BIORAD Polaron Division) with a layer of 25 nm in thickness. Hereupon samples were examined under high vacuum in a field emission scanning electron microscope (JEOL, JSM-5510 SEM) with a working distance of 8 mm. Secondary electron images were acquired at an accelerating voltage of 5 kV. For processing of the images, SEM Control User Interface, Version 5.21, JEOL Technics Ltd. was used.

8.3.10 Statistical analysis

For comparison SigmaPlot (Systat Software Inc, Chicago, Illinois) was used to carry out statistical analysis on the test results. Normality test (Shapiro-Wilk) was followed by an all pair wise multiple comparison procedure (Fisher LSD Method) to evaluate significant differences.

8.4 Results and Discussion

8.4.1 Rheofermentometer analysis

Rheofermentometer analysis is used to gain information on dough rise and gas formation. When evaluating the results it has to be kept in mind, that a weight of 1500 g was applied to the fermentation chamber when analyzing wheat samples, while no weight was applied for gluten free samples. Wheat and oat samples reached a maximum dough development height (Hm) of 49 mm (Table 8.1). These values are unmatched by the gluten free batters or the wholewheat dough, which reached 15 mm (maize) to 28 mm (sorghum). This indicates that the visco-elastic properties of oat and wheat dough are superior. Bran particles present in the wholemeal flour disrupt the gluten network and hence limit the extensibility of the dough. A statistically significant linear correlation between specific volume and maximum dough development height was observed ($P<0.05$). Decrease in dough volume at the end of the test, calculated as percentage of the maximum ((Hm-h)/Hm), was significantly lower for wheat dough compared to gluten free batters. The low value for wheat dough suggests that the combination of gas produced and the rheological properties of the sample were more favorable in sustaining the macrostructure of the proofed dough pieces compared to the other samples. The time for reaching maximum dough rise (T1) was significantly lower in gluten free batters when compared to wheat doughs. The total volume of gaseous release (Vt) of wheat and wholewheat dough was 1366 mL and 1570 mL, respectively. Gas production was highest in teff, buckwheat and quinoa batters (1676 mL, 1670 mL and 1583 mL, respectively).

Table 8.1 Dough development and gaseous release of wheat doughs and gluten free batters determined with the rheofermentometer at 30 °C for 1.5h; values in one column followed by the same letter are not significantly different (p<0.05)

	Dough development			Gas production
	Hm [mm]	T1 [min]	(Hm-h)/Hm [%]	Vt [mL]
Wheat	49 ± 2^a	90 ± 0^a	0.20 ± 0.14^b	1366 ± 10^{cd}
Wholewheat	19 ± 1^{de}	90 ± 0^a	1.30 ± 0.28^b	1570 ± 1^{ab}
Rice	19 ± 1^{de}	46 ± 2^c	27.20 ± 0.42^a	1058 ± 21^e
Oat	49 ± 1^a	67 ± 1^b	25.4 ± 8.20^a	1061 ± 28^e
Quinoa	22 ± 1^{cd}	43 ± 1^c	28.05 ± 4.31^a	1583 ± 39^{ab}
Buckwheat	20 ± 0^b	53 ± 2^b	24.30 ± 5.23^a	1670 ± 78^a
Sorghum	28 ± 2^b	42 ± 0^c	21.50 ± 0.28^a	1329 ± 6^d
Maize	15 ± 1^e	38 ± 1^c	26.20 ± 0.28^a	1466 ± 34^{bc}
Teff	$26 \pm 2b^c$	42 ± 2^c	25.25 ± 0.64^a	1676 ± 18^a

8.4.2 Loaf characteristics

It has been previously reported that these flours are suitable alone or as composites for the production of bread. However, the quality of the resulting end products varies widely, with gluten free breads usually being inferior to wheat bread. The quality evaluation showed that the produced breads differed in final loaf volume, crumb firmness, crumb structure, shelf life and taste attributes. Visual appearance strongly influences consumer liking. Therefore the produced breads as well as slices thereof are shown in Figure 8.1 and 8.2.
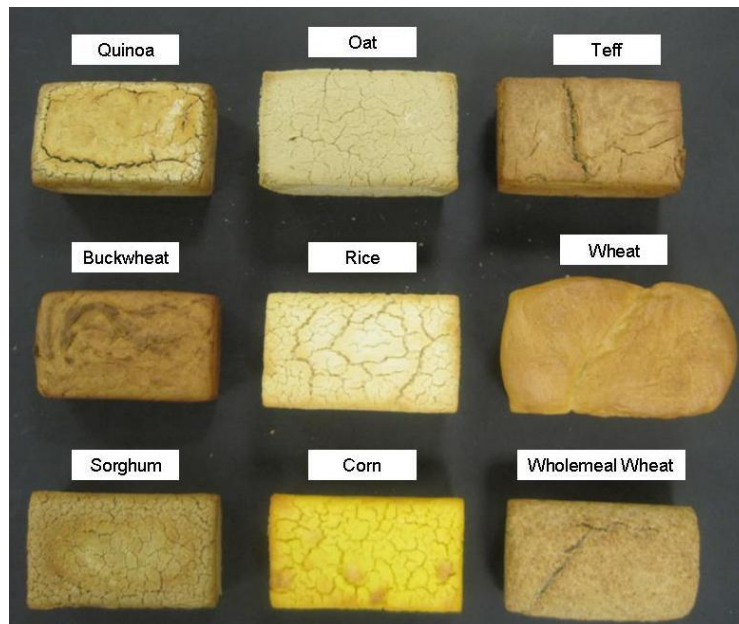
Figure 8.1 Visual appearances of breads produced from different gluten free and wheat flours
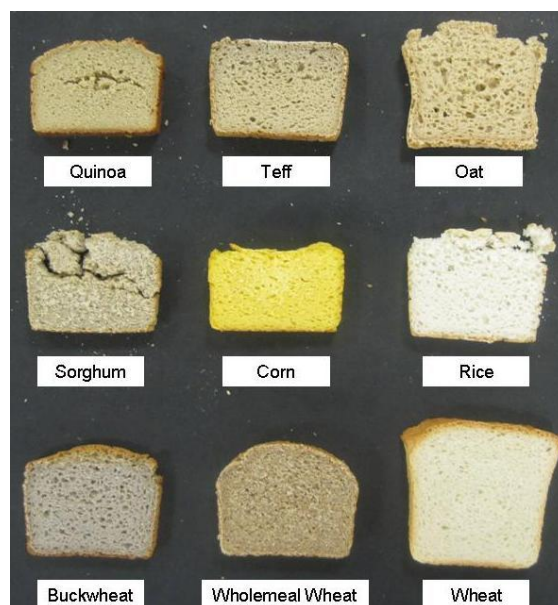
Figure 8.2 Visual appearances of bread slices produced from wheat and gluten free flours

Another important parameter, known to strongly influence consumer's choice is the loaf specific volume. From an economic standpoint, a high ratio of volume per weight is desired. Due to the exceptional visco-elastic properties of gluten, gas retention during proofing and baking is higher in wheat dough as compared to gluten free batters. Therefore, loaf specific volume of white wheat bread is highest (2.6 mL/g) (Table 8.2). However, the bran particles in wholemeal wheat flour puncture and break a high number of these gas bubbles, which results

in a lower specific volume (1.7 mL/g in this study) (Seyer 2009). Due to the lack of a cohesive protein matrix, elasticity and extensibility of the gluten free batters is reduced and loaf volumes are low. With a specific volume of 2.4 mL/g, oat bread showed the highest value close to white wheat bread. All other gluten free loafs had significantly lower specific volumes, with maize having only half the volume of wheat bread (1.3 mL/g). Statistically significant differences were also detected in bake loss (Table 8.2). Again, the breads differed significantly in moisture content. Rice bread showed the highest moisture content and wheat bread the lowest. This was expected due to the different amounts of water added to the batters/doughs. A statistically significant positive correlation between water addition level and moisture content of the final loaves was detected (P<0.05). To describe the texture of the gluten free and wheat breads, crumb hardness, springiness and chewiness are shown in Table 8.2. Oat bread had the softest crumb (4.5 N). Wheat and rice bread had a crumb hardness of 8.5 N and 18.8 N, sorghum bread of 26.3 N. The low values found in these samples are desired, since consumers relate a firm crumb to an old product. Due to its higher fibre content, crumb hardness of wholemeal wheat bread (31.5 N) was about four times higher than that of white wheat bread. Maize bread had significantly higher crumb hardness (66.7 N) than all other breads of this study. Crumb springiness, a value describing the recovery of the sample after compression, is important in separating soft, soggy bread from soft but resilient bread. Oat and wheat bread had the highest crumb springiness (1.08 and 1.00, respectively), whereas sorghum bread showed the lowest crumb springiness (0.88). Chewiness, i.e. the product of hardness, cohesiveness and springiness, gives an indication on the energy required to masticate a solid food. Wheat bread had a chewiness of 7 N, which is significantly lower than wholemeal wheat and most gluten free breads. Sorghum bread had a chewiness value of only 5N and the other gluten free breads ranged from 11 N for rice bread to 36 N for buckwheat bread. Regarding overall mechanical texture, oat bread is the most favourable, even compared to white wheat bread. Its crumb is significantly softer and springier.

Table 8.2 Loaf and slice characteristics of gluten free and wheat breads; values in one column followed by the same letter are not significantly different (p<0.05)

	Specific Volume [ml/g]	Bake loss [g]	Moisture [%]	Hardness [N]	Springiness	Chewiness [N]	Rate of staling	Water activity
Wheat	2.62 ± 0.18 [a]	42.89 ± 2.83 [f]	40.96 ± 1.51 [f]	8.48 ± 1.33 [f]	1.004 ± 0.018 [b]	7.74 ± 1.41 [f]	3.55 ± 0.29 [ab]	0.967 ± 0.002 [d]
Wholewheat	1.70 ± 0.06 [d]	50.18 ± 2.52 [f]	46.28 ± 1.98 [d]	31.52 ± 3.56 [c]	0.913 ± 0.025 [cd]	18.03 ± 4.95 [d]	1.58 ± 0.09 [c]	0.969 ± 0.004 [d]
Rice	1.80 ± 0.05 [cd]	63.89 ± 2.73 [c]	55.20 ± 2.16 [a]	18.79 ± 1.90 [e]	0.953 ± 0.015 [c]	11.04 ± 2.29 [e]	0.83 ± 0.12 [df]	0.987 ± 0.003 [a]
Sorghum	1.85 ± 0.14 [c]	72.46 ± 3.39 [a]	44.29 ± 4.49 [e]	26.28 ± 5.05 [d]	0.878 ± 0.039 [d]	5.26 ± 2.24 [g]	1.59 ± 0.24 [cd]	0.980 ± 0.002 [b]
Oat	2.40 ± 0.12 [b]	66.42 ± 4.04 [b]	49.42 ± 1.80 [bc]	4.47 ± 0.90 [g]	1.083 ± 0.215 [a]	20.37 ± 2.08 [cd]	4.10 ± 1.77 [a]	0.985 ± 0.002 [a]
Quinoa	1.51 ± 0.07 [f]	58.91 ± 3.24 [d]	48.23 ± 2.31 [cd]	31.98 ± 2.50 [c]	0.930 ± 0.030 [c]	32.93 ± 2.47 [b]	0.18 ± 0.07 [gef]	0.974 ± 0.002 [c]
Teff	1.60 ± 0.03 [e]	52.66 ± 5.01 [e]	50.25 ± 1.59 [b]	43.13 ± 5.20 [b]	0.942 ± 0.017 [c]	31.91 ± 7.48 [b]	1.29 ± 0.19 [cd]	0.978 ± 0.002 [b]
Buckwheat	1.69 ± 0.06 [d]	53.89 ± 1.49 [e]	50.64 ± 2.40 [b]	42.92 ± 4.03 [b]	0.952 ± 0.014 [c]	36.25 ± 3.38 [a]	0.82 ± 0.05 [gd]	0.971 ± 0.003 [d]
Maize	1.33 ± 0.10 [g]	60.82 ± 6.41 [d]	46.09 ± 1.78 [d]	66.66 ± 3.56 [a]	0.902 ± 0.018 [cd]	22.70 ± 2.59 [c]	2.41 ± 0.62 [bc]	0.979 ± 0.003 [b]

8.4.3 Crumb grain

Apart from physical texture, described under 3.2, also visual texture of the crumb is an important attribute of bread quality. Digital image analysis was used to quantitatively describe crumb grain and results are shown in Table 8.3. When comparing wheat to wholemeal wheat bread it is apparent that crumb structure and cell characteristics are very distinct. Regarding the number of cells, white wheat bread had the highest (4906 cells), whereas wholemeal wheat bread had the lowest number between all breads of this study (2453 cells). This difference can be explained by the high amount of bran particles present in the dough, which penetrated gas cells and caused leaks (Seyer 2009). Number of cells in gluten free breads was significantly lower than in white wheat bread. Teff and quinoa bread showed a relatively higher number of alveoli (3327 and 3170 cells), whereas buckwheat counted 2985 cells, sorghum 2788 cells and oat 2667 cells. The number of cells in maize and rice is similarly low as in wholemeal wheat flour. The gas cells were incorporated through the mixing process and only their size were influenced by further bread production steps. For the production of all gluten free breads, the same mixing regime was followed. Hence, the different number of cells is due to differences in dough consistency (Schober 2009). The area of cells as a percentage of total slice area is given in Table 8.3. Higher values, as found in oat or rice bread (54.55 % and 53.85 %, respectively) indicate a more open texture. Quinoa, buckwheat, maize and teff breads showed the smallest area of cells (50.58 %, 49.92 %, 47.75 % and 47.76 %) indicating a denser structure. This is reflected in the specific volume, which was lowest for these loaves. However, not the holes themselves are the most significant contributor to mechanical strength of the baked product, but the surrounding matrix referred to as "cell walls" (Cauvain 1999). Cells of rice bread crumb had the highest wall thickness (0.54 mm), followed by oat bread (0.51 mm). Thin cell walls as in wheat and maize bread (both 0.43 mm) are desirable. The mouth feel of bread is known to be strongly influenced by these cell characteristics: finer, thin-walled uniform cells yield a softer and more elastic texture, than coarse, thick-walled cell structures

do (Scanlon and Zghal 2001). Cell elongation is a measure of how far the pore shape differs from a circle, with values close to 1 indicating rounded cells and higher values indicating greater elongation. White wheat bread showed the most elongated cells, whereas voids of wholemeal wheat bread were less elongated. The latter contained a high proportion of dietary fibre which disrupted the starch-gluten matrix and hence restricted gas cell expansion, forcing the alveoli to expand in a certain way. The most rounded cells were observed in rice bread.

Table 8.3 Crumb cell characteristics of gluten free breads compared to wheat bread

	Slice area [mm^2]	Number of cells	Cell elongation	Area of cells [%]	Wall thickness [mm]
Wheat	6847 ± 73 [a]	4906 ± 38 [a]	1.517 ± 0.021[a]	51.29 ± 0.05 [c]	0.427 ± 0.006 [e]
Wholewheat	4760 ± 38 [b]	2453 ± 2 [g]	1.393 ± 0.015[ef]	55.16 ± 0.54 [a]	0.478 ± 0.005 [c]
Rice	5296 ± 125 [b]	2507 ± 170 [g]	1.380 ± 0.000[f]	53.85 ± 0.11 [b]	0.539 ± 0.008 [a]
Sorghum	4840 ± 162 [b]	2788 ± 100 [e]	1.410 ± 0.000[ef]	53.88 ± 1.24 [b]	0.479 ± 0.004 [c]
Oat	5188 ± 32 [b]	2667 ± 39 [ef]	1.440 ± 0.030[cd]	54.55 ± 0.35 [ab]	0.505 ± 0.018 [b]
Quinoa	4006 ± 54 [d]	3170 ± 82 [c]	1.417 ± 0.006[de]	50.58 ± 0.71 [cd]	0.455 ± 0.002 [d]
Teff	4401 ± 45 [c]	3327 ± 21 [b]	1.443 ± 0.015[cd]	47.75 ± 0.41 [e]	0.452 ± 0.005 [d]
Buckwheat	4794 ± 29 [b]	2985 ± 15 [d]	1.477 ± 0.006[b]	49.92 ± 0.09 [d]	0.446 ± 0.002 [d]
Maize	3758 ± 68 [e]	2576 ± 16 [fg]	1.453 ± 0.006[bc]	47.76 ± 0.22 [e]	0.430 ± 0.003 [e]

* Values in one column followed by the same letter are not significantly different (p<0.05)

8.4.4 Shelf life

The shelf life of bread is determined by the staling behaviour of the product and its microbial deterioration. Bread staling involves crumb firming, which has been attributed mainly to recrystallization of amylopectin and water redistribution between crumb and crust. Sciarini *et al*. proposed previously that in wheat breads the gluten network slows down the movement of water, thus gluten free breads are more prone to stale (Sciarini *et al*. 2010). This assumption cannot be confirmed by the data of this study, as staling of most gluten free breads was lower than that of wheat bread (Table 8.2). Maize bread showed the highest value (39.4). Staling of all other breads was much lower. Teff bread showed a staling rate of 13.4, sorghum bread 10.2

and buckwheat bread 8.4. Staling rate of wholewheat bread was higher (9.9) than that of white wheat bread (5.3). In bread made with oat or rice flour, staling was far less pronounced (3.5 and 3.3 respectively). Quinoa breads staled the slowest (1.0). These values show that factors other than the presence or absence of gluten are influencing the staling rate. The changes in compressibility and crumbliness of bread crumb is in large parts attributable to the retrogradation behaviour of cereal starch and hence the ratio of amylose to amylopectin (Sluimer 2005). The significantly lower amylose content in quinoa flour [20] is reflected in a much slower staling of quinoa bread compared to others.

The quality of bread is lost rapidly not only due to staling but also due to microbial spoilage. Under ambient conditions mold grows on well-packaged wheat bread within four to six days (Sluimer 2005). White wheat bread of this study had a microbial shelf life of four days. As expected, shelf life of gluten free breads was lower. With the exception of rice bread, the first mold growth was observed on day four, giving the breads a shelf life of three days. Also wholemeal wheat bread was spoiled on day four. Rice bread had a shelf life of only two days. The microbial stability of gluten free breads is mainly compromised because of the high water activity (a_w). Rice bread has the highest water activity (0.987) (Table 8.2). Wheat bread, having the lowest water activity (0.969), had the longest microbial shelf life. The gluten free flours of this study generally showed significantly higher values for water activity than the two wheat flours (Table 8.2).

8.4.5 Energy content
Studies showed that the exclusion of gluten from the diet very often results in a significant increase of body fat stores and weight gain in coeliac patients (Capristo *et al*. 2000; Smecuol *et al*. 1997). As bread is a major source of energy in our daily diet, the calorie content of gluten free products is of importance. As expected, calorie content was highest in white wheat bread (212 kcal/100 g). Wholemeal wheat bread had 210 kcal/100 g. Due to the fact that the gluten free breads of this study contained significantly higher amounts of water, their calorie content

was lower compared to white wheat bread: oat 194 kcal/100 g, buckwheat 191 kcal/100 g, maize 183 kcal/100 g, sorghum 186 kcal/100 g, teff 188 kcal/100 g and rice 158 kcal/100 g.

8.4.6 Sensory evaluation

The aroma quality of bread crumbs, were evaluated in a first sensory trial by determination of the overall aroma liking. The liking of wheat bread aroma was scored with 6.7 points on the nine-point-scale meaning that it was liked moderately. Almost identical values were determined for oat and wholemeal wheat bread aroma (6.7 and 7.2), showing that oat was equally liked as both wheat crumbs. An indifferent scoring was obtained for sorghum bread crumb (5.5 points) and all other bread crumbs had in part much lower liking scores: 2.7 for quinoa, 3.0 for rice and buckwheat, 3.2 for maize and 3.8 for teff.

Aroma profile analyses were performed in order to characterize and describe the crumb aroma in detail. Therefore, the characteristic odor attributes were identified by the sensory panel, which then also determined the intensities of these odor qualities. Using this approach and by comparing the profiles, specific odor characteristics of each bread crumb were evaluated. The aroma profiles of wholemeal and wheat crumb as well as the seven gluten free flours are shown in Figure 8.3. Wheat bread crumb was dominated by a medium intense yeast-like note and the profile was completed by weak malty and buttery notes. It can be concluded that these attributes were responsible for the positive evaluation of liking. In contrast to wheat, the yeast-like, malty and buttery intensities were in part much lower in the buckwheat crumb where pea-like, moldy and vinegar-like notes with weak intensities were detectable. These attributes can be correlated with the low acceptance of buckwheat crumb evaluated in the liking test. Again, the maize crumb exhibited a reduced yeast-like and malty aroma in comparison to wheat and an undesirable vomit-like note was perceivable, which was responsible for the low liking of maize bread. The aroma profile of oat bread was very similar to the wheat profile. Although the yeast-like note was decreased, the malty intensity in oat was comparable to wheat and the buttery note was even more intense. Undesirable odor

notes like moldy, pea-like and vomit-like, which have been detected in maize and buckwheat, were not perceivable in oat crumb. The high resemblance of the aroma profiles is therefore the reason why oat and wheat crumb had a similar liking. The pea-like odor attribute was the outstanding note of quinoa crumb and its intensity ranged from medium to high. The low acceptance of quinoa aroma is therefore explainable with the dominant presence of this odor note. Additional notes, which were reminiscent of cooked potato and mold, were also perceived with weak to medium intensity. Rice and wheat bread crumb aroma agreed in nearly all the intensities of the attributes yeast, dough-like and malty. However, the low liking of rice crumb was obviously caused by a vomit-like odor with a weak to medium intensity. Low intensities were determined for the evaluated odor characteristics in the sorghum crumb. Although a weak pea-like note was detectable in the crumb, but only a little influence on aroma liking was observed. Teff bread crumb showed reduced intensities of yeast, dough-like, malty and buttery, which have been found as the positive attributes in wheat crumb. The absences of these attributes, in combination with the detected vinegar-like quality, were responsible for the negative evaluation. The comparable liking scores of the crumbs made from wholemeal and baker's flour were correlated with the aroma profiles. The attributes yeast, dough-like, malty and buttery were almost identical. Only the oat flakes-like attributes were higher and vinegar-like notes were lower in wholemeal wheat crumb.
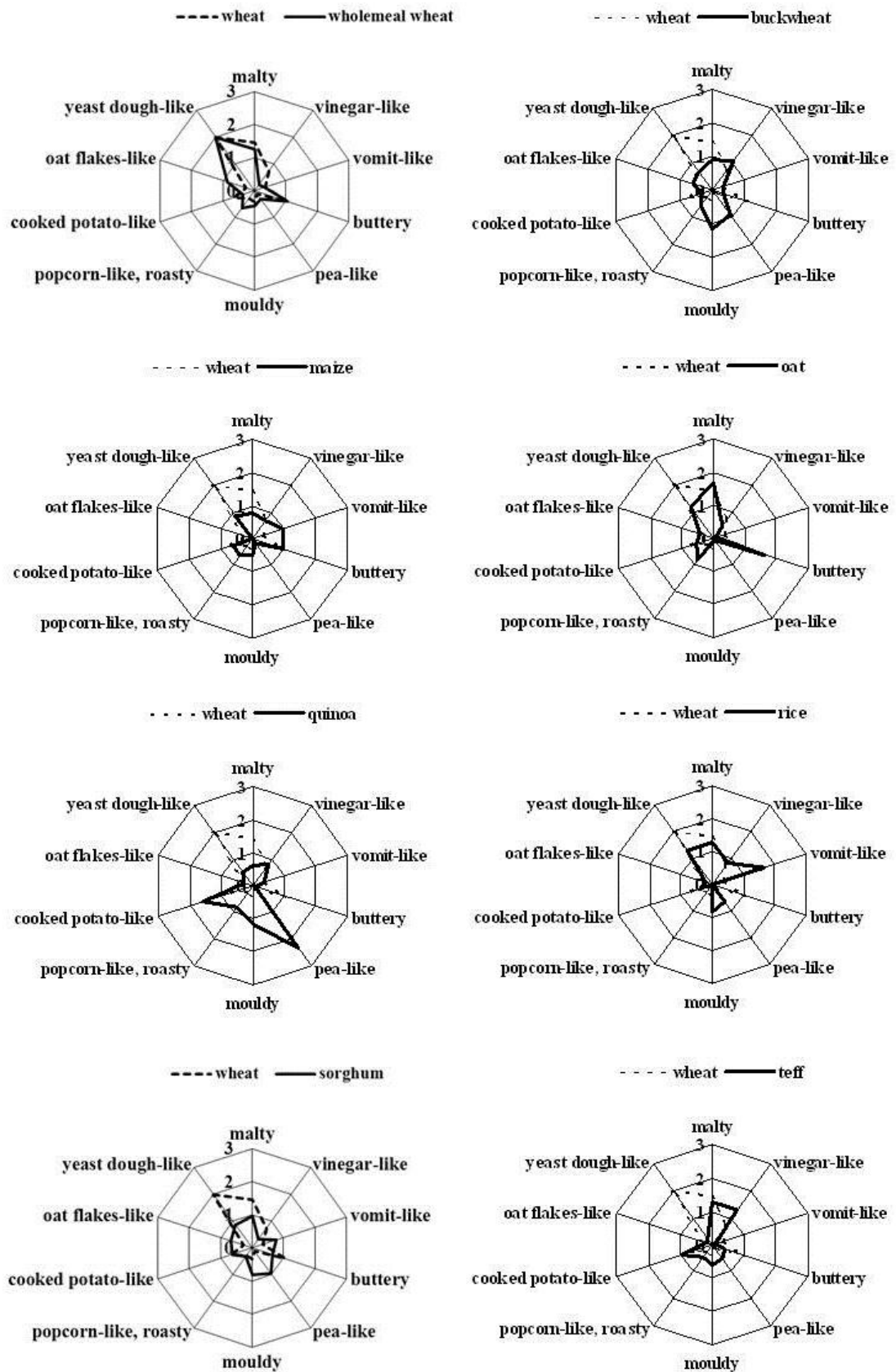
Figure 8.3 Aroma Profile Analysis of bread crumbs made from different flours

