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Fundamental research on gluten-free bread

Thesis presented by

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Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

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Stefan Horstmann

Abbreviations

BU	Brabender units
BV	Breakdown viscosity
CD	Coeliac disease
CMC	Carboxymethylcellulose
DH	Dermatitis Herpetiforms
EPS	Exo poly saccharides
FODMAPS	Fermentable oligo-, di-, mono saccharides and polyols
FV	Final viscosity
GA	Gluten Ataxia
GF	Gluten-free
GLcOS	Gluco- oligo Saccharides
HPMC	Hydroxy propyl-methyl cellulose
IBS	Irritable bowel syndrome
LAB	Lactic acid bacteria
NCGS	Non-coeliac gluten sensitivity
PT	Pasting temperature
PV	Peak viscosity
RH	Relative humidity
SF	Sprouted flour
Slope BP	Slope baking process
Slope FP	Slope fermentation process
SP	Swelling power
TMH	Temperature at maximum height
WA	Wheat allergy
WBC	Water binding capacity
WDEA	Wheat dependent exercise-induced anaphylaxis
WHC	Water holding capacity
WHO	World Health Organisation

Abstract

The interest in gluten-free products has increased drastically over the past decades. This is the result of advanced detection methods for gluten-related disorders and the lifestyle choices of consumers. Gluten plays a key role in the production of bread, due to its viscoelastic properties. The replacement of gluten in bread creates a major challenge for producers and scientists to overcome. A literature review as part of this thesis discussed the current state of gluten-free bread and the efforts made to improve it. The quality of gluten-free bread has improved but is still considered to be of poor quality in regard to texture and nutritional value. Based on this review it was concluded that there is a need for a more fundamental understanding of ingredient interactions in a gluten-free bread system. The gained knowledge could help to improve the quality and nutritional value of gluten-free bread. This thesis addresses this issue by characterising commercially available raw materials and their influence on a model bread system (potato starch, HPMC, salt, sugar, yeast, water). Protein supplementation (pea, carob, lupin, potato, soy) in the model bread system affected bread quality parameters, such as specific volume and crumb hardness. Statistical analysis showed strong correlations between the functional properties (foaming, solubility) of the proteins and the bread quality parameters. In addition, the potential of functional ingredients such as hydrocolloids (HPMC, xanthan gum, guar gum, locust bean gum, sodium alginate, pectin) at different concentrations (0.25,0.5, 1.0,1.5,2.0%) to improve the quality of the model bread system was evaluated. It was observed that the addition of sodium alginate and pectin increased the specific volume of the breads in comparison to the HPMC and offered a more consumer-friendly substitute. Furthermore, beer yeast strains of the species *Saccharomyces cervisiae* were applied to a model bread system (potato starch, pea protein, pectin, salt, sugar, yeast, water). The results generated revealed the potential use of beer yeasts in the model bread system. The activity of yeast, which is affected by temperature and time strongly influenced the size of the baked loaves and correlated with crumb hardness. Lastly, the addition of milled sprouts (amaranth, brown millet, corn, lentil, lupin, pea, quinoa) to improve the nutritional value and its effect on the quality of model-bread system was evaluated. A comprehensive analysis of chemical composition, dough rheology and final bread properties revealed no significant correlations. However, the addition of amaranth caused an improvement of the specific volume and crumb hardness in comparison to a control.

The application and combination of the different ingredient groups showed an improvement of the bread formulation in comparison to the starting formulation. The gained fundamental knowledge about the effect of raw material in a gluten-free model bread system opens new opportunities to improve gluten-free bread. The study further suggests raw materials for the use in gluten-free bread production. It also revealed ingredients which could be used to satisfy the demand by consumers for improved nutritional value.

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Chapter 1 Introduction

Bread derived from wheat (*Triticum aestivum L.*) flour is one of the most common food items consumed all around the world. Gluten, the protein present in wheat, is a key component in flour for the breadmaking process. Indeed, mixing with water allows the gluten to develop a network, which gives a viscoelastic dough with gas retaining ability, mixing tolerance, resistance to stretch and extensibility (Gallagher, Gormley, & Arendt, 2004; Moore, Schober, Dockery, & Arendt, 2004). Thus, gluten is of fundamental importance for the overall appearance and textural properties of wheat-based baked products. In wheat bread, the solid matrix of the crumbs consists of a continuous phase of partially gelatinized starch and a continuous gluten network, which encloses the starch granules and fibre fragments (Dürrenberger, Handschin, Conde-Petit, & Escher, 2001). Nevertheless, there are individuals who cannot consume wheat containing bread. Coeliac disease (CD), also called gluten enteropathy and coeliac sprue, is one of the most common food induced intolerance in humans. This disease is caused by the intolerance to wheat gluten and similar proteins of barley and rye. It is an immune-mediated enteropathy causing inflammation in the small intestine and triggered by the ingestion of the storage protein gluten (Shan et al., 2002). CD is not the only disease that is caused by the ingestion of gluten. The intolerances which also fall in the category have the umbrella term "gluten-related disorders" (GRD) (Sapone et al., 2012). The four main forms besides CD, which are summarized by the umbrella term, are: non-celiac gluten sensitivity, dermatitis herpetiformis, wheat allergy and gluten ataxia. Based on the advanced and evolving technologies in medicine more people are diagnosed with CD and related diseases. The increase of the gluten-free market is also due to the consumption of these products by the family members and friends of coeliac patients and by healthy consumers, who eat gluten-free products as a lifestyle choice (Arranz, Fernández-Bañares, Rosell, Rodrigo, & Peña, 2015).

The avoidance of gluten-containing products is currently the essential treatment of coeliac disease (CD). Patients who suffer from CD have to strictly adhere to the gluten-free diet, hence a re-exposure to gluten would reactivate the disease. In general, a strict gluten-free diet has been recommended for all forms of gluten-related disorders (Pietzak, 2012). Thus, the interest in gluten-free cereal products has increased significantly over the last number of years, which led to a drastic increase of published literature in this area. A literature review as part of this study (**Chapter 2**) gives an

overview of coeliac disease and other gluten-related disorders, the current state of gluten-free bread and the analysis of the most promising approaches for developing gluten-free bread. The lack of gluten in dough results in a less cohesive and elastic system than wheat dough. For this reason, gluten-free doughs are more like a liquid batter, highly smoothly and difficult to handle. As a consequence, the end product shows a crumbly texture, poor colour and many other quality defects (Gallagher, Gormley, & Arendt, 2004). Over the last few decades, the quality, in terms of texture and structure, of gluten-free bread has improved. However, it is still described as a blend of refined or chemically-based food ingredients with unpalatable and frequently chemical flavours (Rosell & Matos, 2015). In addition, it was found that the nutritional value of gluten-free breads is very poor, due to the lack of vital minerals, vitamins, amino acids and an increased amount of fat in comparison to gluten-containing breads (Miranda, Lasa, Bustamante, Churruca, & Simon, 2014; Pellegrini & Agostoni, 2015).

As seen for the commercial products, gluten-free breads present a complex formulation, where a wide range of gluten-free ingredients from different botanical sources are combined. Based on the given situation it is not possible to understand how every single component interacts in a gluten-free system. For this reason, fundamental research on simple gluten-free formulations is necessary. Therefore, research on the application of different starches and their effect on a simple gluten-free formulation has been recently been conducted (Horstmann, Belz, Heitmann, Zannini, & Arendt, 2016) (not part of this study). The results gained from that study, showing potato starch as the most promising starch for the fundamental starch recipe build the basis for this thesis.

Legume proteins, due to their functional properties and high nutritional value, have been under investigation as ingredient in gluten-free products in recent years (Waglay, Karboune, & Alli, 2014). Some of these proteins, such as lupin, pea and soy have already been utilized in complex gluten-free bread formulations (Ziobro, Juszczak, Witczak, & Korus, 2016; Ziobro, Witczak, Juszczak, & Korus, 2013). The use of legume proteins in cereal products has recently been reviewed and recommended for the use in gluten-free formulations, where they have a potential key role to play in terms of their techno-functional properties and the improvement the nutritional profile of gluten-free products (Foschia, Horstmann, Arendt, & Zannini, 2017). Based on the beneficial properties of proteins the established gluten-free base formulation was fortified with

different protein sources (pea, lupin, soy, potato, carob). The correlation analysis between protein properties, dough properties and final bread quality were established and investigated in **Chapter 3**. The knowledge obtained in this study enables a better understanding and application of plant protein in gluten-free bread formulations. This could help the industry to improve gluten-free bread quality, and potentially lead to improvement of the nutritional value.