8.4.7 Microstructure

Batters and breads were investigated by means of scanning electron microscopy. Representative micrographs are shown in Figure 8.2 and 8.3. The batters/doughs preserved some characteristics of the flours (Hager *et al*. 2012): starch granules of various sizes and shapes as well as protein aggregates (Figure 8.2). During mixing of dough the granules swell and get deformed. This is most obvious for maize samples. While granule size in the flour was below 10 μm, their diameter is much larger in the dough. The foam structure of dough consists of a continuous starch-protein matrix containing discrete gas cells, starch granules and in the case of wholemeal, bran particles.
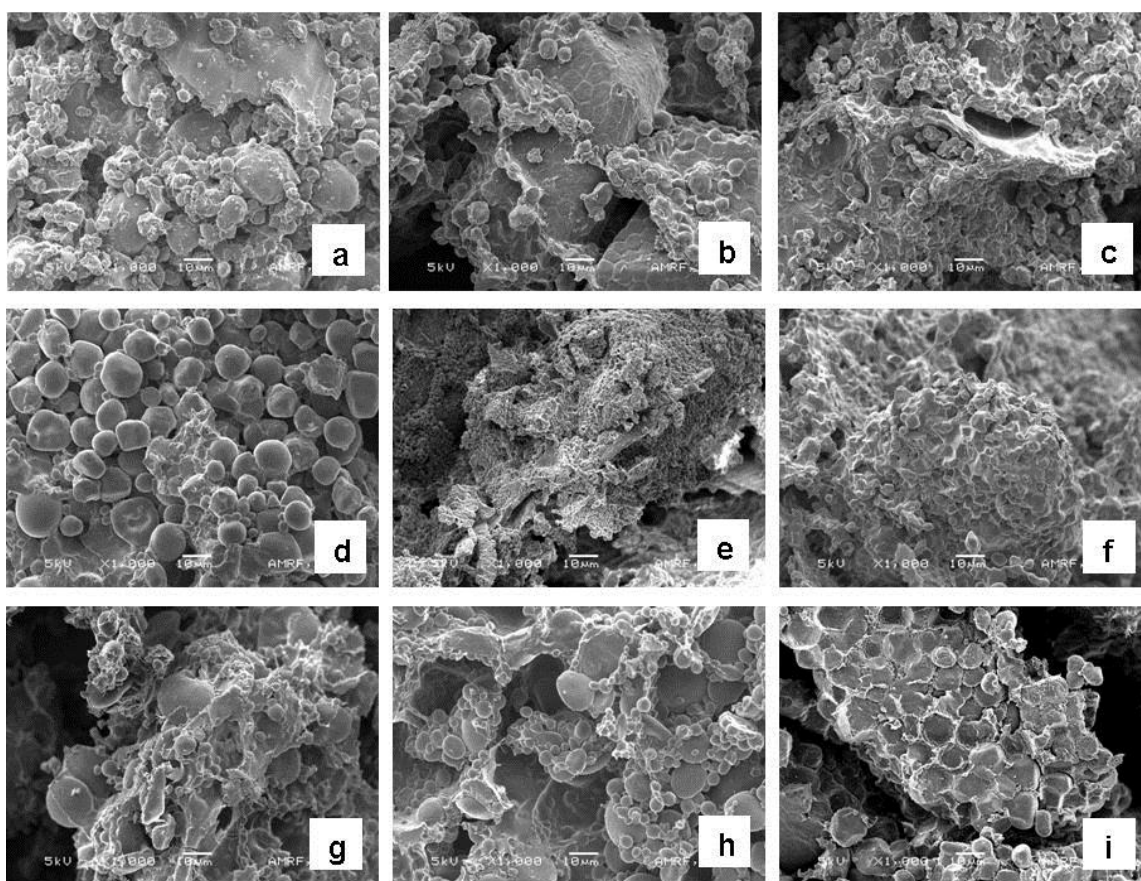
Figure 8.2 Micrographs of gluten free and wheat doughs: (a) oat, (b) buckwheat, (c) teff, (d) maize, (e) quinoa, (f) rice, (g) wheat, (h) wholemeal wheat (i) sorghum

The transformation of dough to bread is a complex process during which several structural changes take place. These changes include the gelatinization of starch, which can be observed

by means of scanning electron microscopy. During baking, the combination of gas production and evaporation turns the foam into a sponge structure with interconnected cells (Rojas *et al*. 2000). Figure 8.3 shows the resulting breads, where compared to flour and dough a reduced number of granular shaped starch is present. Bread dough represents a limited water system and therefore starch cannot fully gelatinize (i.e. not all starch granules loose crystalline structure or leach amylose). Due to partial gelatinization, swollen or distorted granules can be observed. For the examination of bread microstructure, scanning electron microscopy seems unfavourable as gelatinised starch and proteins cannot be distinguished. Therefore the gluten network cannot be visualised and no real structural differences between gluten free and wheat breads can be observed. Compared to all other breads, oat and rice bread seem more aerated. This finding is in accordance with macrostructure observations by image analysis, where these samples showed the highest percentage area of cells.
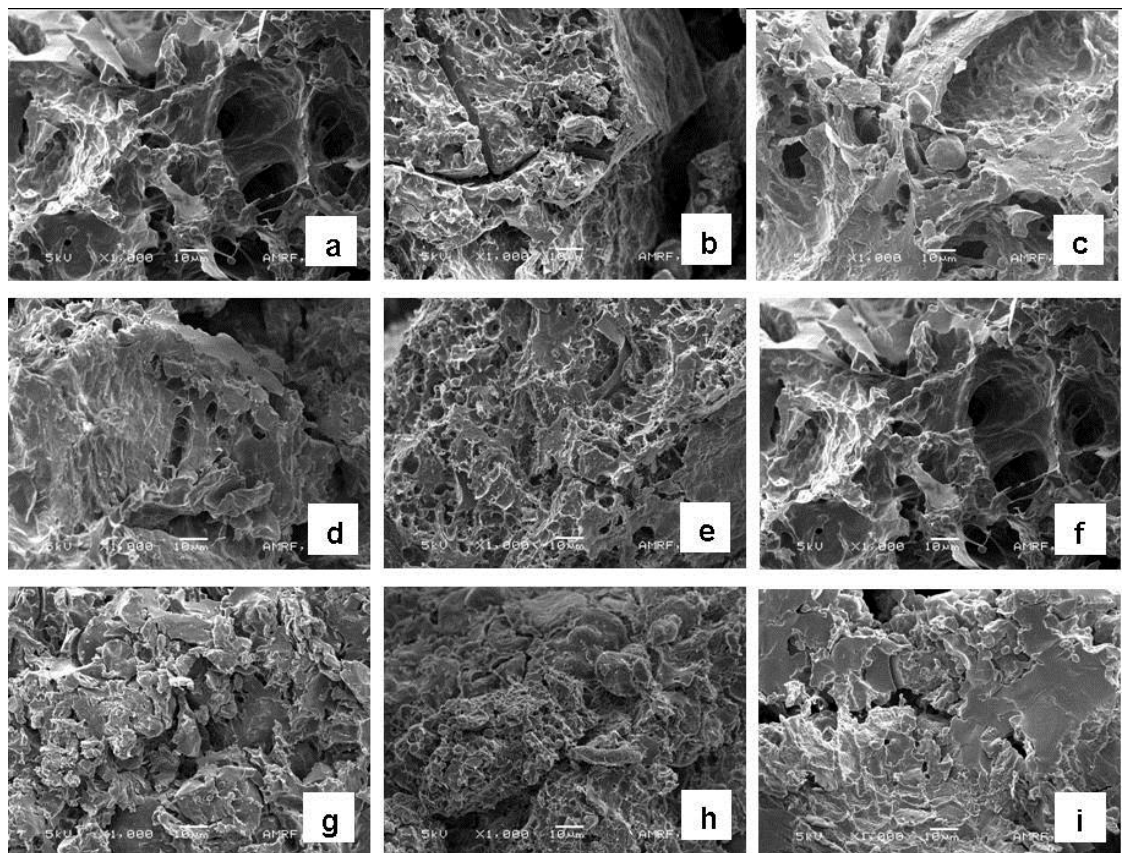
Figure 8.3 Micrographs of gluten free and wheat breads: (a) oat, (b) buckwheat, (c) teff, (d) maize, (e) quinoa, (f) rice, (g) wheat, (h) wholemeal wheat (i) sorghum

8.5 Conclusion

Due to differences in the composition of gluten free flours [20], although nutritionally superior, the loaf quality of gluten free and wholemeal wheat breads is inferior to that of white wheat bread. Oat represents an exception, as the breads produced from this flour were comparable to wheat bread regarding orthonasal liking and loaf quality characteristics such as specific volume, crumb hardness and springiness. In terms of crumb grain characteristics, white wheat bread is dissimilar to the other samples with a high number of thin walled cells. Several gluten free samples including quinoa, teff and buckwheat showed a dense structure indicated by a low area of cells as a percentage of slice area. Oat and rice bread were characterised by an open aerated structure. The microbial shelf life of gluten free and wholemeal wheat breads was lower than that of white wheat bread, which can be explained by the higher water activity of these samples. Rice and maize flour represent economical ingredients and hence are widely used for the production of gluten free foods. This study however showed that compared to other gluten free raw materials, their suitability for the production of bread is reduced. Orthonasal liking of the resulting breads was low and mechanical crumb grain characteristics were unfavourable. In addition, microbial shelf life of rice bread was lowest compared to all other breads of this study. Maize breads were characterised by a strong yellow colour, low specific volume and a dense and firm crumb. While a previous publication showed that the nutritional quality of flours made from pseudocereals or teff is better than that of wheat flour, their bread making properties and sensory characteristics compromise their suitability for the production of gluten free bread somewhat. However, their utilisation as part of a composite formulation could lead to an improvement of products.

8.6 Acknowledgements

8.7 References

Alvarez-Jubete L, Auty M, Arendt, EK, Gallagher E (2010) Baking properties and microstructure of pseudocereal flours in gluten-free bread formulations. European Food Research and Technology, 230(3), 437-445

Brites C, Trigo MJ, Santos C, Collar C, Rosell CM (2010). Maize-Based Gluten-Free Bread: Influence of Processing Parameters on Sensory and Instrumental Quality. Food and Bioprocess Technology, 3(5), 707-715

Bultosa G, Hall AN, Taylor JRN (2002) Physico-chemical Characterization of Grain Tef [Eragrostis Tef (Zucc.) Trotter] Starch. Starch/Staerke, 54, 461-468

Buttery RG, Guadagni DG, Ling LC (1976) Geosmin, a musty off-flavor of dry beans. J Agric Food Chemistry, 24, 419-420

Capristo E, Addolorato G, Mingrone G, De Gaetano A, Greco AV, Tataranni, PA, Gasbarrini, G (2000) Changes in body composition, substrate oxidation, and resting metabolic rate in adult celiac disease patients after a 1-y gluten-free diet treatment. American Journal of Clinical Nutrition, 72(1), 76-81

Cauvain S.P, Whitworth M.B, Alava J.M. (1999) The Evolution of Bubble Structure in Bread Doughs and Its Effect on Bread Structure. American Association of Cereal Chemists, St. Paul, Minnesota

Czerny M, Christlbauer M, Christlbauer M, Fischer A, Granvogl M, Hammer M, Hartl C, Moran-Hernandez N, Schieberle P (2008) Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. European Food Research and Technology, 228, 265-273

Dal Bello F, Clarke CI, Ryan LAM, Ulmer H, Schober TJ, Strom K, Sjogren J, van Sinderen D, Schnurer J, Arendt EK (2007) Improvement of the quality and shelf life of wheat bread by fermentation with the antifungal strain Lactobacillus plantarum FST 1.7. Journal of Cereal Science, 45(3), 309-318

Dickey W and Kearney N (2006) Overweight in celiac disease: prevalence, clinical characteristics, and effect of a gluten-free diet. American Journal of Gastroenterology, 101, 2356-2359

Flander L, Salmenkallio-Martilla M, Suortti T, Autio K. (2007) Optimization of ingredients and baking process for improved wholemeal oat bread quality. LWT - Food Science and Technology 40, 860-870

Gujral H.S, Guardiola I, Carbonell J.V, Rosell C.A. (2003) Effect of cyclodextrinase on dough rheology and bread quality from rice flour. Journal of Agriculture and Food Chemistry 51(13), 3814-3818

Hager AS, Wolter A, Jacob F, Zannini E, Arendt EK (2012) Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours. Journal of Cereal Science, 56, 239-247

Huettner E.K, Dal Bello F, Arendt E.K. (2010) Fundamental study on the effect of hydrostatic pressure treatment on the bread-making performance of oat flour. European Food Res earch and Technology, 230(6), 827-835

Hugo L.F, Rooney L.W, Taylor J.R.N. (2003) Fermented sorghum as a functional ingredient in composite breads. Cereal Chemistry, 80(5), 495-499

Mezaize S, Chevallier S, Le Bail A, de Lamballerie M. (2009) Optimization of Gluten-Free Formulations for French-Style Breads. Journal of Food Science, 74(3), E140-E146

Mohammed M.I.O, Mustafa A.I, Osman G.A.M. (2009) Evaluation of wheat breads supplemented with Teff (Eragrostis tef (ZUCC.) Trotter) Grain flour. Australian Journal of Crop Science, 3(4), 207-212

Moore M.M, Juga B, Schober T.J, Arendt E.K. (2007) Effect of lactic acid bacteria on properties of gluten-free sourdoughs, batters, and quality and ultrastructure of gluten-free bread. Cereal Chemistry, 84(4), 357-364

Moore M.M, Schober T.J, Dockery P, Arendt E.K. (2004) Textural comparisons of gluten-free and wheat-based doughs, batters, and breads. Cereal Chemistry, 81(5), 567-575

Nunes M.H.B, Ryan L.A.M, Arendt E.K. (2009) Effect of low lactose dairy powder addition on the properties of gluten-free batters and bread quality. European Food Research and Technology 229:31-41

Renzetti S, Dal Bello F, Arendt E.K. (2008) Microstructure, fundamental rheology and baking characteristics of batters and breads from different gluten-free flours treated with a microbial transglutaminase. Journal of Cereal Science, 48, 33-45

Rojas J.A, Rosell C.M, de Barber C.B, Perez-Munuera I, Lluch M.A. (2000) The baking process of wheat rolls followed by cryo scanning electron microscopy. European Food Research and Technology 212(1), 57-63

Rosell CM and Santos E (2010) Impact of fibers on physical characteristics of fresh and staled bake off bread. Journal of Food Engineering, 98(2), 273-281

Rosell C.M, and Marco C. (2008) Rice. In: Arendt EK, Dal Bello F (ed) Gluten-free cereal products and beverages. Elsevier Academic Press, Massachusetts

Rosell C.M, Cortez G, Repo-Carrasco R. (2009) Breadmaking Use of Andean Crops Quinoa, Kaniwa, Kiwicha, and Tarwi. Cereal Chemistry, 86(4), 386-392

Sanni A.I, Onilude A.A, Fatungase M.O. (1998) Production of sour maize bread using starter-cultures. World Journal of Microbiology and Biotechnology, 14(1), 101-106

Scalon M.G, Zghal M.C. (2001) Bread properties and crumb structure. Food Research International, 34, 841-864

Schakel S.F, Jasthi B, Van Heel N, Harnack L. (2009) Adjusting a nutrient database to improve calculation of percent calories from macronutrients. Journal Food Composition and Analysis, 22, S32-S36

Schober T.J. (2009) Manufacture of Gluten-Free Specialty Breads and Confectionery Products. In: Gallagher. E. (ed) Gluten-Free Food Science and Technology, Blackwell Publishing Ltd, Chichester

Schober T.J, Bean S.R. (2008) Sorghum and Maize. In: Arendt EK, Dal Bello F (ed) Gluten free Cereal Products and Beverages. Elsevier, Burlington, Elsevier Academic Press, Massachusetts

Schober T.J, Bean S.R., Boyle D.L. (2007) Gluten-free sorghum bread improved by sourdough fermentation: Biochemical, rheological, and microstructural background. J Agric Food Chemistry, 55(13), 5137-5146

Schuh C., Schieberle P. (2006) Characterization of the key aroma compounds in the beverage prepared from Darjeeling black tea: quantitative differences between tea leaves and infusion. Journal of Agriculture and Food Chemistry, 54, 916-924

Sciarini L.S, Ribotta P.D, Leon A.E, Perez G.T (2010) Influence of Gluten-free Flours and their Mixtures on Batter Properties and Bread Quality. Food Bioprocess Technology, 3(4), 577-585

Seyer M.E, Gelinas P. (2009) Bran characteristics and wheat performance in whole wheat bread. International Journal of Food Science and Technology, 44(4), 688-693

Sluimer P. (2005) Principles of Breadmaking, Functionality of Raw Materials and process Steps. American Association of Cereal Chemists. St. Paul, Minnesota

Smecuol E, Gonzalez D, Mautalen C, Siccardi A, Cataldi M, Niveloni S, Mazure R, Vazquez H, Pedreira S, Soifer G, Boerr L.A, Maurino E, Bai J.C. (1997) Longitudinal study on the effect of treatment on body composition and anthropometry of celiac disease patients. American Journal of Gastroenterology, 92(4), 639-643

Thompson T. (2003) Oats and the gluten free diet. Journal of the American Dietetic Association, 103(3), 376-379

Vallons K.J.R, Ryan L.A.M, Koehler P, Arendt E.K. (2010) High pressure-treated sorghum flour as a functional ingredient in the production of sorghum bread. European Food Research and Technology, 231(5), 711-717

Wrigley C, Corke H, Walker C.E. (2004) Encyclopedia of grain science. 2nd edn. Elsevier Academic Press, Oxford

Chapter 9: Development of gluten free fresh egg pasta based on oat and teff flour

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9.1 Summary

Due to increased awareness of consumers about the relationship between food and health as well as the requirements of people following a gluten free diet, the production of cereal products from raw materials other than wheat is of interest. However, the elimination of the visco-elastic gluten protein represents a technological challenge. During this study, response surface methodology was applied to determine optimal formulations for the production of egg pasta from oat and teff flour. Wheat flour was used as a control. The resulting products were characterised regarding firmness and elasticity, stickiness and cooking loss. The results showed that the mechanical texture of oat and teff pasta was comparable to wheat pasta, however elasticity was significantly reduced. Compositional analysis was carried out on flour raw materials as well as on the final pasta products, showing that regarding fiber and mineral content, oat and teff samples are nutritionally superior to wheat. In addition the microstructure was investigated by means of scanning electron microscopy, allowing also the observation of structural changes occurring during cooking. Upon cooking, a distinct outer layer can be observed, resulting from protein denaturation and starch gelatinisation. This structural feature is clearly visible for cooked wheat pasta and but is less apparent for teff and oat pasta.

9.2 Introduction

Pasta is a highly convenient food product, consumed all over the world. The term usually refers to unleavened extruded wheat dough, composed simply of flour and water, sometimes egg. A significant part of the human population however cannot tolerate gluten, a protein composite found in wheat, rye and barley. Hence, it is necessary to develop products based on alternative

cereals or pseudocereals. Also for the non-coeliac population, switching from refined wheat products to nutritionally more valuable grains could bring benefits regarding health and well-being. Oat (*Avena sativa*), an annual plant grown throughout the temperate regions, is an important cereal crop mainly used as livestock feed. Recently, interest in the utilisation of oats for human consumption has increased due to a health claim granted by the European Food Safety Agency (EFSA). Based on scientific evidence the EFSA concluded that a cause and effect relationship exists between the consumption of beta-glucans and the reduction of blood cholesterol concentrations. Therefore a health claim has been granted and the following wording reflects the scientific evidence: "Regular consumption of beta glucans contributes to maintenance of normal blood cholesterol concentrations" (EFSA Panel on Dietetic Products 2009). Besides, wholegrain oats also contain high amounts of unsaturated fatty acids, minerals, vitamins and phytochemicals (Emmons and Peterson 2001). Protein found in oats is known to be nutritionally superior to that of wheat, due to higher lysine contents, a limiting amino acid in most other cereals (Lastizy 1996). Its status in a gluten free diet is however controversial. Most but not all people with intolerance to gluten can include oats in their diet without adverse effect on their health (The Commission of the European Communities 2009). Teff (*Eragrostis tef*), which can be considered a minor crop when compared to the above mentioned, originates from Ethiopia where it is used for the production of several types of flat bread (Tatham *et al*. 1996). It is a small-seeded annual grass, falls into the group of millet and hence is gluten free. From a nutritional point of view, teff is a highly valuable grain as it contains high levels of calcium, magnesium and iron as well as folate (Hager *et al*. 2012). Commercial gluten free pasta often shows significantly lower protein contents compared to wheat containing counterparts. Hence, the utilisation of teff is beneficial as this cereal contains higher amounts of protein than many other flours (Hager *et al*. 2012). Apart from the utilisation of high quality raw materials, enrichment with additional protein ingredients is another approach to improve the nutritional value of gluten free products. An obvious

ingredient to increase the protein content of pasta is egg. Eggs are traditionally used in pasta mainly to achieve flavour effects (Antognelli 1980), but can also aid structure formation. Egg proteins facilitate the formation of a tighter protein network, yielding a harder product, both before and after cooking. In addition, the tighter protein network reduces penetration by water and hence starch granule swelling during cooking (Garcia-Estepa *et al*. 1999). Dehydrated egg products offer many advantages such as reduced microbial hazard and prolonged shelf life, lower storage and transport costs. Research on gluten free pasta is limited. Wang *et al*. used oat flour and egg albumin to produce gluten free noodles (Wang and Tilley 2011). Chillo *et al*. (2009) produced oat pasta, adding carboxymethylcellulose and pregelatinized starch as structuring agents. To the author's knowledge, no publications exist on the production of pasta from teff. The aim of this study was to develop oat and teff pasta of similar quality to wheat pasta. A central composite design and response surface methodology (RSM) was applied to determine the optimal formulation in a minimal number of experimental trials. RSM has been successfully applied previously in order to develop gluten free pasta from quinoa (Caperuto *et al*. 2001), rice brokens (Raina 2005), sweet potato and soy flour (Singh *et al*. 2003) as well as quinoa, maize and soy flour (Mastromatteo *et al*. 2011). The teff, oat and wheat pasta produced during this study were characterised regarding their chemical composition, mechanical texture, stickiness and cooking loss. In addition scanning electron microscopy was used to investigate the microstructure and observe changes occurring during cooking.