The absence of gluten, in breads, with its unique viscoelastic properties results in reduced gas retention and structure formation (Hager & Arendt, 2013). A lot of research has been conducted to tackle this problem by the addition of hydrocolloids. These are water-soluble polysaccharides with varied chemical structures and have a wide range of functional properties that make them suitable for different applications particularly in the area of gluten-free bread (Li & Nie, 2016). In a gluten-free system, the water is added at a much higher concentration and therefore results in the full gelatinization of the starch (Hager, Wolter, Jacob, Zannini, & Arendt, 2012). The amount of water added depends on the various ingredient combinations due to interactions and crosslinking. **Chapter 4** investigated the application of six hydrocolloids (guar gum, locust bean gum, xanthan gum, sodium alginate, pectin) regarding their suitability in a gluten-free formulation. The hydrocolloids were applied at five different concentrations (0.25%, 0.5%, 1%, 1.5%, 2.0% dry basis). The fragile nature of gluten-free dough systems makes it usually difficult to assess the water absorption properties by the use of a farinograph. Thus, the chapter also attempts to find a solution for the different water absorption properties with the aid of an equation which takes the water holding capacity of the ingredient into consideration

The production of CO_2 by yeast is with the retention of produced CO_2 one of the crucial factors in gluten-free bread production. The application of yeast has an influence on the dough and bread parameters, but also on the aroma and flavour profile of breads (Lai & Lin, 2006). During fermentation, yeast ferments available sugars and produces CO_2 as a metabolite. The amount of CO_2 and other metabolites vary between the applied yeast strains. *Saccharomyces cerevisiae* (Baker's yeast) is the most commonly used yeast, which is the primary leavening agent in bread products (Rezaei et al., 2014). However, studies on wheat bread demonstrated positive effects of other *Saccharomyces cerevisiae* yeast strains more commonly used in the production of beer (Heitmann,

Zannini, & Arendt, 2015; Horstmann, Belz, Heitmann, Zannini, & Arendt, 2016). Therefore, the suitability of various yeast strains commonly used in the beer brewing process, to be used in gluten-free dough leavening to enhance bread quality characteristics were investigated in **Chapter 5**. This is the first study to comprehensively determine the effect of different yeast strains on gluten-free dough properties, bread texture, structure, bread aroma and flavour profile in combination with descriptive sensory analysis.

To tackle the current situation of low nutritional value bread, **Chapter 6** comprehensively investigates a wide range of sprouted grains and seeds and their effect on gluten-free bread quality. The inclusion of sprouted grains and seeds in cereal products, based on its health benefits, has been named as one of the major trends in marketing reports (The Washington Post 2017). To improve the nutritional value of wheat-bread the addition of sprouted grains and seeds has become common practice since it can, based on its enzyme activity, make complex minerals, vitamins and amino acids available to the human body. For gluten-free breads, which are reported to have a lack of nutritional value compared to its wheat-containing counterpart, the application of sprouted raw materials hence offers new potential to improve the nutritional value. For this approach flours of sprouted amaranth, brown millet, corn, lentil, lupin, pea and quinoa were implemented in the current gluten-free dough formulation. The sprouts were applied at a concentration of 5% to improve health benefits and to analyse the effect on the dough and bread properties of the established gluten-free system. For this purpose, gained knowledge from **Chapter 3, 4** were utilised.

The aim of this dissertation was to gain a better and more fundamental understanding of gluten-free bread formulations and the interactions of different ingredients to improve the quality. The gained knowledge is believed to contribute to the field of research in the gluten-free area. This generates new opportunities to produce gluten-free breads and further allows producers to better understand the gluten-free system. This knowledge is believed to allow producers to modify their formulation and maintain the structural quality of their baked bread. In addition, this study introduced a range of ingredients suitable for the application in gluten-free formulations, which can be used as a solution to enhance gluten-free breads in terms of structural, nutritional and sensorial aspects.

This doctoral dissertation provides knowledge to satisfy the high demand of the consumers for improved quality of gluten-free bread. It also creates a fundamental understanding and recipe base to conduct future research on.

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Chapter 2 Nutritional therapy – Facing the gap between coeliac disease and gluten-free food

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Abstract

The market for gluten-free bakery products is growing considerably since better diagnostic methods allow the identification of an increasing number of people suffering from coeliac disease and other gluten-related disorders, such as dermatitis herpetiformis, gluten ataxia, wheat allergy and non-coeliac gluten sensitivity. The only safe treatment available for these types of disorders is to follow a strict lifelong gluten-free diet. Besides the people needing to follow a gluten-free diet for health reasons, a new segment of consumers has arisen who consume gluten-free products as a lifestyle choice. Among the bakery products, bread is a major staple food consumed daily all over the world. The dough and bread quality characteristics (such as gas retention, mixing tolerance, resistance to stretch, extensibility and crumb structure) are mostly attributed to the presence of gluten. Despite the improved quality of gluten-free breads in the last number of years, most products on the market are still described as a low-quality product. In addition to the low overall quality of gluten-free products, the nutritional value of many them is quite poor. This review gives an overview of the gluten-related disorders and the gluten-free diet. The trends in this gluten-free bakery segment will also be reviewed. An overview of the major ingredients used in gluten-free bread products will be given, based on the analysis of current marketing studies. The choice of the ingredients discussed in this paper is based on a comprehensive study of the leading gluten-free breads available on the gluten-free market, as well as a detailed study of the scientific literature. The impact of the various ingredients on the bread-making process and bread quality is also part of this review. Major emphasis will be placed on the application of sourdough as a means to improve gluten-free bread quality.