9.3 Materials and Methods

9.3.1 Raw materials

The suppliers for ingredients used were Odlums, Ireland, for white wheat flour (12.7 % moisture); Trouw, The Netherlands, for wholegrain white teff flour (9.5 % moisture) and E. Flahavan & Son Ltd, Ireland, for wholegrain oat flour (10.4 % moisture). Desugared spray dried

egg albumin powder (High gel) was sourced from Ovovita, Poland and monoglycerides of edible fatty acids (MULTEC MONO 9402 sfp KOSHER) were supplied by Puratos, Belgium.

9.3.2 Compositional analysis of raw materials

Crude fat, protein and moisture content of flours were determined according to the AACC methods 30-10, 46-12 and 44-15A, respectively (AACC International 1995a; AACC International 1995b; AACC International 1995c). Protein content was calculated with a protein factor of 6.25, except for wheat flours where 5.83 was used. Ash content was determined according to Matissek (2006). Total starch levels were determined using an enzyme kit (K-TSTA) supplied by Megazyme, Ireland.

9.3.3 Experimental design

Response surface methodology was used to evaluate the effect of the independent variables (level of water, emulsifier and egg white powder) on the dependent variables (firmness, elasticity and cooking loss). Hereupon, optimum ingredient levels could be determined. A circumscribed, two-dimensional central composite design was developed featuring variations in the addition levels of water (ranging from 37.5-47.5 % based on 100 % oat and teff flour, and from 32.5-37.5 % based on 100 % wheat flour), egg white powder (ranging from 12.5-17.5 % based on 100% oat and teff flour, and from 5-10 % based on 100 % wheat flour) and emulsifier (ranging from 0-2 % based on 100% flour). The upper and lower limits of these levels were selected based on preliminary trials conducted (results not shown). A total of 13 trials were carried out, comprising four for the factorial, six for the axial and three as central points. The response of each of the investigated parameters was analysed by fitting quadratic models to the data with least square regression in order to identify significant ($p < 0.05$) effects of the variations in ingredient levels on the responses. Three dimensional graphs for the models were used to visualise overall trends.

9.3.4 Pasta production

Fresh pasta was produced using a benchtop cold extrusion single barrel system (Häussler, Germany) equipped with a spaghetti nozzle (diameter of holes: 2 mm). Dry ingredients were sifted, premixed in the mixing chamber of the pasta machine and water (at 50°C) was added. Total mixing time was 10 min. Every 2 min the mixing process was stopped and the dough was scraped down from the edges of the chamber. Dough was extruded and the first 25 cm of spaghetti were discarded. After that samples of 25 cm or 5 cm length were taken for elasticity or firmness and stickiness measurements, respectively. For determination of pasta composition, spaghetti were stored frozen until analysis.

9.3.5 Cooking process

Optimum cooking time for wheat pasta was the time required for the opaque central core of the noodle to disappear when squeezed gently between two glass plates (Approved Method 16-50) (AACC International 1995d). This method was not applicable for teff and oat samples. Hence, cooking time was chosen based on empirical observations. An optimal cooking time of 10 min was chosen for wheat and oat pasta, while teff pasta was cooked for 12 min. These cooking times were used for determination of elasticity, firmness and imaging using scanning electron microscopy. To compare stickiness, all samples were cooked for 12 min.

9.3.6 Cooking loss

Dry matter losses during cooking were determined by AACC Approved Method 16-50 (AACC International 1995d). Pasta samples (25 g) were cooked to optimum time in 300 mL of distilled water in a beaker, rinsed in a stream of cold water for 30 sec and drained. Cooking and rinse water were collected and the volume made to 500 mL. Three aliquots of 20 mL were evaporated to dryness in an air oven set to 100°C and the average weight of the residues in the glass beaker was calculated. The weight of the residue multiplied by 100 equals percentage cooking loss.

9.3.7 Pasta Texture

Pasta texture was evaluated using a TA.XT2i texture analyzer system (Stable Micro Systems, Surrey, UK). Firmness of the cooked pasta was measured by AACC Method 16-50 (AACC International 1995d) for pasta with modifications. Firmness is defined as the work required to cut a defined amount of pasta. A light knife blade attachment was used to cut 5 strands of cooked pasta and peak force was recorded as firmness (N). Test parameters were set as follows: test speed = 0.17 mm/sec; distance = 4.5 mm. Pasta stickiness was evaluated with a pasta firmness/stickiness rig using the following settings: test speed = 0.5 mm/sec; compression force = 1000 g; compression time = 2 sec; withdrawal distance = 10.0 mm. Stickiness is defined as the maximum peak force to separate the probe from the sample's surface upon probe retraction (the higher the force value, the stickier is the sample). To get an idea of pasta elasticity, force at rupture was recorded. One pasta strand per measurement was fixed onto the rig by wrapping one end around the lower arm of the spaghetti tensile rig. Then the other end was wrapped around the upper arm, resulting in a 15 mm long pasta strand between the two arms. The strand was stretched until breaking by pulling apart the two arms using the following test parameters: test speed = 3.0 mm/sec; distance = 100 mm. Force at rupture was recorded. Due to the high standard deviations obtained for this test, elasticity results are the average of 15 measurements.

9.3.8 Compositional analysis of pasta

Compositional analysis of pasta was carried out on the uncooked extruded products produced using the optimized recipies (Table 9.5). Crude fat, protein and moisture content of pasta were determined according to the AACC methods 30-10, 46-12 and 44-15A, respectively (AACC International 1995a; AACC International 1995b; AACC International 1995c). Protein content was calculated with a protein factor of 6.25, except for wheat samples where 5.83 was used. Carbohydrate contents were calculated by difference [100 – (moisture + fat + protein + ash + fibre)]. For the determination of crude fibre a homogenised portion of the sample was

defatted and treated successively with boiling solutions of sulfuric acid and potassium hydroxide. The residue was separated by filtration on a sintered - glass filter, washed, dried, weighed, and ashed. The fibre was determined gravimetrically. The loss of weight resulting from ashing corresponds to the fibre present in the test sample (International Organisation for Standardisation 2000).

9.3.9 Scanning electron microscopy

Freeze-dried pasta samples were attached onto double-sided carbon tape fixed to an aluminium specimen stub and were preliminary gold-coated in a SEM coating system (BIORAD Polaron Division, UK) with a layer of 30 nm in thickness. Hereupon samples were examined under high vacuum in a field emission scanning electron microscope JSM-5510 SEM (JEOL Technics Ltd., Japan) with a working distance of 8 mm. Secondary electron images were acquired at an accelerating voltage of 5 kV. For the processing of the images, SEM Control User Interface, Version 5.21, (JEOL Technics Ltd., Japan) was used.

9.3.10 Statistical analysis

Design Expert Version 7 (Stat-Ease, U.S.A.) was used to generate surface response plots that permitted evaluation of the linear, quadratic and interactive effects of independent variables on the selected dependent variables ($p < 0.05$) and to optimise pasta formulations. Results of compositional analysis, firmness, elasticity, cooking loss and stickiness of optimised recipes are shown as average ± standard deviation of at least triplicate measurements. Analysis of Variance (One-way ANOVA), followed by Fisher LSD post hoc test, was performed to determine significant differences between pasta samples made from different flours. Statistica 7.1 (StatSoft, U.S.A.) was used for this purpose.

9.4 Results and Discussion

9.4.1 Compositional analysis of raw materials

Raw material characteristics are of primary importance in determining cooking quality of pasta. Table 9.1 shows the compositional data of the flours utilised. Proteins contribute significantly to texture and flavour of a food product. Protein content, one of the first parameters looked at when describing flour quality, is higher in teff flour (12.8 g/100 g) compared to wheat flour (11.5 g/100 g) and is significantly lower for oat flour (6.9 g/100 g). Next to proteins, also starch plays a major role in determining pasta quality. Regarding flour samples of this study, oat flour showed the highest starch level, followed by wheat and teff flour (Table 9.1). Fat content was lowest in wheat flour (1.8 g/100 g), while increased levels were detected in teff and oat flour (4.4 g/100 g and 6.7 g/100 g, respectively). Ash content was highest for teff flour (2.2 g/100 g), compared to 0.9 g/100 g for wheat and 0.8 g/100 g for oat flour.

Table 9.1 Composition of utilised raw materials expressed as g per 100 g flour

	Wheat	Teff	Oat
Protein	11.54 ± 1.07^{b}	12.84 ± 0.51^{a}	6.91 ± 0.08^{c}
Fat	1.81 ± 0.05^{c}	4.39 ± 0.26^{b}	6.74 ± 0.80^{a}
Total starch	68.06 ± 2.34^{ab}	57.77 ± 5.94^{b}	69.38 ± 1.66^{a}
Ash	0.92 ± 0.02^{b}	2.15 ± 0.05^{a}	0.82 ± 0.01^{c}

$ Values in one box followed by the same letter are not significantly different (p <0.05)

9.4.2 Pasta production

The production of pasta based solely on gluten free flours was unsuccessful. This is in agreement with previous authors who also found that additional structuring agents are necessary to obtain extrudable dough. Chillo *et al*. (2009) were not successful in the production of oat pasta unless carboxymethylcellulose and pregelatinised starch were added. Schoenlechner *et al*. (2010) screened different protein ingredients and found that egg white powder was superior for the improvement of gluten free pasta. During this study preliminary trials were carried out, aiming at evaluating sensory and textural properties of pasta produced with egg albumin, whole egg and egg yolk powders as well as fresh eggs. Albumin powder

showed the most significant textural improvement and hence was included into the formulations of this study. Sabanis and Tzia (2011) previously showed that a major role in the formation of a tight protein network is played by ovalbumin, the main protein of albumin, while an increase in yolk content worsens the structure of pasta.This is probably due to a dilution effect as the egg yolk contains mainly fat. Following preliminary trials determining the ingredient ratios necessary to obtain extrudable doughs, optimisation of the formulation was carried out using response surface methodology. The analysis of variance for quality of pastas is presented in Tables 9.2 to 9.4.

Table 9.2 Analysis of variance for evaluation of models for quality parameters of wheat pasta

Response		Source	df	Sum of Squares	F value	P value
Firmness		Model	3	11.84	48.36	<0.0001
		Residual	9	0.73		
R^2	0.94	Lack of fit	7	0.66	2.69	0.2975
CV, %	6.08	Pure error	2	0.070		
Elasticity		Model	3	1903.86	49.93	<0.0001
		Residual	9	114.38		
R^2	0.94	Lack of fit	7	101.89	2.33	0.3328
CV, %	6.51	Pure error	2	12.49		
Cooking loss		Model	9	3.60	7.84	0.0587
		Residual	3	0.15		
R^2	0.96	Lack of fit	1	0.077	2.02	0.2911
CV, %	6.22	Pure error	2	0.076		

Table 9.3 Analysis of variance for evaluation of models for quality parameters of oat pasta

Response		Source	df	Sum of Squares	F value	P value
Firmness		Model	3	6.63	46.98	<0.0001
		Residual	7	0.33		
R^2	0.95	Lack of fit	5	0.28	2.06	0.3587
CV, %	5.93	Pure error	2	0.054		
Elasticity		Model	3	677.12	20.00	0.0008
		Residual	7	78.98		
R^2	0.90	Lack of fit	5	52.86	0.81	0.6335
CV, %	9.13	Pure error	2	26.12		
Cooking loss		Model	6	0.62	1.31	0.4154
		Residual	4	0.32		
R^2	0.66	Lack of fit	2	0.075	0.31	0.7625
CV, %	9.14	Pure error	2	0.24		

Table 9.4 Analysis of variance for evaluation of models for quality parameters of teff pasta

Response		Source	df	Sum of Squares	F value	P value
Firmness		Model	9	6.24	31.45	0.0082
		Residual	3	0.066		
R^2	0.99	Lack of fit	1	0.062	30.52	0.0312
CV, %	4.76	Pure error	2	0.000		
Elasticity		Model	3	690.97	18.89	0.0005

		Residual	8	97.55		
R^2	0.88	Lack of fit	6	91.63	5.16	0.1713
CV, %	10.37	Pure error	2	5.92		
Cooking loss		Model	9	4.39	3.02	0.1971
		Residual	3	0.49		
R^2	0.90	Lack of fit	1	0.35	5.14	0.1516
CV, %	9.29	Pure error	2	0.14		

Significance of the lack-of-fit error term, R^2 value, coefficient of variation, and model significance were used to judge adequacy of model fit. The predictive models developed for firmness and elasticity of wheat and oat pasta was considered adequate because they possessed a non-significant lack of fit and had satisfactory levels of R^2, CV and model significance. The developed model for cooking loss for all three pasta products was less predictive because the model was not significant at the 5% level. Even though the model was not significant, explanatory analysis of data was performed in order to verify the tendency of this parameter. The predictive model for elasticity of teff pasta was adequate. However, regarding firmness, the model was less suitable as the lack of fit was significant. Nevertheless, a surface plot was generated for this model, because it offered a reasonable initial solution for describing the quality response of this parameter.

The detailed outcomes of the RSM trials are shown in Appendix Ib. To visualise the combined effects of egg white powder and water level, three-dimensional surface plots at an emulsifier level of 1%, were generated (Figure 9.1). Egg white powder had a positive linear effect on firmness and elasticity of wheat and oat pasta, while the water level had a negative linear effect on these parameters. Response surface plots for teff pasta show the same trends. This was observed previously by Wang and Tilley (2011), which found a significant increase in oat noodle firmness upon addition of egg albumin. The proteins form a network within the dough and thus albumin condensation occurring during the cooking process imparts firmness to the product (Schoenlechner 2010). Emulsifiers are commonly used for the production of gluten free pasta. Their fatty nature enables them to act as a lubricant in the extrusion process, resulting in less nozzle wear and tear and thus making production easier (Lai 2002). Nazarov

found that mono- and diglycerides of fatty acids form complexes with amylose, thereby preventing the passage of starch into the cooking water, reducing cooking loss and stickiness (Nazarov 1977). However, in this study emulsifier addition did not show a significant effect on any of the dependent variables.
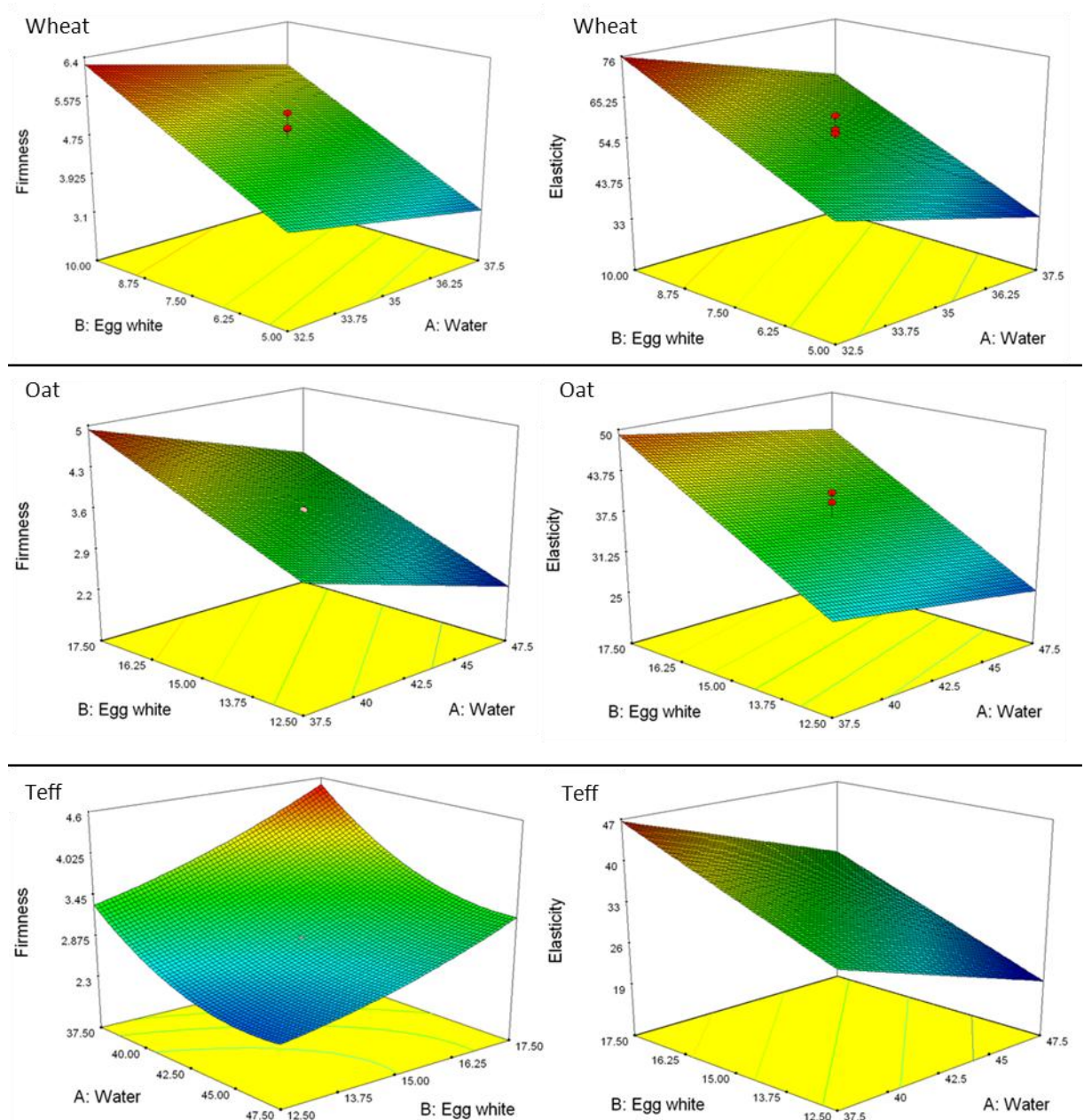
Figure 9.1 Effect of egg white powder and water level on firmness and elasticity of wheat, oat and teff pasta (at an emulsifier level of 1%)

Models were useful in indicating the direction in which to change variables in order to get optimum responses for texture and cooking quality. The multiple regression equations were solved for maximum firmness and elasticity and minimum cooking loss and the resulting

optimum ingredient ratios are shown in Table 9.5. Even though, egg white powder addition also improved pasta made from wheat flour, this effect was more pronounced for teff and oat pasta. Hence, the incorporation of higher levels of this ingredient into the latter two formulations was suggested by the software. Sufficiently high moisture content is required to ensure that the viscosity of the dough is low enough to avoid excessive pressure within the extruder (Kent and Evers 1994). The water addition levels required for optimal dough formation were higher for the teff and oat pasta, probably due to the increased egg white powder addition level of these formulations.

Table 9.5 Optimum ingredient levels in percent as predicted from response surface methodology

	Wheat	Teff	Oat
Flour	69.6	62.8	64.7
Water	22.8	25.1	24.3
Egg white powder	7.0	11.0	9.7
Emulsifier	0.6	1.1	1.3

Texture is a main concern of consumers, with sticky, soft pasta being generally unacceptable. A firm but elastic product is desired, i.e. pasta that is "al dente". In addition, low cooking loss is required. Predicted optimum responses for firmness, elasticity and cooking loss are shown in Table 9.6. These were experimentally tested, obtaining a firmness of 6 N (wheat), 5 N (teff) and 4 N (oat), a force at rupture of 75 g (wheat), 43 g (teff) and 41 g (oat), and cooking loss of 4 % (teff) and 3 % (wheat and oat). The experimental values at the optimum compositions were in general in good agreement with the predicted values. However, as mentioned above the fitted model for cooking loss was less descriptive and therefore for wheat pasta this value was underestimated by the statistics program. Pasta firmness is defined as the work (g*cm) required for the pasta blade to cut five strands of spaghetti positioned adjacent to each other. The value was highest for wheat pasta and comparable for oat and teff pasta. However, the problem with only recording this force is that it is possible for a sample to be firm and yet lack elasticity. Hence, end of elastic limit was determined by pulling apart a pasta strand using an extensibility rig. As expected, force at rupture was significantly higher for the wheat sample.

This is due to the unique visco-elastic properties of gluten. Several publications on gluten free pasta or pasta from non-traditional ingredients report that cooking loss is increased for these products, due to the absence or interruption of the gluten network (Schoenlechner *et al.* 2010). In this study however, cooking loss was not significantly different between oat and wheat pasta but was significantly higher for teff pasta. Contrary to general trends, the teff and oat samples did not show increased stickiness compared to wheat pasta. The value for teff pasta (0.075 N) is even significantly reduced when compared to oat and wheat (0.207 N and 0.209 N, respectively).

Table 9.6 Confirmation of pasta quality characteristics as predicted by response surface methodology compared to experimental values[\$]

		Predicted values	Measured values
Firmness [N]	Wheat	6.24	5.98 ± 0.20a
	Teff	3.99	4.52 ± 0.21b
	Oat	4.17	4.16 ± 0.15c
Elastic limit [g]	Wheat	75.30	74.53 ± 3.62a
	Teff	41.90	43.18 ± 10.72b
	Oat	38.24	40.66 ± 4.96b
Cooking loss [%]	Wheat	2.39	3.27 ± 0.40b
	Teff	4.02	4.06 ± 0.31a
	Oat	3.09	3.27 ± 0.04b
Stickiness [N]	Wheat	-	0.209 ± 0.059a
	Teff	-	0.075 ± 0.018b
	Oat	-	0.207 ± 0.030a

\$ Values in one box followed by the same letter are not significantly different (p <0.05)

9.4.3 Compositional analysis of pasta

Pasta was produced using the optimal formulations described in Table 9.5 and compositional analysis was carried out on these samples. The resulting data is shown in Table 9.7. Gluten free pasta often contains less protein than wheat pasta. Due to the addition of egg white powder as well as the utilisation of protein rich gluten free flours, contents were equal or much higher for the samples of this study (18 g/100 g for teff and 13 g/100 g for oat pasta) compared to the wheat pasta of this study (14 g/100 g) as well as commercial samples (3-10 g/100 g). Also crude fibre contents of teff (0.55 g/100 g) and oat (1.00 g/100 g) samples were significantly

higher than that of wheat pasta (<0.01 g/100 g). However, these values are still relatively low

and should be increased in order to get a nutritionally more balanced product; especially since

it was shown previously that the fibre consumption of coeliac patients as well as the general

public is often too low (Hager *et al.* 2011). Fat content was significantly lower for the wheat

pasta sample with 1.3 g/100 g compared to 2.0 g/100 g for teff and 4.8 g/100 g for oat pasta.

Mineral content was significantly higher in teff pasta (1.64 g/100 g), i.e. ash content is twice as

high as in oat and wheat pasta (0.81 and 0.74 g/100 g, respectively).

Table 9.7 Composition of uncooked pasta samples[$]

Sample	Protein [g/100g]	Moisture [g/100g]	Carbohydrate [g/100g]	Fat [g/100g]	Ash [g/100g]	Total dietaryfibre [g/100g]
Wheat	13.45±0.11b	32.86±0.18a	50.65	1.33±0.22c	0.74±0.01b	< 0.1
Teff	17.85±0.11a	31.59±0.06c	46.88	2.04±0.01b	1.64±0.04a	0.55 ± 0.14a
Oat	13.02±0.25c	32.01±1.58b	48.49	4.85±0.01a	0.81±0.07b	1.00 ± 0.49a

$ Values in one column followed by the same letter are not significantly different (p <0.05)

9.4.4 Scanning electron microscopy

Pasta samples before and after cooking were observed by means of scanning electron

microscopy. Freshly extruded pasta showed a compact internal structure with slightly swollen

starch granules embedded into the protein matrix (Figure 9.2-9.4, top rows). Great differences

existed in the starch granule morphology between the different raw materials used. Figure 9.2

shows wheat pasta, containing two types of starch granules: large lenticular (> 15 µm) and

smaller spherical ones (5 – 15 µm). Oat starch is a compound starch, comprised by granules of

up to 10 µm (Figure 9.3). Teff granules are polygonal in shape and between 2 and 7 µm in

diameter (Figure 9.4). A local increase in temperature during the extrusion process might have

led to starch gelatinisation in certain areas of the uncooked product, as reported by Petitot *et

al.* (2009), and this was particularly evident in the case of oat and teff pasta. The structural

changes occurring during the cooking process, mainly starch gelatinisation and protein

coagulation, are well reflected in the micrographs (Figure 9.2-9.4, bottom rows). Upon

cooking, a distinct outer layer can be observed. In the interspaces between granules, protein

denaturation and interaction formed a continuous and strengthened network, trapping

granular starch. The latter, by swelling and gelatinising, also obstructs interspaces. This structural feature is clearly visible for cooked wheat pasta and is responsible for the characteristic mouth feel referred to as "al dente". The distinct outer layer is less apparent for teff and oat pasta, explaining the failure of cooking time determination by compressing the spaghetti between two glass plates (AACC approved Method 16-50) (AACC International 1995d). The core areas of all samples show starch granules with a limited degree of gelatinisation, due to limited water absorption.
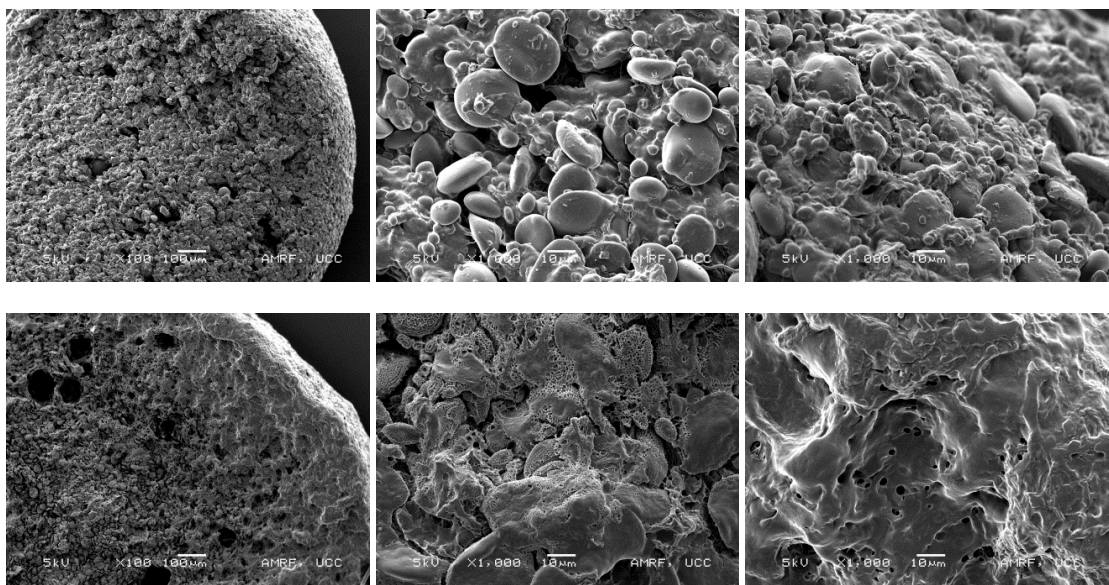
Figure 9.2 Scanning electron micrographs of uncooked (top row) and cooked (bottom row) wheat pasta: overview (left); core area (middle) and outer area (right)
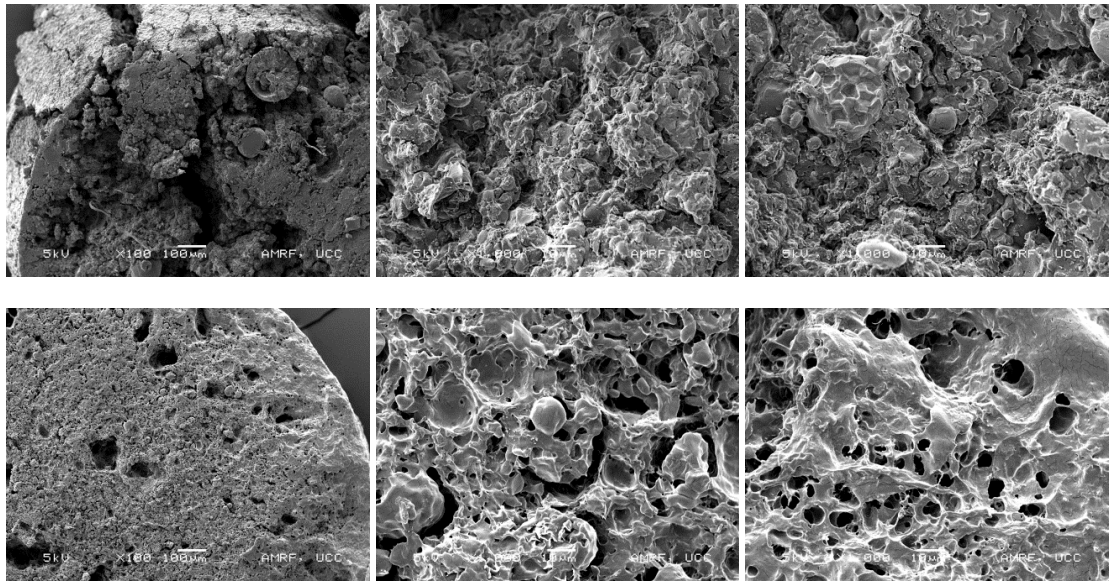
Figure 9.3 Scanning electron micrographs of uncooked (top row) and cooked (bottom row) oat pasta: overview (left); core area (middle) and outer area (right)
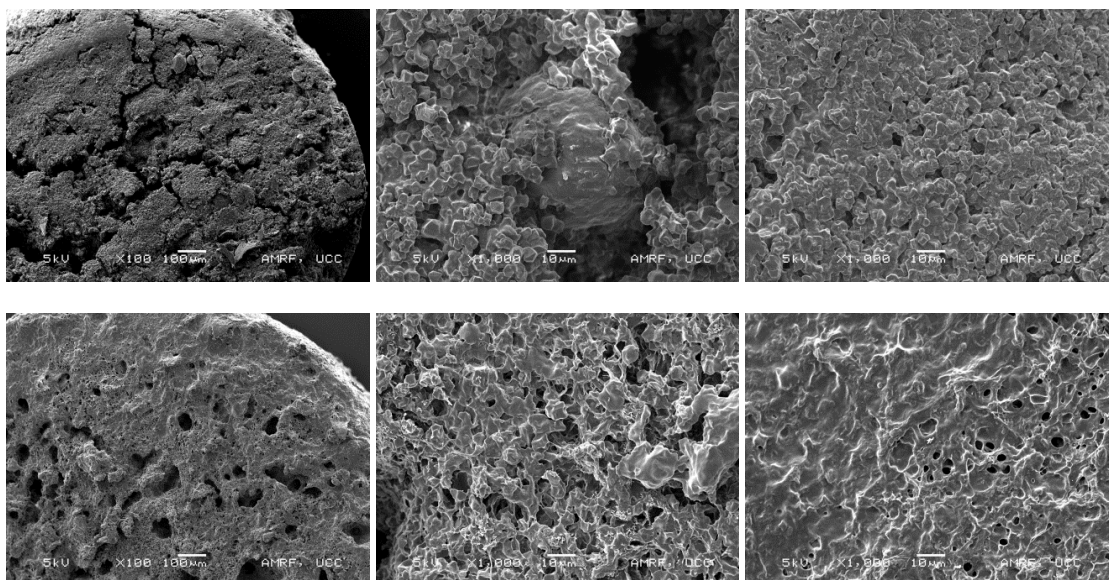
Figure 9.4 Scanning electron micrographs of uncooked (top row) and cooked (bottom row) teff pasta: overview (left); core area (middle) and outer area (right)

9.5 Conclusions

In this study, RSM was successfully used to identify the optimal formulations for egg pasta from wheat, oat and teff flours. Textural properties as well as protein content of resulting gluten free products were shown to be comparable to the produced wheat pasta. Pasta firmness and cooking loss were similar among the three samples. Force at rupture was highest for wheat pasta, indicating that the gluten free samples are less elastic. Stickiness of oat and wheat pasta was in the same range. Teff pasta showed significantly lower stickiness values.

The results clearly showed that teff and oat flour can be used to produce gluten free pasta of acceptable texture. However, also sensorial aspects and nutritional characteristics of the produced pasta have to be considered when evaluating food quality. Scanning electron microscopy represents a well suited tool to study the ultra-structure of pasta, allowing also the observation of structural changes occurring during cooking. Upon cooking, a distinct outer layer can be observed, resulting from protein denaturation and starch gelatinisation. This structural feature is clearly visible for cooked wheat pasta but is less apparent for teff and oat pasta.

9.6 Acknowledgements

9.7 References

AACC International (1995a) Approved Methods of Analysis, 9th Ed. Method 30-10 Crude Fat. In: Flour, Bread, and Baked Cereal Products Not Containing Fruit. AACC International, St. Paul, MN, U.S.A.

AACC International (1995b) Method 46-12 Crude Protein—Kjeldahl Method, Boric Acid Modification. In: Approved Methods of Analysis, 9th Ed. AACC International, St. Paul, MN, U.S.A.

AACC International (1995c) Method 44-15A Moisture—Air-Oven Methods. In: Approved Methods of Analysis, 9th Ed. AACC International, St. Paul, MN, U.S.A.

AACC International (1995d) Method 16-50 Pasta Cooking Quality - Firmness. In: Approved Methods of Analysis, 9th Ed. AACC International, St. Paul, MN, U.S.A.

Antognelli C. (1980) The manufacture and applications of pasta as a food and as a food ingredient – a review. Journal of Food Technology, 15(2), 125-145

Caperuto L.C, Amaya-Farfan J, Camargo C.R.O (2001) Performance of quinoa (Chenopodium quinoa Willd) flour in the manufacture of gluten free spaghetti. Journal of the Science of Food and Agriculture, 81(1), 95-101

Chillo S, Civica V, Iannetti M, Suriano N, Mastromatteo M, Del Nobile M.A. (2009) Properties of quinoa and oat spaghetti loaded with carboxymethylcellulose sodium salt and pregelatinized starch as structuring agents. Carbohydrate Polymers, 78(4), 932-937

EFSA Panel on Dietetic Products (2009) Scientific Opinion on the substantiation of health claims related to beta-glucans and maintenance of normal blood cholesterol concentrations (ID 754, 755, 757, 801, 1465, 2934) and maintenance or achievement of a normal body weight (ID 820, 823). EFSA Journal, 7, 1254

Emmons C.L. and Peterson D.M. (2001) Antioxidant activity and phenolic content of oat as affected by cultivar and location. Crop Science, 41(6), 1676-1681

Garcia-Estepa R.M, Guerra-Hernandez E, Garcia-Villanova B. (1999) Phytic acid content in milled cereal products and breads. Food Research International, 32(3), 217-221

Hager A.S, Axel C, Arendt E. (2011) Status of Carbohydrates and Dietary Fiber in Gluten-free Diets. Cereal Foods World, 56(3), 109-114

Hager A.S, Wolter A, Jacob F, Zannini E, Arendt E.K. (2012) Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours. Journal of Cereal Science, 56, 239-247

International Organisation for Standardisation (ISO) (2000) EN ISO 6865 Animal feeding stuffs – Determination of crude fibre content – Method with intermediate filtration

Kent N.L, Evers A.D, (1994) Technology of Cereals. An Introduction for Students of Food Science and Agriculture 4th edn, Woodhead Publishing, Cambridge

Lai H.M. (2002) Effects of rice properties and emulsifiers on the quality of rice pasta. Journal of the Science of Food and Agriculture, 82(2), 203-216

Lastizy R. (1996) The Chemistry of Cereal Proteins. 2nd Edition. CRC Press, Florida

Manthey F.A, Hall C.A. (2007) Effect of processing and cooking on the content of minerals and protein in pasta containing buckwheat bran flour. Journal of the Science of Food and Agriculture, 87(11), 2026-2033

Mastromatteo M, Chillo S, Iannetti M, Civica V, Del Nobile M.A. (2011) Formulation optimisation of gluten-free functional spaghetti based on quinoa, maize and soy flours. International Journal of Food Science and Technology, 46(6), 1201-1208

Matissek R. and Steiner G. (2006) Bestimmung des Gluehrueckstandes durch direkte Veraschung (Aschegehalt). In: Lebensmittelanalytik –Grundzuege, Methoden, Anwendungen. Springer-Verlag, Berlin

Nazarov I.N. (1977) Technology and Equipment for the Food Industry, Food Industry Press, Moscow

Petitot M, Abecassis J, Micard V. (2009) Structuring of pasta components during processing: impact on starch and protein digestibility and allergenicity. Trends in Food Science and Technology, 20, 521-532

Raina CS, Singh S, Bawa A S, Saxena DC (2005) Formulation of pasta from rice brokens: optimization of ingredient levels using response surface methodology. Eur Food Res Technol 220(5-6):565-574

Sabanis D and Tzia C (2011) Selected Structural Characteristics of HPMC-Containing Gluten Free Bread: A Response Surface Methodology Study for Optimizing Quality. Int J Food Properties 14(2):417-431

Schoenlechner RJ, Drausinger J, Ottenschlaeger V, Jurackova K, Berghofer E (2010) Functional Properties of Gluten-Free Pasta Produced from Amaranth, Quinoa and Buckwheat. Plant Food Hum Nutr 65(4):339-349

Singh N, Singh J, Kaur L, Sodhi NS, Gill BS (2003) Morphological, thermal and rheological properties of starches from different botanical sources. Food Chem 81(2):219-231

Tatham AS, Fido RJ, Moore CM, Kasarda DD, Kuzmicky DD, Keen JN, Shewry PR (1996) Characterisation of the major prolamins of tef (Eragrostis tef) and finger millet (Eleusine coracana). J Cereal Sci 24(1):65-71

The Commission of the European Communities (2009) Commission Regulation (EC) No 41/2009 concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten

Wang F, Tilley M (2011) Effects of transglutaminase on the rheological and noodle-making characteristics of oat dough containing vital wheat gluten or egg albumin. J Cereal Sci 54(1):53-59

Chapter 10: Starch properties, *in vitro* digestibility and sensory evaluation of fresh egg pasta produced from oat, teff and wheat flour

Anna-Sophie Hager, Michael Czerny, Jürgen Bez, Emanuele Zannini, Elke K. Arendt (2012)

10.1 Abstract

Specific dietary requirements, e.g. coeliac disease, as well as increased consumer demand for products of high nutritional value, makes the production of pasta from alternative cereals interesting. Raw material characterisation showed that the utilisation of oat and teff flour is beneficial as these ingredients contain higher levels of fibre and mineral composition is superior to that of wheat. Starch properties significantly influence pasta quality and therefore damaged starch level, amylase activity, pasting properties and gelatinisation temperatures of the flours were investigated. Fresh egg pasta based on wheat, oat and teff flour was produced. Sensory properties of oat spaghetti were found to be very close to that of wheat pasta but improvement of smoothness and aroma is necessary, while teff spaghetti showed reduced sensory quality. An *in vitro* enzymatic digestion was performed using a dialysis system to mimic the behaviour of pasta as eaten and make predictions on the glycemic index (GI). The predicted GI was highest for wheat pasta, followed by teff and oat. Ultra structure was studied using confocal laser scanning microscopy, allowing the visualisation of differences in starch granule size and shape as well as gelatinisation occurring during the cooking process.

10.2 Introduction

Coeliac disease is an immune mediated disease causing inflammation in the small intestine. The condition is triggered by the ingestion of gluten, a storage protein found in wheat, rye and barley (Hill *et al*. 1995). Currently, the only available treatment is the exclusion of gluten from the diet. This makes the availability of high quality cereal products produced from alternative raw materials necessary. The peptides triggering coeliac disease are contained in the prolamin fraction of wheat proteins. Even though also panicoideae such as teff contain significant amounts of prolamins, this group of storage proteins has separate evolutionary origins of those in triticeae and this grain is therefore not coeliac-toxic (Shewry and Halford 2002). The status of oat in a gluten free diet is controversial because although composition and amino acid sequence of its storage proteins (avenins) differ from those found in wheat, it is not clear yet, if oat elicits an immune response in coeliac disease patients (The Commission of the European Communities 2009). The production of foods from alternative grains represents a challenge, as wheat gluten possesses exceptional structure forming potential unmatched by any other cereal protein. In a previous study (Hager *et al*. 2012b) response surface methodology was used to develop optimal formulations for the production of teff, oat and wheat pasta. Literature on the production of pasta from gluten free raw materials is scarce. Wang *et al*. (2011) used oat flour and egg albumin to produce gluten free noodles. Chillo *et al*. (2009) produced oat pasta, adding carboxymethylcellulose and pregelatinized starch as structuring agents. To the author's knowledge, no publications exist on the production of pasta from teff. Regarding wheat pasta, usually semolina is used and only little information on pasta made from wheat flour is available (Majzoobi *et al*. 2012; Vernaza *et al*. 2012). In a previous study response surface methodology was used for the first time to develop optimal formulations for the production of fresh egg pasta based on teff, oat and wheat flour (Hager *et al*. 2012b). Egg white powder was incorporated into the formulations to improve firmness and elasticity as well as protein content of the resulting spaghetti. The chosen egg addition level

represents a compromise between improvement of functional and nutritional properties and achievement of satisfactory sensory properties. Composition and texture of the resulting products were compared to each other, showing that the produced gluten free samples were of similar quality to that of wheat pasta. However, when discussing the quality of a food product, also its nutritional and sensory characteristics are of importance. Hence, these were evaluated as part of this work.

Gluten-free pasta is often characterised by low palatability, exhibiting poor mouth feel and flavour. To gain information on the aroma profile and overall liking of the spaghetti produced during this study, sensory evaluation was carried out by a trained panel.

Many of the screened commercial products show undesirable colouring. The bright yellow colour of conventional pasta products is one of the main quality characteristics and visual appearance of the uncooked product plays a key role in consumer's choice. These are often compromised when using non-traditional raw materials for pasta making; hence colour measurements have been carried out during this study.

Pasta is a carbohydrate based food, with conventional wheat semolina pasta containing approximately 70 % starch. Starch is an important part of a balanced diet and degree of digestion and absorption is affected by a number of factors. The Glycemic index (GI) is a model ranking carbohydrate containing foods from 0-100 based on their postprandial blood glucose response. Not all carbohydrates illicit the same response: highly refined grains have a high GI, whereas wholegrain products tend to have a low GI. Pasta is considered a source of slowly released carbohydrates, therefore possessing a low GI. Starch that is rapidly digested and absorbed in the small intestine produces undesirably high blood-glucose and insulin levels after a meal. The short duration of the absorptive phase stimulates the release of free fatty acids from the liver, which in turn can cause insulin resistance upon consumption of the following meal. Insulin resistance is considered a potential risk factor for the development of a

number of diseases such as diabetes, atherosclerosis and obesity. Simultaneous occurrence of type 1 diabetes mellitus and coeliac disease can be observed frequently (Walker-Smith *et al*. 1969). This makes the control of blood glucose response in the gluten-free diet an important issue (Franzese *et al*. 2011). The GI represents the relative rate of entry of glucose in the bloodstream compared with a reference carbohydrate source (white wheat bread). Previous research has demonstrated that the rate of *in vitro* amylolysis using a multi-enzyme dialysis system corresponds well with the rate of starch uptake *in vivo* as judged from the postprandial blood glucose response (Singh *et al*. 2010). Hence, this method has been applied in the course of this study to predict and compare the GI of the different pasta samples.

Ultrastructure of the pasta samples was investigated in a previous publication by means of scanning electron microscopy (SEM) (Hager *et al*. 2012b). The resulting micrographs provide valuable information on the differences in starch granule size and shape and illustrate well the processes happening during cooking. However, in the external region, starch granules are largely deformed, swollen and it is difficult to differentiate them from proteins when viewed under SEM (Petitot 2009). Therefore, confocal laser scanning microscopy was chosen in this study, where compounds can be selectively stained.

10.3 Materials and Methods

10.3.1 Raw materials
The suppliers for the ingredients used were Odlums, Ireland, for wheat flour (12.7 % moisture); Trouw, The Netherlands, for wholegrain white teff flour (9.5 % moisture) and E. Flahavan & Son Ltd., Ireland, for wholegrain oat flour (10.4 % moisture). Desugared spray dried egg albumin powder (High gel) was sourced from Ovovita, Poland and monoglycerides of edible fatty acids (MULTEC MONO 9402 sfp KOSHER) were supplied by Puratos, Belgium.

10.3.2 Pasta production

Pasta was produced as described by Hager *et al*. (2012b) using a benchtop cold extrusion single barrel system (Häussler, Germany) equipped with a spaghetti nozzle. The formulations consisted of the following ingredients: 69.6 % wheat flour, 22.8 % water, 7.0 % egg white powder, 0.6 % emulsifier for wheat pasta; 62.8 % teff flour, 25.1 % water, 11.0 % egg white powder, 1.1 % emulsifier for teff pasta; 64.7 % oat flour, 24.3 % water, 9.7 % egg white powder, 1.3 % emulsifier for oat pasta. Dry ingredients were sifted, premixed in the mixing chamber of the pasta machine and water (at 50°C) was added. Total mixing time was 10 mins. Dough was extruded and pasta was stored frozen until analysis.

10.3.3 Composition of flour raw materials

Crude fat, protein and moisture content of flours were determined according to the AACC methods 30-10, 46-12 and 44-15A, respectively. Protein content was calculated with a protein factor of 6.25, except for wheat flours where 5.7 was used. Ash content was determined according to Matissek (2006). Dietary fibre and phytate levels were determined using enzyme kits (K-TDFR, K-PHYT) supplied by Megazyme, Ireland. Polyphenol content was determined according to Alvarez-Jubete *et al*. (2010). Minerals were analyzed by ICP-AES following the method EN ISO 11885 E22. The chloride concentration of flours was determined according to the MEBAK Analysenkommision (1996).

10.3.4 Amylase activity and starch properties of flour raw materials

Total and damaged starch levels, as well as α-amylase activity ratio were determined using enzyme kits (K-TSTA, K-SDAM, and K-CERA, respectively) supplied by Megazyme, Ireland. Pasting properties were determined using a Rapid Visco Analyser (Newport Scientific, Warriewood, Australia). Measurements were performed using approximately 3 g of sample (moisture content of flours was used to calculate exact weight of powder to be used in order to keep solid-to-liquid ratio constant). The profile was held at 50°C for 1 min, ramp to 95°C over 3.8 min, held at 95°C for 2.5 min, cooled back to 50°C over 3.8 min and held at 50°C for

1.4 min each analysis took 12.5 min and was done in triplicate. The gelatinisation

temperatures were determined by differential scanning calorimetry (DSC). Experiments were

carried out on a Mettler Toledo DSC821^e instrument. Flour samples of 3-5 mg were weighed

directly into aluminium pans and 10 mg of water were added. An empty container was used as

reference. Samples were heated from 25°C to 105°C at a rate of 5°C/min and onset

temperatures (T_O), peak temperature (T_P) as well as end temperature (T_E) were recorded.

Triplicate measurements were conducted.

10.3.5 *In vitro* digestibility of starch

In vitro digestibility of starch was evaluated according to Brennan and Tudorica (2008) by a

multi-enzymic digestion within dialysis tubing followed by analysis of reducing sugars released

to permeate. In addition to the three pasta samples, a white wheat bread (prepared as

described in Hager *et al*. (2012a) using 2% salt, 2% sugar, 3% yeast and 63% water based on

flour) was included into the study as reference. Aliquots of 4 g cooked pasta or bread were

mixed with 20 mL sodium potassium phosphate buffer (0.2 M, pH 6.9), pH was adjusted to 1.5

and 5 mL pepsin solution was added (pepsin 115 U ml^{-1}, EC 3.4.23.1, 674 U mg^{-1} solid, Sigma

Aldrich, Ireland). The mixture was incubated for 30 min at 37°C. Hereupon pH was readjusted

to 6.9 with 6 N NaOH and 1 mL of porcine pancreatic α-amylase solution was added (α-

amylase 110 U ml^{-1}, EC 3.2.1.1, 22 U mg^{-1} solid, Sigma Aldrich, Ireland). The mixture was filled

up to 50 mL and then transferred into dialysis tubes (Sigma Aldrich, Ireland). Glass beads were

added to mimic peristalsis. The tubes were placed into glass beakers containing 450 mL

sodium potassium phosphate buffer (pH 6.9) and incubated for 300 min at 37°C. Tubes were

inverted every 15 min. Every 30 min aliquots of 1 mL dialysate were withdrawn and the

volume was replaced each time with 1 mL sodium potassium phosphate buffer. The reducing

sugar content of the withdrawn dialysates was determined using the 3,5 dinitrosalicilic acid

method. Therefore, 100 µL dialysate together with 100 µL 3,5-dinitrosalicylic acid (DNS)

reagent were boiled for 10 min. The reaction tubes were cooled immediately on ice and

diluted with 1 mL distilled H$_2$O. Absorbance was measured at 546 nm. A standard curve using maltose (Sigma Aldrich, Ireland) was prepared. The DNS solution was prepared by combining solution A (10 g of 3,5 dinitrosalicilic acid powder in 200 mL 2 N NaOH) and solution B (300 g potassium sodium tartrate tetrahydrate in 500 mL distilled water) and adjusting the volume to 1 L with distilled water. The following equations were used for calculation of reducing sugars released (RSR) (dialysed fragments of digested starch plus native reducing sugars calculated as maltose equivalents as percentage of the total available carbohydrates in 4g sample), hydrolysis index (HI) (area under the curve from 0-180 min as a percentage of the corresponding area of the reference white wheat bread) and the predicted GI:

% RSR = A$_{sample}$ x 500 x 0.95 / A$_{maltose}$ x carbohydrate x 100

A$_{sample}$ = sample Absorbance at 546 nm

A$_{maltose}$ = Absorbance of solution containing maltose (1mg/ml)

Carbohydrate = mg starch and sugars contained in 4g sample (1326 mg/4 g wheat bread, 762 mg/4g wheat, 836 mg/4g teff and 764 mg/4g oat pasta)

500 = total volume (mL)

0.95 = conversion factor from maltose to starch

HI = AUC(0-180min)sample / AUC(0-180min)wheatbread x 100

GI$_{predicted}$ = 0.862HI + 8.189

The amount of carbohydrates available in 4 g of pasta was calculated by difference [100 − (moisture + fat + protein + fibre + ash)]. Crude fat, protein and moisture content of pasta were determined according to the AACC methods 30-10, 46-12 and 44-15A, respectively. For the determination of crude fibre a homogenised portion of the sample was defatted and treated successively with boiling solutions of sulphuric acid and potassium hydroxide. The residue was

separated by filtration on a sintered glass filter, washed, dried, weighed, and ashed. The fibre was determined gravimetrically. The loss of weight resulting from ashing corresponds to the fibre present in the test sample.

10.3.6 Sensory analysis

All sensory analyses were performed as detailed by Hager *et al*. (2012a) using a trained panel consisting of 22 members (5 male, 17 female, aged 22 – 44 years). At least ten assessors participated in each sensory session. Sensory analyses were performed in a sensory panel room at 21 ± 1 °C over three different sessions. Pasta (500 g) were cooked freshly in water (2 L) for 12 min. Cooked pasta were given on a plate and the samples were presented to the sensory panel immediately after cooking. For aroma profile analysis, the assessors had to sniff the freshly cooked pasta. The characteristic odour attributes were determined in a group discussion. In a separate session, freshly cooked pasta samples were presented to the panel, which evaluated the attribute intensities on a scale from 0 (not detectable) to 3 (high intensity). The assessors were trained immediately prior to analysis with aqueous odorant solutions in defined concentrations (Czerny *et al*. 2008). The odorant solutions reflected the evaluated characteristic odour attributes of the flours: buttery (butane-2,3-dione; 120 µg/L), cooked potato-like (3-(methylthio-)propanal; 140 µg/L), fatty ((E,E)-deca-2,4-dienal, 7.7 µg/L), putrid (sodium sulfide 1000 µg/L), popcorn-like (2-acetyl-1-pyrroline; 12 µg/L). Boiled eggs and hay, purchased from local supermarkets, were used as references for the attributes boiled egg-like and hay-like, respectively because no odorant reference was available.

For taste profile analysis the sensory panel tasted the fresh pasta samples and scored the intensities of the taste attributes on a scale from 0 (not detectable) to 10 (high intensity). The assessors were trained on the taste attributes immediately prior to analysis with following taste solutions: sweet (sucrose, 6.0 g/L), salty (NaCl, 1.3 g/L), sour (citric acid, 0.4 g/L), bitter (caffeine, 0.3 g/L) and umami (monosodium glutamate, 0.5 g/L). The mentioned compounds were purchased from Sigma-Aldrich (Taufkirchen, Germany) and aromaLab AG (Freising,

Germany). Sensory textural properties were evaluated by the sensory panel by tasting the pasta. The assessors scored the juiciness, firmness and stickiness of the samples on the following eleven-point scales: juiciness: 0 (dry) to 10 (juicy); firmness: 0 (hard) to 10 (soft); stickiness: 0 (smooth) to 10 (sticky). In order to evaluate aroma liking, the panel had to sniff the pasta samples and to evaluate the liking of the samples on a nine-point-scale from 1 (dislike very much) over 5 (neither like nor dislike) to 9 (like very much).

10.3.7 Visual appearance

Evaluation of colour of the uncooked pasta samples was performed using a Colour Meter, CR-300 (Minolta Camera Co. Ltd., Japan). Six random readings were taken on the surface of several aligned spaghetti strands before and after cooking. Results were expressed in the CIE L*a*b* space as L* (lightness; 0 = black, 100 = white), a* (+a* = redness, -a* = greenness) and b* (+b* = yellowness, -b* = blueness) values (CIE 1986).

10.3.8 Confocal Laser Scanning Microscopy

A Periodic acid Schiff's kit (Sigma, Germany) was used as staining method to detect polysaccharides. Pasta samples before and after cooking (12 min) were immersed for 10 min into periodic acid, washed with distilled water and then immersed in Schiffs acid for 20 min. Thereafter the samples were washed twice for 5 min in 1.2 M HCl in ethanol. An MRC-1024 confocal laser-scanning system (Biorad, Herts, UK) mounted on an upright microscope (Axioskop, Zeiss, Germany) with 20x objectives was used. Fluorescence images of a number of optical sections were acquired by scanning the sample along the optical axis in 1 µm steps. The multi line Ar laser (488 nm) was used and signals were collected through a BA510IF filter.

10.3.9 Statistical analysis

Results are shown as average ± standard deviation of at least triplicate measurements. Analysis of Variance (One-way ANOVA), followed by Fisher LSD post hoc test, was performed using Statistica 7.1 (StatSoft, U.S.A.).

10.4 Results and Discussion

10.4.1 Composition and nutritional value of flour raw materials

The nutrient content of pasta is determined by that of the utilised raw materials. Even though from a technological standpoint, wheat is certainly the most suitable raw material for pasta production, cereals such as teff and oat are characterised by a higher nutritional value. Protein content, one of the first parameters looked at when describing flour quality, is higher in teff flour (12.8 ± 0.5 g/100 g) compared to wheat flour (11.2 ± 1.1 g/100 g). This value is significantly lower for oat flour (6.9 ± 0.1 g/100 g) ($p < 0.05$). However, not only the amount but also the quality has to be considered. Protein found in oats is known to be superior to that of wheat, due to higher lysine contents, a limiting amino acid in cereals (Lasztity 1996). Compositional analysis showed that the utilisation of teff and oat flours for pasta production would result in products of increased fibre content. Teff and oat flour contain significantly more fibre (4.5 ± 0.6 g/100 g and 4.1 ± 0.4 g/100 g, respectively) than refined wheat flour (3.4 ± 0.1 g/100 g). In comparison to refined wheat flour, oat and teff flours show higher ash contents and favourable mineral composition (Table 10.1).

Table 10.1 Ash content (% w/w based on fresh weight) and mineral composition (mg/kg) of flour raw materials[$]

	Wheat	Oat	Teff
Ash	0.92 ± 0.02[b]	0.82 ± 0.01[c]	2.15 ± 0.05[a]
Calcium	1797.7 ± 10.5[a]	224.3 ± 2.1[c]	1543.0 ± 2.0[b]
Magnesium	244.0 ± 1.0[c]	392.7 ± 4.5[b]	1689.7 ± 14.5[a]
Sodium	38.1 ± 3.7[b]	16.7 ± 3.5[c]	59.8 ± 2.1[a]
Potassium	1520.3 ± 8.5[c]	1743.7 ± 16.5[b]	3827.7 ± 4.5[a]
Iron	13.4 ± 0.1[c]	16.4 ± 0.1[b]	85.3 ± 2.0[a]
Copper	1.51 ± 0.0[c]	2.7 ± 0.1[b]	9.3 ± 0.1[a]
Manganese	8.25 ± 0.1[c]	27.7 ± 0.4[b]	34.5 ± 0.4[a]
Zinc	7.59 ± 0.00[c]	11.3 ± 0.1[b]	41.5 ± 0.1[a]
Chloride	825.6 ± 42.6[a]	670.0 ± 48.5[b]	481.0 ± 39.1[c]
Phosphorus	908.7 ± 3.5[c]	1476 ± 25[b]	3617.0 ± 11.0[a]

[$] Values in one row followed by the same letter are not significantly different ($p < 0.05$)

Minerals are important for various physiological functions in the human body and per day, more than 100 mg of the major minerals (Na, Mg, K, Ca, P, and Cl) and less than 100 mg of

trace elements (Fe, Cu, Zn) are required. Teff flour can be considered a good source of calcium. The high amount of calcium in white wheat flour of this study is due to the fact that calcium carbonate is added to this product. Magnesium levels are relatively higher in oat flour compared to wheat, but are highest in teff flour. Wheat is known to be a source of iron. This study however showed that flours made from oat and especially teff are even higher in their iron content. Also copper, magnesium and zinc levels are higher in oat and teff than in wheat flour. Cereal flours being an important source of minerals also contain phytic acid. It is considered an anti-nutritional factor as it has a high chelating activity, which may decrease the bioavailability of certain elements such as calcium, magnesium, iron and zinc. Phytate also adversely affects the absorption of other nutrients such as amino acids, proteins and starch. In this study, teff flour contained high amounts of phytate (1.52 ± 0.21 g/100 g), while wheat and oat flour showed low phytate concentrations (0.16 ± 0.03g/100 g and 0.27 ± 0.01 g/100 g). A possible way to counteract this high anti nutrient content is the application of sourdough in pasta production, as lactic acid bacteria produce significant amounts of phytase (Capozzi *et al*. 2012). Polyphenols are a heterogeneous group of molecules (large multiples of phenol structural units) produced as secondary plant metabolites, which affect nutritional and sensory properties. Among the different flours this value was significantly higher in teff flour (176 ± 2 mg/100 g) compared to oat and wheat flour (22 ± 0 mg/100 g and 13 ± 0 mg/100 g). Although lipids comprise only about 1.5-7.0 % of cereal grains, they are of physiological importance as a source of essential fatty acids. In pasta, lipids affect colour and affect cooking loss. Fatty acids can complex with amylose, which reduces its water solubility and lowers cooking loss (Manthey and Twombly 2005). High fat contents as found in oat flours can cause a reduced shelf life, due to oxidation. Compared to wheat flour (1.81±0.05 g/100 g), fat content was significantly higher in oat and teff flour (6.74±0.80 g/100 g and 4.39 g/100 g). However, the fat of the latter two is characterised by a high content of nutritionally valuable unsaturated fatty acids such as linoleic and α-linolenic acid.

10.4.2 Amylase activity and starch properties of flour raw materials

Apart from its role as a major food reserve providing a bulk nutrient and energy source in the human diet, starch determines cooking quality and pasta texture. Variations in starch properties impact on water uptake, gel consistency and integrity of the protein matrix (Manthey and Twombly 2005). Total starch contents of oat and wheat flour are comparable (69 ± 2 g/100 g and 68 ± 2 g/100 g, respectively). This value is significantly lower for teff flour (58 ± 6 g/100 g). Milling of grains causes physical damage to a proportion of the starch granules. Their altered properties are of nutritional and technological significance, as damaged starch granules show increased water absorption and are more susceptible to enzyme hydrolysis. High levels of damaged starch in pasta are associated with increased cooking loss (Manthey and Twombly 2005). In this study, the highest amount of damaged starch was found in white wheat flour (7.9 ± 0.4 g/100 g), followed by oat flour (4.9 ± 0.1 g/100 g). Teff flour contained the lowest amounts (2.1 ±0.2 g/100 g). This difference is probably due to differences in endosperm hardness of the grains and milling procedures used to obtain the flours of this study. Milling of grains causes physical damage to a proportion of the starch granules. It is likely that due to the bigger size of wheat starch, granules are damaged to a greater extent during milling.

High alpha-amylase activities negatively influence pasta quality, with the resulting products showing softer texture and increased solid loss during cooking. Activities of this enzyme were significantly different among the flours of this study. In wheat flour, an activity of 0.67 ± 0.06 IU was detected, while the value was lower for teff flour (0.45 ± 0.02 IU). Due to the heat treatment as part of oat flour production, no alpha-amylase activity was detected in this sample.

Viscosity behaviour during heating from 50°C to 95°C gives an indication of the capacity of the different starches to retain water and swell, which in turn significantly influences pasta quality. Peak viscosity is known to give an indication. Oat flour had the highest peak viscosities (2189 ±

18 cP), followed by wheat flour (1599 ± 8 cp), and teff flour (735 ± 5 cP). During the holding period, i.e. constant high temperature and shear, the granules absorb water until rupture. Hereon the viscosity decreases to a minimum. This breakdown in viscosity was lowest for teff flour (49 ± 2 cP), while oat and wheat had significantly higher values (608 ± 7 cP and 588 ± 2 cP, respectively). This indicates that teff starch is more resistant to heating and shear stress. When the gelatinized starch cools, amylose and amylopectin chains realign (retrogradation) and the viscosity increases again until a gel is formed at the end. Final viscosity gives information on the strength of this gel. It is highest for oat flour (3010 ± 53 cP), followed by wheat (2094 ± 12 cP) and lowest for teff flour (1406 ± 21 cP). Setback is the difference between final and peak viscosity and is related to the retrogradation behaviour of starch. A lower setback as in wheat (504 ± 6 cP) compared to teff (680 ± 7 cP) and oat (820 ± 35 cP), indicates that the starch retrogrades less during cooling. Apart from viscosity measurements, differential scanning calorimetry is of great value in studying the loss of crystalline order during gelatinization, which occurs when starchy materials are heated in the presence of water as during cooking of pasta. The temperatures at onset (T_O), peak (T_P) and end of gelatinisation (T_E) vary between the different samples. Gelatinisation of oat starch commences at 51°C, peaks at 56°C and ends at 62°C. These parameters are comparable for wheat flour (T_O is 55°C, T_P is 61°C and T_E is 65°C). Teff flours showed relatively higher transition temperatures (T_O is 66°C, T_P is 71°C and T_E is 77°C). When discussing results of viscosity measurements and differential scanning calorimetry it has to be kept in mind, that the conditions are different to those of pasta production. During analysis of flours an excess of water is present, while pasta represents a limited-water system.

10.4.3 *In vitro* digestibility of starch

An *in vitro* enzymatic digestion was performed using a dialysis system in order to mimic the behaviour of pasta as eaten. This allowed making predictions on the GI of the spaghetti developed in this study. As expected, there were significantly more reducing sugars released

from the control wheat bread than from the pasta samples. Jenkins *et al*. (1983) previously

showed that blood glucose response of diabetic subjects was reduced upon consumption of

spaghetti compared to wheat bread. As bread and wheat pasta of this study were produced

from the same flour, this result confirms that food form rather than raw material used

determines glycaemic response. Complete gelatinisation is important in order to obtain highly

digestible starch. When starch molecules are heated in excess water, the crystalline structure

is disrupted and water molecules become linked to the exposed hydroxyl groups of amylose

and amylopectin. Therefore, water availability, which is higher in bread than in pasta dough, is

an important factor determining starch enzymatic digestibility. The principal process

facilitating starch availability for water penetration and subsequent α-amylase attack is heating

to 100°C for several minutes. However, optimally cooked spaghetti "al dente" should possess a

firm core, thus not allowing enough cooking time to achieve easily digestible starch. As

observed by scanning electron microscopy (Hager *et al*. 2012b), a large amount of starch is still

present in its granular form and is hence resistant to enzymatic attack. The hydrolysis curves

(Figure 10.1) show the reducing sugars released as a percentage of total available

carbohydrates over a period of 300 min.
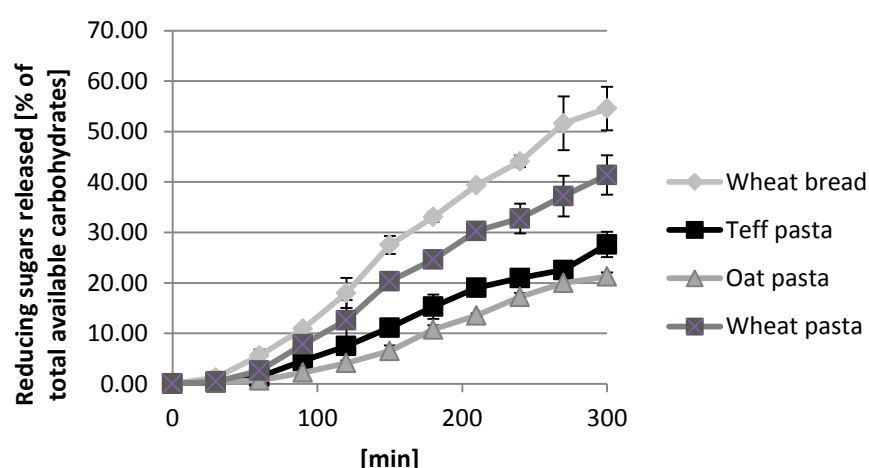
Figure 10.1 Dialysed fragments of digested starch plus native reducing sugars released during incubation with alpha-amylase calculated as maltose equivalents as percentage of the total available carbohydrates in 4 g sample.

The proportion of starch digested at different time points is significantly lower in oat and teff pasta compared to wheat. This may be due to the higher fibre content (1.0 and 0.6 g/100 g for uncooked oat and teff pasta, respectively compared to <0.1 g/100 g uncooked wheat pasta). Also Brennan and Tudorica (2008) observed decreased starch digestion in samples with increased fibre content. Another possible explanation is the higher addition level of egg white powder. It was previously described that the presence of protein in the food matrix influences the rate of starch digestion (Kim *et al*. 2008). The higher amount of protein possibly creates a stronger network, hence reducing the starch availability to enzymatic attack. Hydrolysis index was significantly different for the three samples (p<0.05). The value was highest for wheat pasta (68±8), followed by teff (43±2) and oat (28±2). This value is based on the ratio of area under the curve (0-180 min) for a pasta product compared to that of a reference white wheat bread and indicates a significant reduction of starch digestion in teff and oat pasta compared to wheat. Berti *et al*. (2004) used a similar dialysis system to evaluate *in vitro* digestibility of starch in gluten free and wheat samples and proposed that gluten free foods tend to have higher glycaemic indices than their wheat containing counterparts. The present study however shows that this is not necessarily true, but depends on the raw materials used. Clemente *et al*. (2001) showed *in vivo*, that although gluten free egg pasta induced an earlier plasma glucose response in the first 2 hours post consumption, the overall postprandial plasma glucose response was comparable to that of the wheat product. Regarding pasta of the current study, predicted GI of wheat spaghetti was 67±7. This value is higher than previously published results for conventional durum wheat pasta (Brennan *et al*. 2004; Tudorica *et al*. 2002). A possible explanation for this divergence is the use of wheat flour, which has a reduced particle size compared to the commonly used semolina. Predicted GI for teff and oat pasta was significantly (p<0.05) lower than for wheat pasta (45±1 and 32±2, respectively). As described above, the utilised teff flour contains large amounts of phytic acid and polyphenols. This might explain the reduced predicted GI of teff compared to wheat pasta, as it was previously shown

that these antinutrient compounds reduce starch digestibility (Thompson and Yoon 1984). Numerous publications report the beneficial effects of soluble fibres on the glucose response (Biörklund *et al*. 2005; Brennan *et al*. 2004; Jenkins *et al*. 2002; Tudorica *et al*. 2002). Among cereals, especially oat contains high amounts of soluble fibre, mainly β-glucan, possibly explaining the low predicted GI of the oat sample. Low-glycemic-index carbohydrates are generally considered to be those with a GI below 40 and include for example pumpernickel bread, legumes and parboiled rice. Those that have a GI between 40 and 70 are considered to have a moderate glycemic response and those greater than 70 have a high glycemic response (Bell and Sears 2003). Therefore, oat pasta can be considered a product of low glycaemic index, while the teff and wheat spaghetti of this study elicit moderate glycaemic response.

10.4.4 Sensory analysis

Initially, the overall sensory characteristics were investigated by evaluating the liking of the cooked spaghetti. Wheat pasta scored the highest aroma liking value (orthonasal evaluation) of 6.8 meaning that the pasta was liked moderately. The aroma of oat pasta was liked slightly (value 5.8) and teff pasta was evaluated as neither like nor dislike (value 4.6)

Wheat pasta and both gluten free counterparts were further investigated by profiling their aroma (Figure 10.2).
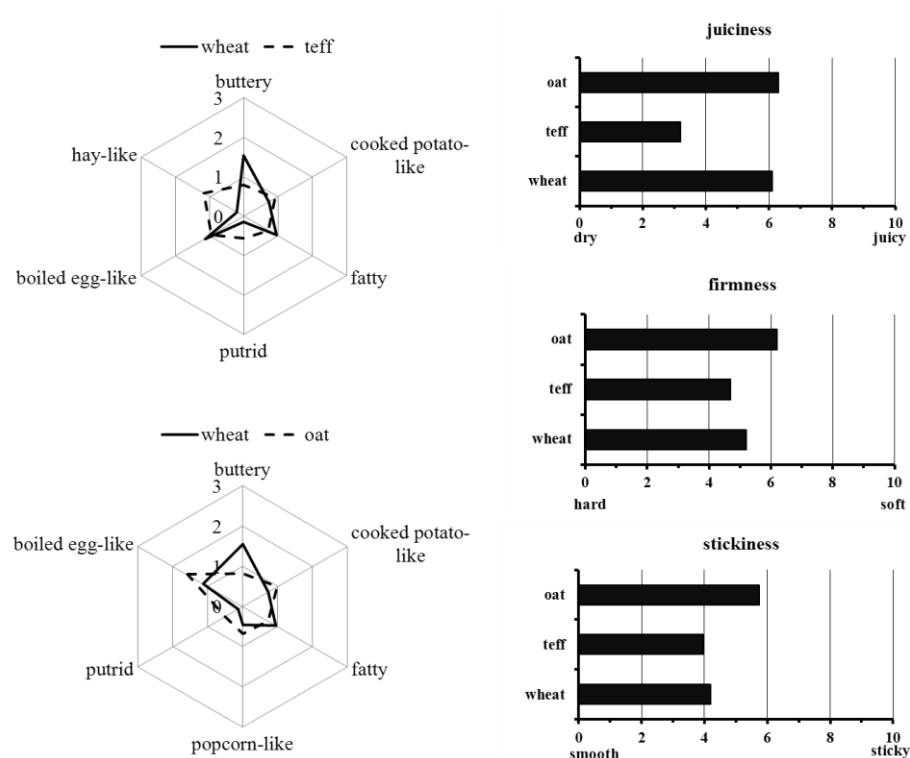
Figure 10.2 Orthonasal aroma profile analysis and sensory evaluation of pasta texture

The aroma of wheat pasta was described as buttery with weak boiled egg-like, fatty and boiled potato-like attributes. The aroma profiles of oat and teff spaghetti differed from wheat by a lower buttery intensity observed in both pasta and by the presence of a weak hay-like note, which was detectable in teff spaghetti. The reduced liking in aroma can therefore be attributed to the changes in the intensities of buttery and hay-like odour notes.

The investigation of retronasal liking demonstrated that in particular the liking of oat and teff pasta decreased in comparison to aroma liking (3.7 and 3.0, respectively). Because aroma, taste and texture were evaluated in parallel by retronasal liking, this loss of sensory quality can be attributed to reduced taste and/or texture properties. Therefore, taste and texture properties were investigated in further experiments. Taste profiles of the three pasta showed that this criterion was not responsible for the decreased qualities because all samples were very low in all five taste attributes (≤ 2.0 on the used 11-point-scale). In contrast, the assessment of texture properties (Figures 10.2) demonstrated that oat spaghetti were as juicy

as wheat spaghetti whereas teff spaghetti were much drier than oat wheat. On the other hand the stickiness of teff and wheat pasta was comparable while oat pasta was stickier than teff and wheat. The texture properties of oat pasta might be explained by the β-glucan fraction, which is able to bind higher levels of water. This may cause a relatively high juiciness but stickiness is affected negatively. Firmness was almost comparable for all three pasta samples. Summarizing the sensory results of gluten-free and the reference pasta, oat spaghetti was found to be similar to wheat pasta regarding sensory quality but improvement of smoothness and aroma is required. The low sensory quality of teff pasta was attributed in particular to a hay-like aroma and a high dryness.

10.4.5 Visual appearance

The visual appearance of spaghetti before and after cooking is certainly one of the main factors responsible for consumer liking. Regarding the samples of this study, it can be observed that the colouring of oat and wheat pasta is relatively homogeneous, while teff pasta shows dark specs (Figure 10.3).

Figure 10.3 Visual appearance of pasta samples before and after cooking for wheat (left), teff (center) and oat egg pasta (right).

Appearance of the uncooked product is strongly influencing the customer's choice. Pasta is expected to show a homogenous glossy surface of clear, bright yellow. Regarding the pasta samples of this study, colour was significantly different (p<0.05), depending on the raw material used (Table 10.2). Brightness (L* value) is a measure of the amount of light reflected

from the surface of the spaghetti relative to that reflected from a white surface, with higher values indicating a lighter product. While uncooked oat and wheat pasta show comparable L* values, teff pasta is significantly darker. Positive a* values, as found for all of the uncooked samples, give an indication on the redness of a sample. As expected, wheat pasta, being the most yellow product, shows the highest b* value, followed by oat pasta. While an amber colour is desired for conventional wheat pasta, teff and oat samples of this study are produced from wholegrain flours. Therefore, brown pigmentation is acceptable. Cooking not only influences textural properties of pasta, but also alters the colour of pasta products. Cooked spaghetti are lighter than their uncooked counterparts. Interestingly, also redness is reduced during cooking. Cooked wheat pasta even shows a negative a* value, indicating a green hue. Regarding yellowness (b* value), cooked wheat and teff pasta do not differ significantly. Oat pasta shows a higher b* value. Overall, it can be said that even though the uncooked products differ significantly in their visual appearance, upon cooking all samples are similar.

Table 10.2 Colour (L, a and b values) for uncooked and cooked pasta samples[$]

Sample	$L_{uncooked}$	$a_{uncooked}$	$b_{uncooked}$	L_{cooked}	a_{cooked}	b_{cooked}
Wheat	67.59±2.12a	2.03±0.34c	29.23±0.82a	74.59±1.11a	-1.05±0.38b	16.43±0.46b
Teff	57.98±1.07b	2.96±0.24b	19.10±1.00c	62.32±1.51c	1.63±0.26a	15.62±1.41b
Oat	67.07±2.01a	4.13±0.31a	23.17±1.53b	68.51±0.95b	1.35±0.31a	17.86±0.77a

[$] Values in one row followed by the same letter are not significantly different ($p < 0.05$)

10.4.6 Confocal Laser Scanning Microscopy

Microscopy is a tool well suited for the visualisation of processes happening during food production. In this particular study, changes in the starch structure occurring during cooking of pasta were observed by Confocal Laser Scanning Microscopy. The advantage of this microscopy technique is the possibility to selectively stain certain compounds. The periodic acid Schiff reaction was used to visualise polysaccharides. Carbohydrates are first oxidised by periodic acid, resulting in the formation of aldehyde groups. These can then react with the

Schiff's reagent. A colourless, unstable dialdehyde compound is formed and then transformed to a coloured final product by restoration of the quinoid chromophoric grouping. Upon excitation with the laser, a fluorescent signal corresponding to the starch matrix of the pasta is obtained (Figure 10.4). This signal was stronger in the outer area of pasta samples and no signal was obtained from the core area of the samples. The micrographs of the uncooked samples demonstrate well the differences in starch granule morphology. The wheat sample shows two populations of starch granules: large lenticular and small granular ones. Starch granules of oat and teff are relatively smaller and are sometimes organised in bigger spherical structures, the so called compound starch granules. Significant changes can be observed upon cooking. Pasta represents a limited-water system and hence, even after cooking for 12 min, a great proportion of starch is still present in its granular form. Interestingly, even upon gelatinisation, the starch matrix differs significantly between the three samples. Figure 10.4b shows cooked wheat pasta. It can be seen that in the outer layer gelatinisation had occurred. Also the micrographs of cooked oat pasta (Figure 10.4d) show a continuous mass of gelatinised starch, but no clear outer layer can be observed. Compared to the other two samples, starch granules of teff pasta were only minimally influenced by the cooking process (Figure 10.4f). This is in agreement with the rapid visco analyser results, where a lower break down viscosity indicated that teff starch is more resistant to heating and shear stress. During the scanning of different locations in the spaghetti samples, a higher number of air holes and cracks was observed in oat and teff pasta compared to the wheat sample, which can be explained by the lack of the visco-elastic gluten protein. This confirms previous findings obtained from scanning electron microscopy (Hager *et al*. 2012b).
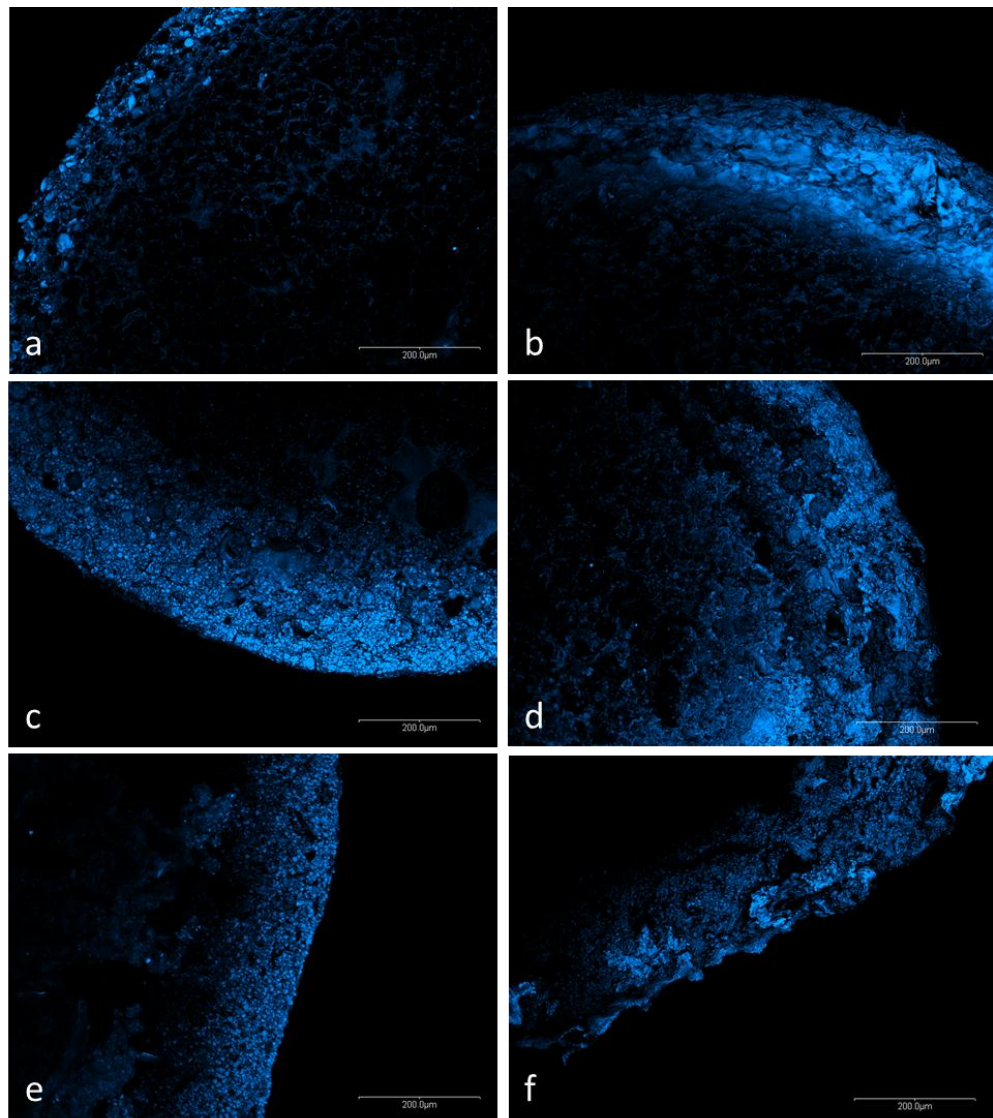
Figure 10.4 Cofocal laser scanning micrographs of pasta, where starch was stained with Periodic acid-Schiff. a) wheat pasta uncooked b) wheat pasta cooked c) oat pasta uncooked d) oat pasta cooked e) teff pasta cooked f) teff pasta uncooked

10.5 Conclusion

Compared to wheat, cereals such as teff and oat are characterised by a higher nutritional value. Flours milled from these alternative grains contain higher levels of fibre and minerals composition is superior, when compared to white wheat flour. Pasting properties and gelatinisation temperatures of cereals are diverse and these differences are possibly in part responsible for the reduced predicted GI of the gluten free samples. Texture and taste of teff pasta should be improved while oat pasta already shows similar sensory quality to wheat pasta. In conclusion, it can be said that the slowly digestible carbohydrates together with the

high protein content make pasta samples of this study an energy food to be consumed before exercise. Oat and teff pasta are nutritionally superior to many gluten free pasta products currently on the market.

10.6 Acknowledgements

10.7 References

Alvarez-Jubete L, Wijngaard H, Arendt E.K, Gallagher E. (2010) Polyphenol composition and *in vitro* antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. Food Chemistry, 119(2), 770-778

Analysenkommision (1996) MEBAK Brautechnische Analysenmethoden III. Selbstverlag der Mebak, Freising-Weihenstephan

Bell S.J. and Sears B. (2003) Low-glycemic-load diets: impact on obesity and chronic diseases. Critical Reviews in Food Science and Nutrition, 43, 357-377

Berti C, Riso P, Monti L.D, Porrini M. (2004) *In vitro* starch digestibility and in vivo glucose response of gluten-free foods and their gluten containing counterparts. European Journal of Nutrition, 43(4), 198-204

Biörklund M, Van Rees A, Mensink R.P, Onning G. (2005) Changes in serum lipids and postprandial glucose and insulin concentrations after consumption of beverages with beta-glucans from oats or barley: a randomised dose-controlled trial. European Journal of Clinical Nutrition, 59, 1272-1281

Brennan C.S, Kuri V, Tudorica C.M. (2004) Inulin-enriched pasta: effects on textural properties and starch degradation. Food Chemistry, 86,189-193

Brennan C.S. and Tudorica C.M. (2008) Evaluation of potential mechanisms by which dietary fibre additions reduce the predicted glycaemic index of fresh pastas. International Journal of Food Science and Technology, 43(12), 2151-2162

Capozzi V, Russo P, Fragasso M, De Vita P, Fiocco D, Spano G. (2012) Biotechnology and pasta-making: lactic acid bacteria as a new driver of innovation. Frontiers in Microbiology 3, Article 94

C.I.E. (International Comission on Illumination) (1986) Colorimetrie, 2nd ed. Publication C.I.E. No. 15, Vienna

Clemente G, Giacco R, Lasorella G, Coppola S, Trapanese E, Torre P, Greco L. (2001) Homemade gluten-free pasta is as well or better digested than gluten-containing pasta. Journal of Pediatric Gastroenterology and Nutrition, 32(1), 110-113

Czerny M, Christlbauer M, Christlbauer M, Fischer A, Granvogl M, Hammer M, Hartl C, Moran-Hernandez N, Schieberle P. (2008) Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. European Food Research and Technology, 228, 265-273

Franzese A, Iafusco D, Spadaro R, Cavaliere O, Prisco F, Auricchio R, Troncone R, Valerio G. (2011) Potential celiac disease in type 1 diabetes: A multicenter study. Diabetes Research and Clinical Practice, 92(1), 53-56

Hager A.S, Wolter A, Czerny M, Bez J, Zannini E, Arendt E.K, Czerny M. (2012a) Investigation of product quality, sensory profile and ultra-structure of breads made from a range of commercial gluten free flours compared to their wheat counterparts. European Food Research and Technology, 235(2), 333-344

Hager A.S, Lauck F, Zannini E, Arendt E.K. (2012b) Development of gluten free fresh egg pasta based on oat and teff flour. European Food Research and Technology, 235(5), 861-871

Hill I.D., Horvath K., Fasano A. (1995) Epidemiology of Coeliac Disease. American Journal of Gastroenterology, 90(1), 163-164

Jenkins A.L, Jenkins D.J.A, Zdravkovic U, Wursch P, Vuksan V. (2002) Depression of the glycemic index by high levels of beta-glucan fiber in two functional foods tested in type 2 diabetes. European Journal of Clinical Nutrition, 56, 622-628

Jenkins D.J.A, Wolever T.M.S, Jenkins A.L, Lee R, Wong G.S, Josse R. (1983) Glycemic response to wheat products – reduced response to pasta but no effect of fibre. Diabetes Care, 6(2), 155-159

Kim, E.H.J, Petrie J.R, Motoi L, Morgenstern M.P, Sutton K.H, Mishra S, Simmons L.D. (2008) Effect of structural and physicochemical characteristics of the protein matrix in pasta on *in vitro* starch digestibility. Food Biophysics, 3(2), 229-234

Lasztity, R. (1996) The Chemistry of Cereal Proteins. CRC Press, Boca Raton, Florida.

Manthey F. A. and Twombly W. (2005) Extruding and Drying of Pasta. In: Hui, Y.H. and Sherkat F., Handbook of Food Science, Technology, and Engineering. CRC Press, Melbourne

Matissek R. and Steiner G. (2006) Bestimmung des Gluehrueckstandes durch direkte Veraschung (Aschegehalt). In: Lebensmittelanalytik –Grundzuege, Methoden, Anwendungen. Springer-Verlag, Berlin, Germany

Petitot M, Abecassis J, Micard V. (2009) Structuring of pasta components during processing: impact on starch and protein digestibility and allergenicity. Trends Food Science and Technology, 20, 521-532

Shewry P.R. and Halford N.G. (2002) Cereal seed storage proteins: structures, properties and role in grain utilization. Journal of Experimental Botany, 53(370), 947-958

Singh J, Dartois A, and Kaur L. (2010) Starch digestibility in food matrix: a review. Trends in Food Science and Technology, 21, 168-180

The Commission of the European Communities (2009) Commission Regulation (EC) No 41/2009 concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten.

Thompson L.U, Yoon J.H. (1984) Starch Digestibility as Affected by Polyphenols and Phytic Acid. Journal of Food Science, 49(4), 1228-1229

Tudorica C.M, Kuri V, Brennan C.S. (2002) Nutritional and Physicochemical Characteristics of Dietary Fiber Enriched Pasta. Journal of Agricultural and Food Chemistry, 50, 347-356

Walker-Smith J.A, Vines R, Grigor W (1969) Coeliac disease and diabetes. Lancet, 2(7621), 650

Chapter 11: General discussion

Bread and pasta represent two staples consumed daily all over the world. However, an increased number of individuals follow certain diets, either prescribed by the doctor (e.g. coeliac disease, wheat allergy) or voluntarily as a life style choice. This makes the availability of specially developed products such as gluten free bread and pasta important. A market overview was conducted as part of this thesis, including 95 bread and 33 pasta products from seven European countries and the U.S.A. This study showed that more and more acceptable products can be found in supermarkets, online stores and whole salers. However, most products show common problems and none of the currently available gluten free breads or pastas are equal to wheat containing counterparts in terms of nutritional value, texture and taste. High sugar and fat contents as well as low levels of protein and fibre were frequently observed. Gluten free breads often show strong off flavours as well as dense, crumbly and dry texture (Chapter 3). Another common issue in terms of gluten free bread is the reduced shelf life. Due to the fact that many products consist mainly of starch, their rate of staling is higher and breads often harden within less than a day. Mariotti *et al*. (2013) reported up to almost 20 times higher crumb hardness values on day 3 of storage compared to measurements on day 0. Besides increased mold growth is a problem; often overcome by the addition of chemical preservatives or MAP packaging, which in turn cause reduced sensory quality. Also Gallagher *et al*. (2002) reported short shelf-lifes, dry and crumbly mouthfeel and unsatisfying taste of gluten free products. Now ten years later, products have certainly improved but the described defects can still be observed in the vast majority of commercial gluten free breads as shown by the current market study (Hager *et al*. 2011).

Regarding the nutritional value, especially the low fibre contents detected in the majority of commercial bread products are of concern as cereal foods are a major contributor to the human fibre intake. As part of this study the available publications on the total carbohydrate as well as the fibre intake of coeliac disease patients compared to that of the non-coeliac

general public were reviewed, showing that the fibre intake was too low in both groups (Castetbon *et al.* 2009; Elmadfa and Freisling 2003; Fukuda *et al.* 2007; Galvin *et al.* 2001; Grehn *et al.* 2001; Hopman *et al.* 2006; Lohiniemi *et al.* 2000; Oehlund *et al.* 2010; Wild *et al.* 2010). Therefore possible ways of increasing the uptake of this highly important nutrient have to be considered.

Looking at the 33 commercial gluten free pasta products, texture issues such as reduced elasticity, increased cooking losses and stickiness were detected (Chapter 4). Besides, atypical colouring was frequently observed. While many of the commercial samples show firmness values equal to or higher than wheat pasta, the elasticity is often significantly lower. Firmness values of the gluten free samples ranged from 149 – 1264 g, while the value of wheat control was 503 g. Regarding elasticity, gluten free samples ranged from 11-71 g and only eight of 33 samples showed values higher or equal to wheat pasta (45 g). Stickiness values for the commercial pasta samples vary widely from 10 to 55 g. The value for commercial wheat pasta was 22.93 g. Higher cooking losses of gluten free pasta compared to wheat pasta are frequently reported in literature (Chillo *et al.* 2007; Chillo *et al.* 2008; Lucisano *et al.* 2012; Susanna and Prabhasankar 2013). Lucisano *et al.* (2012) determined the cooking behaviour of fourteen different commercial gluten free spaghetti samples and concluded, in agreement with the present study, that none of the gluten free samples showed the same viscoelastic and textural characteristics than durum wheat pasta. During preparation and tasting of commercial gluten free pasta samples, it was observed that the typical mouthfeel expected from spaghetti ("al dente"), cannot be achieved when cooking these products. Comparing the nutritional value, it was shown that none of the commercial products showed protein contents comparable to standard wheat pasta and about half the products were higher in fat (Hager *et al.* 2012a). Another observation made during the market study was the high price of gluten free products. When purchasing gluten free bread and pasta for the market overview, products cost up to 4 times as much as standard wheat products. This is in agreement with

Singh and Whelan (2011), Stevens and Rashid (2008) and Lee *et al.* (2007). These authors found that generally gluten free products are more expensive than their gluten containing counterparts.

Scanning electron microscopy was used to visualise structural changes occurring during the cooking process, mainly starch gelatinisation and protein coagulation. Upon cooking and sectioning a spaghetti strand, a distinct outer layer of denatured protein and gelatinised starch can be observed (Petitot *et al.* 2009). This continuous and strengthened network entraps granular starch and prevents it from being washed out into the cooking water. Micrographs of commercial wheat and gluten free pasta (Chapter 4) and pasta produced in this study (Chapter 9) showed in case of wheat samples a strong outer layer of coagulated protein that entraps the ungelatinized starch granules. This layer was less pronounced in case of gluten free samples, explaining the higher cooking loss and stickiness observed in these products.

The majority of commercial gluten free breads and pastas are based on rice and maize. However, as Chapter 7 shows, more nutritious gluten free raw materials are available. While endosperm-derived white wheat flour, maize and rice flour contain only 3.4%, 2.6% and 0.4% fibre, quinoa flour contains a much higher amount (7.1%). Also regarding protein content, maize and rice flour are poor when compared to teff or pseudocereal flours such as buckwheat and quinoa. In this study the latter contained nearly twice as much protein (13.5% for quinoa, 12.8% for teff, 12.2% for buckwheat) when compared to maize and rice flour (5.5% and 7.3%) and exceeded even the protein level of bakers flour (11.5%). Quinoa flour contained much higher fat contents compared to all other flours, but the fatty acid composition was shown to be superior (high levels of α-linolenic acid, low omega-6/omega-3 ratio). Quinoa and buckwheat contained high amounts of folate, which is of interest as a low intake of this vitamin is linked to neural tube defects in the foetus. Also mineral content is higher in teff and pseudocereals but in the same time higher levels of anti-nutrients such as mineral chelating

phytate and polyphenols were detected in these flours. When looking at the results of the current study it has to be kept in mind, that the chemical composition is characteristic of species but also of variety. In the course of this study commercial flours were evaluated which are often composed of a mixture of varieties. Nevertheless, the present study gives a good idea of the general characteristics of these flours and helps with the selection of more nutritious raw materials for the development of gluten free products (Hager *et al*. 2012b).

Nutritional properties are not the only characteristics that clearly differentiate alternative cereals from wheat. Also starch granule size and shape are very different, as shown by scanning electron microscopy in Chapter 7. While wheat starch is made of two populations, large lenticular and small spherical granules, the gluten free flours of this study consist of much smaller starch granules, in case of rice and oat organised as compound starch. Also damaged starch values are significantly different, being highest in rice and wheat flour. These altered starch properties are partly the reason for the altered technological properties observed in Chapter 8.

When working with gluten free flours much higher water addition levels are required. While only 63% water was added in case of wheat, water addition levels of up to 120% (rice) were necessary to obtain an acceptable crumb in case of gluten free flours (percentages based on flour weight). As previously described by Cauvain (1998), if only flour and water and no additional ingredients such as psyllium are used for gluten free bread making, a liquid cake like batter is obtained rather than a formable dough. Therefore, tins have to be used for baking and the development of products such as rolls, baguette or petit pain is especially challenging. Altered dough characteristics were also reflected in the results of fermentability trials (Rheofermentometer). Refined white wheat and oat flour samples reached a maximum dough development height unmatched by the gluten free batters or the wholewheat dough, indicating superior visco-elastic properties of oat. Gas production was highest in teff,

buckwheat and quinoa batters. Quality evaluation of resulting loaves showed big differences not only in visual appearance, but also in final loaf volume, crumb firmness, crumb structure, shelf life and taste attributes. Due to the lack of a cohesive protein matrix, elasticity and extensibility of the gluten free batters is reduced and loaf volumes are low. With a specific volume of 2.4 mL/g, oat bread showed the highest value close to white wheat bread. All other gluten free loafs had significantly lower specific volumes, with maize having only half the volume of wheat bread (1.3 mL/g). It has to be mentioned, that due to the fact that no bread improvers were used, the wheat bread of this study shows only a volume of 2.6 mL/g while values of commercial breads range between 3 and 5 mL/g. Low crumb hardness and high springiness values as measured for oat and white wheat bread are desired, because consumers relate a firm crumb to a staled product. Especially the maize crumb was characterized by high hardness and low springiness values. When comparing white wheat bread to all other breads it becomes apparent that crumb structure and cell characteristics are very different. Quinoa, buckwheat, maize, teff and wholemeal wheat breads show very dense crumbs while oat and rice breads have a more open texture when compared to wheat bread. As the same mixing regime was followed for the production of all gluten free breads, it can be assumed that this is due to the differences in dough consistency. Comparing the outcomes of the conducted baking trials with literature is difficult, because in most studies mixtures of different gluten-free flours and starches were used. However, Renzetti *et al*. (2008) used similar simple recipies based on corn, buckwheat, teff, sorghum and oats and results compare well, with the exeption of oat breads. These authors reported as low specific volumes for oat breads as for maize breads, while oat breads in this study were characterized by much higher volumes. This difference in volume of oat breads might be explained by the fact that in the publication of Renzetti at al. (2008) a constant water level of 125 % (based on flour weight) was used for all breads, while in the present study the water addition levels were optimized for each flour.

Scanning electron micrographs of batters and bread crumbs revealed that distinct starch granules are still visible in wheat and oat breads; while starch is gelatinized to a much higher extent in case of all other gluten free flours (Chapter 8).

The results obtained for wholemeal wheat breads show that the absence of gluten is not the only factor responsible for the reduced loaf quality of gluten free breads. Due to the small grain size, many gluten free flours are milled from the whole grain and the bran particles present are believed to pierce and burst gas bubbles and hence limit the reachable volume (Seyer and Gelinas 2009). In addition, the bran fraction strongly affects colour and sensory properties.

As mentioned above, obtaining an acceptable shelf life that would allow selling fresh gluten free bread represents one of the biggest challenges. Due to high a_w values and the high moisture contents resulting from the increased water addition levels, mold growth commences earlier. The results of shelf life trials presented in Chapter 8 show that on average, mold growth commences on day 4 for most gluten free breads and for rice bread already on day 3. In order to place a bread product in the supermarket, 8 to 10 days of shelf life are required. Apart from microbial deterioration, shelf life of bread is also determined by the staling behavior. Crumb firming, attributed mainly to recrystallization of amylopectin and water redistribution between crumb and crust, was especially pronounced in oat, white wheat and maize bread. Staling rate of teff bread was significantly lower than for the other gluten free flours. This was expected since teff starch has a lower tendency to retrograde, as indicated by lower setback values obtained using the Rapid Viscoanalyser (Chapter 10). Sciarini *et al.* (2010) proposed previously that in wheat breads the gluten network slows down the movement of water, thus gluten free breads are more prone to stale. This assumption cannot be confirmed by the data of this study, as the rate of staling of most gluten free breads is lower than that of wheat bread.

Summarizing the results it can be said that especially oat and teff as well as the pseudocereals quinoa and buckwheat are characterised by a high nutritional value when compared to rice and maize but also compared to wheat flour. Hence, a shift away from wheat flour towards alternative grains would not only benefit coeliac patients but also the general public. However, baking trials conducted with these flours showed reduced loaf quality when compared to wheat. Hence, much research effort is still needed to adapt milling and baking processes to these grains. Another limitation of alternative cereals is their distinct aroma profile (Chapter 8). In wheat bread, yeast-like, malty and buttery notes were predominant. The aroma profile of oat bread was very similar to the wheat profile, while these attributes were less pronounced in gluten free products. In the buckwheat crumb weak pea-like, moldy and vinegar-like notes were detectable and maize and rice showed vomit-like notes. Quinoa showed a strong pea-like aroma and weak cooked potato and mold notes. Regarding the aromas perceived it is not surprising that the gluten free breads reached lower liking scores when compared to wheat.

As mentioned above, the dietary fibre intake of coeliac disease patients as well as the non-coeliac population is generally too low. Chapter 5 deals with the fibre enrichment of wheat and gluten free bread, as an alternative to breads produced from the whole grain, which are poorly accepted by consumers. The soluble fibres inulin and oat β-glucan were chosen, as additional health benefits are related to these ingredients such as a cholesterol lowering effect to β-glucan (EFSA 2009, FDA 1996) and prebiotic properties to inulin (Gibson *et al*. 1995; Tuohy *et al*. 2007). However, their incorporation can compromise loaf quality (Brennan and Cleary 2007; Cavallero *et al*. 2002; Cleary *et al*. 2007; Lazaridou *et al*. 2007). In accordance with Lazaridou *et al*. (2007), gluten free bread showed a lighter crust colour upon addition of oat β-glucan compared to controls. As also observed by Poinot *et al*. (2010) incorporation of inulin resulted in a darkening of the crust. The addition of inulin to bread had unfavourable effects on crumb hardness and the rate of staling in both bread types. Also Capriles and Arêas (2013) described higher firming rates with increasing addition of inulin type fructans to gluten free

breads. In order to keep negative effects as limited as possible, it is crucial to adjust the water level of the dough upon addition of polysaccharides. For wheat breads this is done using the Farinograph. This method however is not applicable to gluten free systems and preliminary baking trials have to be carried out instead (Gujral *et al*. 2003; Hager *et al*. 2012c; Haque and Morris 1994). Alternatively, in certain cases rheology can be used for this purpose as demonstrated by Nunes *et al*. (2009). Thus in chapter 5 the level of water addition required was determined based on small deformation rheological measurements at constant strain (0.01 %) and frequency (10 Hz). The complex moduli G* of the controls were determined, and the amount of water added to the fibre containing batters and doughs was altered until the same G* was reached.

Several studies report on the partial breakdown of β-glucan during processing and hence reduced physiological function (Andersson *et al*. 2004; Lazaridou and Biliaderis 2007; Tiwari and Cummins 2009; Trogh *et al*. 2004). Also size exclusion chromatography and enzymatic testing performed in this study showed that during the bread making process β-glucan is partly degraded. This reduction has to be kept in mind and dosages have to be adjusted when claiming a cholesterol lowering effect on the packaging.

Confocal laser scanning microscopy is an interesting tool for ultra structure evaluation of foods as components can be stained selectively. In Chapter 5, Aniline blue was used for the visualisation of β-glucan, and Fluorescein isothiocyanate for the bread matrix. In wheat bread the formation of a gluten network was observed while the proteins in gluten free bread appeared cloud like. In Chapter 10, changes in the starch structure occurring during cooking of pasta were observed using a Periodic Schiff's acid kit as staining technique. Significant changes were observed upon cooking. Pasta represents a limited-water system and hence, even after cooking for 12 min, a great proportion of starch is still present in its granular form.

The market overview conducted on gluten free bread showed that a high number of products contain xanthan or celluloses and their derivatives. Also a high number of publications exist on

the incorporation of HPMC and xanthan into complex gluten free formulations as these substances can improve textural properties. This work is the first fundamental study using basic formulations solely based on flour, water, salt, sugar and yeast to evaluate the influence of HPMC on volume, hardness and crumb grain of buckwheat, maize, rice and teff breads. Response surface methodology was used as it does not only allow the evaluation of the relative effect of predictor variables (e.g. hydrocolloid and water level) on response variables (e.g. loaf volume, crumb hardness, area of cells and wall thickness) but also allows the determination of optimum ingredient levels. It is generally believed that the increased viscosity of gluten free batters upon xanthan addition help to keep gas bubbles from rising and prevents their coalescence, keeping the system homogeneous until starch gelatinization (Lazaridou *et al*. 2007; Schober 2009). The results of this study contradict this theory somewhat as xanthan addition reduced loaf volume as well as area of cells. Several publications report a volume increase upon HPMC addition (Haque and Morris 1994; Mezaize *et al*. 2009; Sabanis and Tzia 2011a, Sabanis and Tzia 2011b). This study showed that the influence of HPMC depends on the flour matrix. HPMC had negative linear effect on this parameter in rice breads, while the volume of buckwheat bread did not change and volumes of teff and maize breads were increased. HPMC addition decreased crumb hardness of all breads in this study. The hydrocolloid forms a thermo-reversible gel which strengthens when heated and reverts back to weak entanglement after cooling, thereby stabilizing the gelatinizing crumb structure during baking and softening the resulting crumb (Crockett *et al*. 2011). Also in a recent publication by Mariotti *et al.* (2013) a decrease in crumb hardness was observed upon addition of HPMC to two commercial gluten free formulations. The same authors also observed a reduced staling rate upon incorporation of HPMC. Unfortunately, no general rules or guidelines for the application of HPMC and xanthan can be drawn from the results, because this study clearly showed that the effect of hydrocolloids strongly depends on the formulation used. In preliminary trials it was observed, that not only the type of hydrocolloid but also the grade (i.e.

degree of substitution) are of importance. In addition, obtaining hydrocolloids with the same specification from different producers as well as batch-to-batch variations cause varying results. Another issue with HPMC and xanthan is declaration as clean labelling increasingly becomes important also to producers of gluten free foods. This means that upon incorporation of HPMC and xanthan into commercial bread formulations, their use, the dosage level as well as the hydrocolloid grade has to be well thought through and baking trials have to be carried out.

Chapter 9 and 10 aim at the development of high quality pasta from cereals other than wheat. Due to outstanding nutritional characteristics, teff and oat flour were chosen. In addition, teff is generally regarded as gluten free and a large majority of coeliac patients can tolerate oats (Richman 2012). As mentioned above, commercial gluten free pasta is generally low in protein. An obvious ingredient for increasing the protein content of pasta is egg, because eggs are traditionally used for pasta making and the taste is not perceived as off-flavour (Antognelli 1980). However, like gluten, egg protein is also on the allergen list and it remains questionable if the replacement of one allergen by another is advisable. As described in literature, alternative high protein flours used for pasta making are soy, chickpea and sorghum flour as well as whey protein concentrate, soy protein isolate and casein (Schoenlechner *et al*. 2010; Susanna and Prabhasankar 2013). Results showed that egg white powder not only increased the nutritional value of gluten free pasta, but also improved textural properties such as elasticity, firmness and stickiness. Looking at the results of texture profile analysis as well as sensory evaluation by a trained panel, it could be seen that when using oat flour, spaghetti comparable to wheat based products can be obtained. Teff pasta was characterised by high cooking loss and a dry mouthfeel. To allow predictions on the GI of the spaghetti developed in this study, an *in vitro* enzymatic digestion was performed using a dialysis system in order to mimic the behaviour of pasta as eaten (Singh *et al*. 2010). Pasta produced from wheat flour showed the highest values for GI followed by teff pasta. Oat pasta showed the lowest GI value,

possibly due to the high soluble fibre content of this cereal. Also Brennan and Tudorica (2008) observed decreased starch digestion in samples with increased fibre content. Another possible explanation is the higher addition level of egg white powder. It was previously described that the presence of protein in the food matrix influences the rate of starch digestion (Kim *et al*. 2008). The higher amount of protein possibly creates a stronger network, hence reducing the starch availability to enzymatic attack. Differences in pasting properties and gelatinisation temperatures of oat, teff and wheat were measured and these differences are in part responsible for the reduced predicted GI of the gluten free samples. The developed oat and teff pasta are nutritionally superior to many gluten free pasta products currently on the market. As common pasta is usually prepared from only semolina and water, it would be interesting to produce semolina from gluten free grains and evaluate their potential for pasta making.

In conclusion it can be said, that research and development is still needed to ameliorate gluten free breads and pasta. Typical nutritional issues are high fat contents and low levels of protein and fibre, but from a consumer point of view the improvement of texture and taste certainly has priority. Also a wider range of gluten free products would be desirable.

References

Andersson A, Armo E, Grangeon E, Frederiksson H, Andersson R, Aman P. (2004) Molecular weight and structure units of (1-3, 1-4)-β-glucans in dough and bread made from hull-less barley milling fractions. Journal of Cereal Science, 40, 195-204

Antognelli C. (1980) The manufacture and applications of pasta as a food and as a food ingredient – a review. Journal of Food Technology, 15(2), 125-145

Brennan C, Cleary L. (2007) Utilisation Glucagel® in the β-glucan enrichment of breads: A physicochemical and nutritional evaluation. Food Research International, 40(2), 291-296

Capriles V.D. and Arêas J.A.G. (2013) Effects of prebiotic inulin-type fructans on structure, quality, sensory acceptance and glycemic response of gluten-free breads. Food & Function, 4, 104-110

Castetbon K, Vernay M, Malon A, Salanave B, Deschamps V, Roudier C, Oleko A, Szego E, Hercberg S. (2009) Dietary intake, physical activity and nutritional status in adults: the French nutrition and health survey (ENNS, 2006-2007). British Journal of Nutrition, 102, 733-743

Cauvain S.P. (1998) Other Cereals in Breadmaking. In: Technology of Breadmaking. Blackie Academic and Professional, London, pp. 330-346

Cavallero A, Empilli S, Brighenti F, Stanca A. (2002) High (1→3,1→4)-Glucan Barley Fractions in Bread Making and their Effects on Human Glycemic Response. Journal of Cereal Science, 36, 59-66

Chillo S, Civica V, Iannetti M, Suriano N, Mastromatteo M, Del Nobile M.A. (2009) Properties of quinoa and oat spaghetti loaded with carboxymethylcellulose sodium salt and pregelatinized starch as structuring agents. Carbohydrate Polymers, 78(4), 932-937

Chillo S, Laverse J, Falcone P.M. and Del Nobile M.A. (2007) Effect of carboxymethylcellulose and pregelatinized corn starch on the quality of amaranthus spaghetti. Journal of Food Engineering, 83(4), 492-500

Chillo S, Laverse J, Falcone P.M, and Del Nobile M.A. (2008) Quality of spaghetti in base amaranthus wholemeal flour added with quinoa, broad bean and chick pea. Journal of Food Engineering, 84(1), 101-107

Cleary L, Andersson R, Brennan C. (2007) The behaviour and susceptibility to degradation of high and low molecular weight barley β-glucan in wheat bread during baking and *in vitro* digestion. Food Chemistry, 102, 889-897

Crockett R, Ie P, Vodovotz Y (2011) How Do Xanthan and Hydroxypropyl Methylcellulose Individually Affect the Physicochemical Properties in a Model Gluten-Free Dough? Journal of Food Science, 76(3), E274-E282

EFSA Panel On Dietetic Products (2009) Scientific Opinion on the substantiation of health claims related to beta-glucans and maintenance of normal blood cholesterol concentrations (ID 754, 755, 757, 801, 1465, 2934) and maintenance or achievement of a normal body weight (ID 820, 823). EFSA Journal, 7, 1254

Elmadfa I, Freisling H. (2003) Zufuhr von Makronaehrstoffen bei verschiedenen Bevoelkerungsgruppen in Oesterreich. Ernahrungsumschau 50(12), 464-468

FDA (1996) Food Labeling: Health claims; Oat and coronary heart disease. Federal register 61

Fukuda S, Saito H, Nakaji S, Yamada M, Ebine N, Tsushima E, Oka E, Kumeta K, Tsukamoto T, Tokunaga S. (2007) Pattern of dietary fiber intake among the Japanese general population. European Journal of Clinical Nutritrition, 61, 99-103

Gallagher E and Gormley T.R. (2002) The quality of gluten free breads produced at retail outlets. Research Report, Teagasc, The National Food Centre, Dublin 15, Republic of Ireland

Galvin M.A, Kiely M, Harrington K.E, Robson P.J, Moore R, Flynn A. (2001) The North/South Ireland Food Consumption Survey: the dietary fibre intake of Irish adults. Public Health and Nutrition, **4**, 1061-1068

Gibson GR, Beatty ER, Wang XI, Cummings JH (1995) Selective Stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology, 108:4, 975-982

Grehn S, Fridell K, Lilliecreutz M, Hallert C. (2001) Dietary habits of Swedish adult coeliac patients treated by a gluten-free diet for 10 years. Scandinavian Journal of Nutrition, 45, 178-182

Gujral H.S, Guardiola I, Carbonell J.V, Rosell C.M. (2003) Effect of cyclodextrinase on dough rheology and bread quality from rice flour. Journal of Agricultural and Food Chemistry, 51, 3814-3818

Hager A.-S, Axel C, Arendt E.K. (2011) Status of Carbohydrates and Dietary Fiber in Gluten free Diets, Cereal Foods World, 56(3), 109-114

Hager A.-S, Zannini E, Arendt EK (2012a) Gluten-free Pasta—Advances in Research and Commercialization. Cereal Foods World, 57(5), 225-229

Hager A.-S, Wolter A., Jacob F., Zannini E., Arendt E.K. (2012b) Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours. Journal of Cereal Science, 56, 239-247

Hager A.-S, Wolter A, Czerny M, Bez J, Zannini E, Arendt E.K, Czerny M (2012c) Investigation of product quality, sensory profile and ultrastructure of breads made from a range of commercial

gluten-free flours compared to their wheat counterparts. European Food Research and Technology, 235(2), 333-344

Haque A. and Morris E.R. (1994) Combined use of Ispaghula and HPMC to replace or augment gluten in breadmaking. Food Research International, 27(4), 379-393

Hopman E.G, le Cessie S, von Blomberg B.M, Mearin M.L. (2006) Nutritional management of the gluten-free diet in young people with celiac disease. Journal of Pediatric Gastroenterology and Nutrition, 43, 102-108, 2006

Kim E.H.J, Petrie J.R, Motoi L, Morgenstern M.P, Sutton K.H, Mishra S, Simmons L.D. (2008) Effect of structural and physicochemical characteristics of the protein matrix in pasta on *in vitro* starch digestibility. Food Biophysics, 3(2), 229-234

Lazaridou A, Biliaderis C (2007) Molecular aspects of cereal β-glucan functionality: Physical properties, technological applications and physiological effects. Journal of Cereal Science, 46, 101-118

Lazaridou A, Duta D, Papageorgiou M, Belc N, Biliaderis C. (2007) Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. Journal of Food Engineering, 79, 1033-1047

Lee A.R, Ng D.L, Zivin J, Green P.H. (2007) Economic burden of a gluten-free diet. Journal of Human Nutrition and Dietetics, 20, 423-430

Lohiniemi S, Mäki M, Kaukinen K, Laippala P, Collin P. (2000) Gastrointestinal Symptoms Rating Scale in Coeliac Disease Patients on Wheat Starch-based Gluten-free Diets. Scandinavian Journal of Gastroenteroly, 9, 947-949

Lucisano M, Cappa C, Fongaro L, Mariotti M. (2012) Characterisation of gluten-free pasta through conventional and innovative methods: Evaluation of the cooking behaviour. Journal of Cereal Science, 56, 667-675

Mariotti M, Pagani MA, Lucisano M. (2013) The role of buckwheat and HPMC on the breadmaking properties of some commercial gluten free bread mixtures. Food Hydrocolloids, 30, 393-400

Majzoobi M, Ostovan R, Farahnaky A. (2012) Effect of Gluten Powder on the Quality of Fresh Spaghetti Made with Farina. International Journal of Food Engineering, 8(1), 7

Mezaize S, Chevallier S, Le Bail A, de Lamballerie M. (2009). Optimization of gluten-free formulations for French-style breads. Journal of Food Science, 74(3), E140-E146

Nunes M.H, Ryan L.A.M, Arendt E.K. (2009) Effect of low lactose dairy powder addition on the properties of gluten-free batters and bread quality. European Food Research and Technology, 229, 31-41

Öhlund K, Olsson C, Hernell O and Öhlund I. (2010) Dietary shortcomings in children on a gluten-free diet. Journal of Human Nutrition and Dietetics, 23, 294-30022

Petitot M, Abecassis J, Micard V. (2009) Structuring of pasta components during processing: impact on starch and protein digestibility and allergenicity. Trends in Food Science and Technology, 20, 521-532

Poinot P, Arvisenet G, Grua-Priol J, Fillonneau C, Le-Bail A, Probst C. (2010) Influence of inulin on bread : kinetics and physicochemical indicators of the formation of volatile compounds during baking. Food Chemistry, 232, 1474-1484

Renzetti S, Dal Bello F. and Arendt E.K. (2008) Microstructure, fundamental rheology and baking characteristics of batters and breads from different gluten-free flours treated with a microbial transglutaminase. Journal of Cereal Science, 48 (1), 33-45

Richman E. (2012) The safety of oats in the dietary treatment of coeliac disease. Proceedings of the Nutrition Society, 71, 534-537

Sabanis D, Tzia C. (2011a) Effect of hydrocolloids on selected properties of gluten-free dough and bread. Food Science and Technology International, 17(4), 279-291

Sabanis D, Tzia C. (2011b) Selected Structural Characteristics of HPMC-Containing Gluten Free Bread: A Response Surface Methodology Study for Optimizing Quality. International Journal of Food Properties, 14(2), 417-431

Schober T.J. (2009) Manufacture of gluten-free speciality breads and confectionary products. In: Gallagher, E. (Ed.) Gluten-free Food Science and Technology. Oxford: Blackwell publishing Ltd.

Schoenlechner R.J, Drausinger J, Ottenschlaeger V, Jurackova K, Berghofer E. (2010) Functional Properties of Gluten-Free Pasta Produced from Amaranth, Quinoa and Buckwheat. Plant Foods for Human Nutrition, 65(4), 339-349

Sciarini L.S, Ribotta P.D, Leon A.E, Perez G.T. (2010) Influence of Gluten-free Flours and their Mixtures on Batter Properties and Bread Quality. Food Bioprocess Technology, 3(4), 577-585

Seyer ME, Gelinas P. (2009) Bran characteristics and wheat performance in whole wheat bread. International Journal of Food Science and Technology, 44(4), 688-693

Singh J, Whelan K. (2011) Limited availability and higher cost of gluten-free foods. Journal of Human Nutrition and Dietetics, 24, 479-486

Singh J, Dartois A, and Kaur L. (2010) Strach digestibility in food matrix: a review. Trends in Food Science and Technology, 21, 168-180

Stevens L, Rashid M. (2008) Gluten-free and regular foods: a cost comparison. Canadian Journal of Dietetic Practise and Research, 69, 147-150

Susanna S, Prabhasankar P. (2013) A study on development of Gluten free pasta and its biochemical and immunological validation. LWT – Food Science and Technology, 50(2), 613-621

Tiwari U, Cummins E. (2009) Factors influencing β-glucan levels and molecular weight in cereal based products. Cereal Chemistry, 86, 290-301

Trogh I, Courtin CM, Andersson AA, Aman P, Sorensen JF, Delcour JA (2004) The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley flour breads with increased arabinoxylan and (1➔3,1➔4)-β-D-glucan levels. Journal of Cereal Science, 40, 257-267

Tuohy K.M, Kolida S, Lustenberger A.M, Gibson G.R (2007) The prebiotic effects of biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides – a human volunteer study. British Journal of Nutrition, 86, 341-348

Vernaza M.G, Biasutti E, Schmiele M, Jaekel L.Z, Bannwart A, Chang Y.K. (2012) Effect of supplementation of wheat flour with resistant starch and monoglycerides in pasta dried at high temperatures. International Journal of Food Science and Technology, 47(6), 1302-1312.

Wild D, Robins G.G, Burley V.J, Howdle P.D. (2010) Evidence of high sugar intake, and low fibre and mineral intale, in the gluten-free diet. Alimentary Pharmacology & Therapeutics, 32, 573-581

Appendix I: Additional tables

Appendix Ia: Nutritional characterisation of gluten free breads of different categories

Brown Bread	Country	kJ/100g	Kcal/100 g	Carbohydrates [g/100 g]	Sugars	Fibre [g/100g]
Meingast Krustenbroetchen	Austria	1092	261	54.4	3.7	2.3
Minderleinsmuehle rolls	Austria	1167	279	43.8	n.a.	n.a.
Minderleinsmuehle sunflower bread	Austria	1301	311	43.3	n.a.	n.a.
Tattarileipä Buckwheat bread	Finland	1059	253	52.8	2.5	2.5
Tumma leipä Dark bread	Finland	1088	260	56.5	5.5	2.6
Hapan mausteleipä sourdough bread	Finland	1096	262	54.2	3.1	2.7
Pirjon Pakari	Finland	841	201	41.9	3.9	4.1
Schnitzer spezial Bio Landbrot	Germany	803	192	36.6	2.0	4.4
Schnitzer spezial Bio Buchweizenbrot	Germany	895	214	30.1	2.8	5.8
3 Pauly Schwarzbrot mit Teff	Germany	933	223	38.2	2.0	3.6
3 Pauly Vollkorn Schnittbrot	Germany	820	196	38.0	1.0	5.0
Schaer Sunna Vollkornbroetchen	Germany	1088	260	35.6	4.2	8.1

Schaer Landbrot	Germany	937	224	44.7	3.5	5.2
Schaer Ciabatta rustica	Germany	1067	255	39.6	3.7	8.9
Schaer Rustico	Germany	958	229	40.8	3.0	5.7
ener Bio Buchweizenbrot	Germany	895	214	30.1	2.8	5.8
Hammermuehle Bio Landbrot	Germany	933	223	49.0	n.a.	n.a.
Kelkin Sliced Brown Bread	Ireland	933	223	47.8	2.6	1.3
ENER G Gluten-Free Flax Loaf	Ireland	1105	264	41.0	2.9	3.4
Genius Gluten Free Fresh Brown Bread	Ireland	1159	277	42.2	5.1	9.5
Kelkin Multiseed Brown Bread	Ireland	908	217	37.4	2.0	2.3
Marks & Spencer 4 soft brown baps	Ireland	1046	250	31.6	0.4	9.3
NUTRIFREE PanFette Integrale	Italy	1184	283	60.0	2.4	5.6
Semper Toasty Fibre	Sweden	879	210	41.0	1.0	6.5
Semper Toasty pure oats	Sweden	920	220	45.0	0.8	5.5
Semper Minibaguette Fibre	Sweden	920	220	46.0	6.0	4.0
GLUTINO Wholegrain Brown Rice Bread	USA	1046	250	49.9	4.5	4.0
Ener G brown Rice Loaf	USA	1100	263	42.1	5.3	2.6
Ener G Brown Rice Yeast-free Loaf	USA	929	222	37.7	0.0	4.4

Food for Life Brown fruit juice rice bread	USA	1071	256	48.9	7.0	4.7
Average gluten free brown bread		**1006**	**240**	**43.3**	**3.1**	**4.8**
Standard brown bread (whole-wheat)*		**1033**	**247**	**41.3**	**5.6**	**6.8**

				Carbohydrates		Fibre
Pumpernickel	**Country**	**kJ/100g**	**Kcal/100 g**	**[g/100 g]**	**Sugars**	**[g/100g]**
Brotmix - sunflower bread	Austria	782	187	33.0	1.0	4.0
Brotmix - linseed bread	Austria	866	207	36.0	2.0	6.0
PEMA Reisbrot mit Sonnenblumenkernen	Germany	757	181	33.0	1.0	4.0
Schnitzer spezial Bio Inkabrot mit Amaranth	Germany	900	215	30.8	2.9	6.9
Solena wholemeal bread with buckwheat	Germany	874	209	31.0	1.0	7.0
Hammer Muehle Vitalbrotmix sunflower seeds	Germany	757	181	33.0	1.0	4.0
Glutano Dreikornbrot	Germany	812	194	38.0	n.a.	n.a.
Sibylle Diat Buckwheat-linseed bread	Germany	799	191	26.0	n.a.	n.a.
Sibylle Diat sunflower bread	Germany	1017	243	35.0	n.a.	n.a.
Hammer Muehle Bio-Hirsebrot	Germany	1105	264	47.2	n.a.	n.a.
Hammer Muehle Bio-Inkabrot	Germany	1008	241	37.0	n.a.	n.a.
Hammer Muehle Bio-Lupinenbrot	Germany	1029	246	38.3	n.a.	n.a.

	Country	kJ/100g	Kcal/100 g	Carbohydrates [g/100 g]	Sugar	Fibre [g/100g]
Hammer Muehle Vitalbrot-Mix linseeds	Germany	824	197	36.0	2.0	5.0
Average Gluten free breads		**887**	**212**	**34.9**	**1.6**	**5.3**
Standard bread pumpernickel style*		**1046**	**250**	**47.5**	**0.5**	**6.5**

				Carbohydrates		Fibre
Multi-seed bread	**Country**	**kJ/100g**	**Kcal/100 g**	**[g/100 g]**	**Sugar**	**[g/100g]**
Revola GlutaBye Savoureux aux cereales	France	1050	251	30.5	n.a.	10.1
Revola GlutaBye Baguette sesame pavot	France	891	213	45.2	n.a.	n.a.
Schaer Ertha Unser Koernerbrot	Germany	745	178	33.0	1.8	11.6
ener BIO Sesambrot	Germany	1013	242	26.3	1.6	5.0
3Pauly Winzerbrot mit Teff	Germany	891	213	39.2	n.a.	n.a.
Livwell Sliced Multi-Seeded Bread	Ireland	1151	275	44.9	2.1	3.9
Marks & Spencer Brown seeded loaf	Ireland	1025	245	19.3	0.5	14.2
Ds Dietary Specials multigrain sliced loaf	Ireland	987	236	42.6	2.3	5.4
Semper Toasty Trio	Sweden	962	230	40.0	1.1	6.5
Semper Trio Rolls	Sweden	1172	280	45.0	4.0	5.0
Average gluten free multi-seed bread		**989**	**236**	**36.6**	**1.9**	**7.7**
Standard multi-seed wheat bread		**1109**	**265**	**43.3**	**6.4**	**7.4**

White bread	Country	kJ/100g	Kcal/100 g	Carbohydrates [g/100 g]	Sugars	Fibre [g/100g]
Kaisersemmeln - white rolls	Austria	1075	257	56.0	2.3	1.9
Meingast Landbroetchen	Austria	1297	310	62.7	5.5	4.1
Meingast Ciabatta	Austria	1184	283	57.7	0.3	2.4
Porkkanaleipä - Carot bread	Finland	908	217	43.7	2.2	2.3
Kauraleipä - Oatbread	Finland	1046	250	50.4	2.3	6.0
Rouheleipä - white bread with flaxseeds	Finland	941	225	48.8	1.6	4.7
Vuokaleipä pieni Toast	Finland	n.a	n.a	n.a.	n.a	n.a.
Marika-leipä Oat bread	Finland	n.a	n.a	n.a.	n.a	n.a.
Les Recettes de Celiane Pain Campagnard	France	1130	270	52.4	5.5	4.5
Valpiform Pain Campagnard	France	1033	247	50.3	n.a	n.a.
France aglut Le Grand Bio	France	1025	245	45.9	n.a	n.a.
France aglut Pain Biologique	France	1075	257	48.1	n.a	n.a.
Schnitzer spezial Bio feines Reisbrot	Germany	824	197	42.9	7.1	4.2
Schaer Bon matin suesse Broetchen	Germany	1205	288	52.2	12.4	3.0
Schaer Ciabatta	Germany	820	196	40.9	2.7	8.3

Schaer Pan carré	Germany	900	215	39.7	3.8	6.3
Schaer Panini	Germany	891	213	44.6	3.3	4.5
Schaer Mini-Baguette Duo	Germany	908	217	45.3	3.0	6.3
Naturkornmuhle Werz Braunhirse Toast	Germany	1184	283	43.8	n.a.	n.a.
Naturkornmuhle Werz	Germany	1219	291	56.9	n.a.	n.a.
Sibylle Diat chestnut bread	Germany	1017	243	35.0	n.a.	n.a.
Kelkin Gluten Free White Rolls	Ireland	1029	246	53.0	2.9	0.1
Juvela gluten-free White Sliced Loaf	Ireland	971	232	47.8	5.4	3.3
Juvela Gluten-Free Bread Rolls	Ireland	1301	311	58.0	8.1	1.5
Livwell Sliced White Bread	Ireland	1130	270	47.6	trace	4.0
Genius Gluten Free Fresh White Bread	Ireland	1238	296	41.1	3.0	9.7
Juvela Fibre Sliced Loaf	Ireland	858	205	42.1	4.4	5.0
Livwell Sliced White Bread	Ireland	1130	270	47.6	trace	4.0
Juvela white rolls	Ireland	1071	256	46.7	8·8	3.5
Livwell 4 white rolls	Ireland	1172	280	47.7	3.4	4.1
Ds Dietary specials white sliced loaf	Ireland	962	230	41.6	3.1	5.6
Kelkin Sliced White Bread	Ireland	1029	246	53.0	2.9	0.1

Livwell 4 White Pitta Breads	Ireland	1096	262	61.7	4.3	3.9
Marks & Spencer White Loaf	Ireland	1088	260	34.9	0.5	8.0
Marks & Spencer 4 soft white baps	Ireland	1025	245	37.5	0.5	6.7
BiAglut Sforna Gusto Pan carre	Italy	1134	271	50.2	n.a.	n.a.
Semper Toasty white	Sweden	837	200	42.0	4.8	4.1
Semper Minibaguette	Sweden	1130	270	52.0	6.2	2.3
Ener G White Rice Loaf	USA	1100	263	44.7	5.3	2.6
Ener G Tapioca Loaf	USA	1197	286	39.3	3.6	7.1
Udi's Gluten Free Foods Sandwich Bread	USA	1025	245	35.0	3.5	1.8
Average gluten free white bread		**1056**	**253**	**47.0**	**4.0**	**4.0**
Standard wheat white bread*		**1113**	**266**	**50.6**	**4.3**	**2.4**

*Source: USDA National Nutrient Database for Standard Reference; accessed 18/06/2010

Appendix Ib: Worksheets of the central composite experimental design for gluten free pasta

Worksheet of the central composite experimental design for gluten free pasta based on wheat flour

Trial	Independent variables			Dependent variables		
	Water	Egg white	Emulsifier	Cooking loss	Elasticity	Firmness
1	35	7.5	2.41	4.04	56.02	4.42
2	35	7.5	1	3.58	60.79	5.25
3	37.5	5	2	3.37	33.48	2.6
4	35	7.5	1	3.22	56.03	4.93
5	37.5	10	0	3.7	59.12	5.47
6	35	11.04	1	2.39	75.27	6.2
7	35	3.96	1	4.16	32.71	3.19
8	32.5	5	0	4.46	52.38	4.17
9	35	7.5	0.5	3.08	55.37	4.66
10	35	7.5	1	3.27	57.09	4.92
11	38.54	7.5	1	3.79	39.85	4.19
12	32.5	10	2	4.13	72.84	6.08
13	31.46	7.5	1	3.98	61.03	5.03

Worksheet of the central composite experimental design for gluten free pasta based on oat flour

Trial	Independent variables			Dependent variables		
	Water	Egg white	Emulsifier	Cooking loss	Elasticity	Firmness
1	47.50	12.50	2.00	3.43	22.38	2.21
2	49.57	15.00	1.00	n.d.	n.d.	n.d.
3	42.50	15.00	1.00	2.50	39.12	3.59
4	47.50	17.50	0.00	n.d.	n.d.	n.d.
5	37.50	17.50	2.00	3.27	50.01	4.98
6	35.43	15.00	1.00	3.43	36.92	4.35
7	42.50	15.00	1.00	3.07	40.61	3.37
8	42.50	18.54	1.00	2.92	51.80	4.74
9	42.50	15.00	1.00	3.13	33.74	3.27
10	42.50	15.00	0.50	3.01	34.03	3.93
11	42.50	11.46	1.00	3.35	26.67	2.64
12	42.50	15.00	2.41	2.62	35.54	3.78
13	37.50	12.50	0.00	3.14	34.06	3.34

Worksheet of the central composite experimental design for gluten free pasta based on teff flour

Trial	Independent variables			Dependent variables		
	Water	Egg white	Emulsifier	Cooking loss	Elasticity	Firmness
1	47.5	17.5	0	4.32	37.22	3.15
2	42.5	15	1	4.3	31.35	2.86
3	37.5	12.5	0	4.42	37.86	2.7
4	42.5	15	1	3.81	28.21	2.82
5	47.5	12.5	2	4.66	22.5	2.12
6	42.5	11.46	1	5.65	21.1	2.46
7	35.43	15	1	4.49	41.76	4.4
8	42.5	15	0.5	5.02	34.05	2.99
9	49.57	15	1	2.97	n.d.	2.89
10	37.5	17.5	2	4.58	47.52	4.53
11	42.5	15	1	3.9	31	2.91
12	42.5	15	2.4	4.22	26.57	2.75
13	42.5	18.53	1	3.96	44.87	3.93

Appendix II: Publications, Oral and Poster Presentations

First author publications

Hager, A-S., Ryan, L.A.M., Schwab, C., Ganzle, M.G., O'Doherty, J.V., Arendt, E.K. 2011. Influence of the soluble fibres inulin and oat beta-glucan on quality of dough and bread, European Food Research and Technology, 232(3):405-413

Hager A-S., Axel C., Arendt E. K. 2011. Status of Carbohydrates and Dietary Fiber in Gluten free Diets, Cereal Foods World, 56(3):109-114

Hager, A-S., Zannini E., Arendt, E.K. 2012. Formulating breads for specific dietary requirement, In Stanley Cauvain: Breadmaking 2nd edition

Hager, A-S., Vallons K.J.R., Arendt, E.K. 2012. Influence of Gallic Acid and Tannic Acid on the Mechanical and Barrier Properties of Wheat Gluten Films, Journal of Agricultural and Food Chemistry, 60(24):6157-6163

Hager, A-S., Wolter, A., Czerny, M., Bez, J., Zannini, E., Arendt, E.K., Czerny, M. 2012. Investigation of product quality, sensory profile and ultrastructure of breads made from a range of commercial gluten-free flours compared to their wheat counterparts, European Food Research and Technology, 235(2):333-344

Hager, A-S., Wolter, A., Jacob, F., Zannini, E., Arendt, E.K. 2012. Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours, Journal of Cereal Science 56:239-247

Hager, A.-S., Lauck, F., Zannini, E., Arendt, E.K. 2012. Development of gluten-free fresh egg pasta based on oat and teff flour. European Food Research and Technology, 235(5):861-871

Hager, A.-S., Zannini, E., Arendt, E.K. 2012. Gluten-free Pasta—Advances in Research and Commercialization. Cereal Foods World, 57(5):225-229

Hager, A.-S., Arendt, E.K. 2013. Influence of hydroxypropylmethylcellulose (HPMC), xanthan gum and their combination on loaf specific volume, crumb hardness and crumb grain characteristics of gluten-free bread. Food Hydrocolloids, 32, 195-203

Hager, A.-S., Zannini, E., Bez, J., Czerny, M., Arendt, E.K. 2013. Sensory properties, microstructure and *in vitro* digestibility of egg pasta produced from oat, teff and wheat flour. Journal of Cereal Science, In Press

Other

Wolter, A., Hager, A.-S., Zannini, E., Galle, S., Gänzle, M., Arendt, E.K. 2013 Evaluation of the EPS producing strain Weisella cibaria MG1 for the production of sourdough from various flours – a comparative study. Journal of Food Microbiology, In Press

Wolter, A., Hager, A.-S., Zannini, E., Arendt, E.K. 2013 *In vitro* starch digestibility and estimated glycaemic indexes of various gluten-free breads. Journal of Cereal Sciencce, Submitted.

Anna-Sophie Hager, Kaisa Poutanen, Laura Flander, Kati Katina, Elke K. Arendt 2013 Bedeutung des Sauerteiges für ernährungsphysiologische Eigenschaften von Backwaren, In: Markus Brandt und Michael Gänzle: Handbuch Sauerteig (7. Auflage)

Prof. Dr. Elke K. Arendt und Dipl.-Ing. Anna-Sophie Hager 2010 Fortschritt in der Herstellung glutenfreier Backwaren; Zoliakie (Mitgliederbulletin der IG Zoliakie der Deutschen Schweiz) Nr.4

Anna-Sophie Hager, Claudia Axel, Elke K. Arendt 2011 Apporti di carboidrati e fibre alimentari nella dieta senza glutine; Traduzione a cura di Simona Gatti; Celiachia Notizie (Notiziario dell'Associazione Italiana Celiachia) Nr.2

Anna-Sophie Hager, Claudia Axel, Elke K. Arendt 2011 Status of Carbohydrates and dietary fibre in the gluten-free diet; Coeliac Ireland (Coeliac Society Ireland) Issue 5

Anna-Sophie Hager, Emanuele Zannini, Elke K. Arendt 2012 La pasta gluten-free: composizione e qualità: Una revision dei progressi compiuti nella ricerca e nell'industria; CeliachiaNotizie(Notiziario dell'Associazione Italiana Celiachia) Nr.2

Oral and Poster Presentations

Anna-Sophie Hager; Liam A.M Ryan, John V. O'Doherty, Elke K. Arendt (2010) Influence of Beta-glucan from Different Origins on the Quality of Gluten Free Breads. Oral presentation: 2nd International Symposium on Gluten-free Cereal Products and Beverages, Tampere, Finland

Anna-Sophie Hager, Liam A.M Ryan, Clarissa Schwab, Michael G. Gänzle, Elke K. Arendt (2010) Influence of the soluble fibres inulin and oat-β-glucan on quality of dough and bread. Poster presentations: 3rd C&E Springmeeting, Freising-Weihenstephan, Germany and 40th Annual UCC Food Research Conference, Cork, Ireland; 5th International Dietary Fibre Conference, Rome, Italy

Anna-Sophie Hager, Anika Wolter, Emanuele Zannini, Elke K. Arendt (2011) The nutritional profile of different gluten-free flours and their potential use for the production of bread. Oral presentations: 3rd C&E Springmeeting, Freising-Weihenstephan, Germany and 40th Annual UCC Food Research Conference, Cork, Ireland

Anna-Sophie Hager, Elke K. Arendt (2011) Neueste Entwicklungen bei der Herstellung von glutenfreien Getreideprodukten. Oral presentation: DLG e.V. Symposium Lebensmittel für Zöliakie-Betroffene, Muenchen, Germany

Anna-Sophie Hager, Elke K. Arendt (2012) Neueste Entwicklungen im Bereich von gluten-freien Getreideprodukten. Oral presentation: Frühjahrstagung des Weihenstephaner Instituts für Getreideforschung, München, Germany

Anna-Sophie Hager, Elke K. Arendt (2012) The role of dietary fibre in gluten-free bread making. Oral presentation: 5th International Dietary Fibre Conference, Rome, Italy

Anna-Sophie Hager, Fabian Lauck, Anika Wolter, Emanuele Zannini, Elke K. Arendt (2012) The application of Scanning Electron Microscopy (SEM) and Confocal Laser Scanning Microscopy (CLSM) for the investigation of pasta ultrastructure. Poster presentation: The Microscopy Society of Ireland Annual Symposium 2012, Cork, Ireland

Cristiana Garofalo, Manuela Mariotti, Lucia Aquilanti, Andrea Osimani, Lorenzo Fongaro, Anna-Sophie Hager, Emanuele Zannini, Elke Karin Arendt, Francesca Clementi (2012)

Sourdoughs for bread-making with barley flour: sensory and technological evaluation of barley breads. Poster presentation: 5th Symposium on Sourdough, Helsinki, Finland

Anna-Sophie Hager, Emanuele Zannini, Elke K. Arendt (2012) Gluten-free Pasta. Oral presentation: Industry Workshop Gluten-free Foods, University College Cork, Ireland

Anna-Sophie Hager, Elke K. Arendt (2013) Gluten-free Cereal products - Recent advances in the production of bread, pasta and beer. Oral presentation: C&E Springmeeting, Leuven, Belgium

Anna-Sophie Hager, Elke K. Arendt (2013) Recent developments in the area of gluten free malt, beer and non-alcoholic beverages. Oral presentation: 3rd international Symposium on Gluten Free, Vienna, Austria

Anna-Sophie Hager, Fabian Lauck, Anika Wolter, Emanuele Zannini, Michael Czerny, Elke K. Arendt (2013) Production of fresh egg pasta based on oat, teff and wheat flour: Sensory properties, in-vitro digestibility and microstructure. Poster presentation: 3rd international Symposium on Gluten Free, Vienna, Austria

Anna-Sophie Hager, Elke K. Arendt (2013) Influence of hydroxypropylmethylcellulose (HPMC), xanthan gum and their combination on loaf specific volume, crumb hardness and crumb grain characteristics of gluten-free bread. Poster presentation: 3rd international Symposium on Gluten Free, Vienna, Austria

Outi Mäkinen, Anna-Sophie Hager, Elke K. Arendt (2013) Mobilisation of starch and protein reserves and the development of related enzyme activities in Chenopodium quinoa. Poster presentation: 3rd international Symposium on Gluten Free, Vienna, Austria

Anika Wolter, Anna-Sophie Hager, Emanuele Zannini, Elke K. Arendt (2013) Can sourdough influence starch digestibility and in vitro glycaemic index of gluten-free breads? Poster presentation: 3rd international Symposium on Gluten Free, Vienna, Austria

Anika Wolter, Anna-Sophie Hager, Emanuele Zannini, Elke K. Arendt (2013) Exopolysaccharide producer as sourdough starter in gluten-free flours? Poster presentation: 3rd international Symposium on Gluten Free, Vienna, Austria

Awards

Best student poster presentation at the Microscopy Society of Ireland Annual Symposium 2012, Cork, Ireland

EMBARK postgraduate scholarship awarded by IRCSET (Irish Research Council for Science, Engineering and Technology) in 2009