Introduction

Coeliac disease (CD) has become an intensively researched topic over the last few years. In fact, a search on "Google Scholar" with the topic "Coeliac disease" resulted in 15,900 articles in the period between 2000 and 2010. The same search for the period between 2010 and 2015 resulted in 17,500 articles, indicating that more research was conducted in the last five years than in the previous ten years. Based on the advanced diagnostic technologies more people are diagnosed with CD and related diseases.

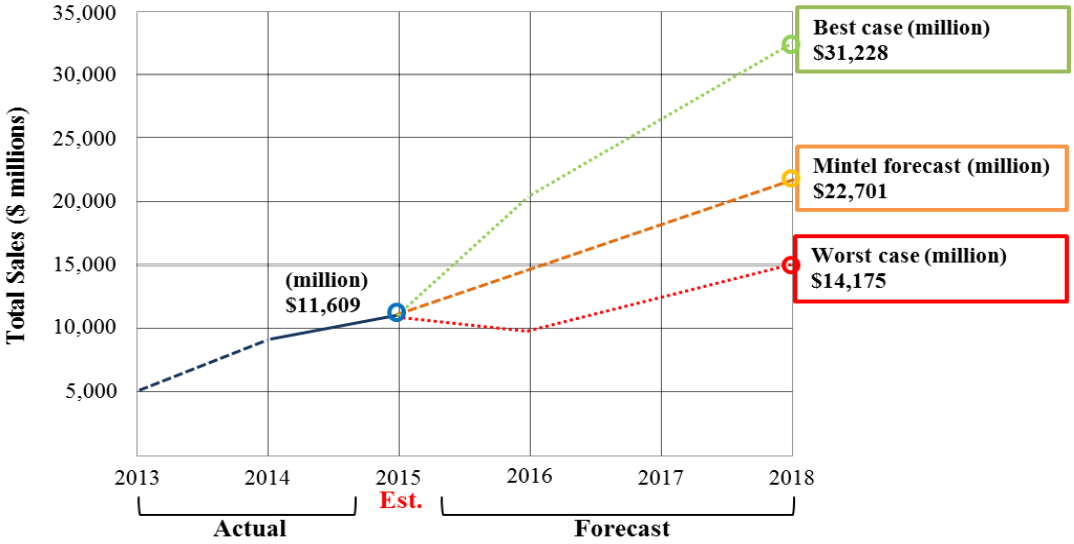

Figure 2-1 Sales* and fan chart forecast of gluten-free foods in the US, at current prices, rolling 52 weeks June 2013–June 2018.

*Sales through MULO, natural supermarket, and specialty gourmet stores; does not include private label items or sales through Whole Foods Market.

Source: SPINS; Information Resources, Inc./(Mintel, 2015).

The increase of the gluten-free market is also due to the consumption of these products from the family members and friends of coeliac patients and otherwise healthy consumers, who eat gluten-free products as a lifestyle choice (Arranz, Fernández-Bañares, Rosell, Rodrigo, & Peña, 2015). While patients suffering from gluten-related diseases rely on a gluten-free diet, other consumers choose to follow it as a lifestyle choice, as it evokes a cultural-, ecological, civic-, historical-, ethnical- or health-based interest of quality (Worosz & Wilson, 2012). All these factors promote the gluten-free

market and its continuing growth. A recent report (Mintel, 2015) on the growing gluten-free market revealed that the expected sales were reached and are still growing. The report stated gluten-free food category growth of 136% between 2013 and 2015, reaching $11 billion, 2015. Based on the fact that food manufacturers introduce new products to the market and start to label their existing ones the share of gluten-free foods in the food category continues to grow. The Mintel report also offers predictions of total sales in the US until 2018 (Figure 2-1), with the worst-case scenario for the gluten-free industry still reaching an increase of almost a fifth (18.1%). In this context, the present review will present a general overview of CD and other gluten-related disorders moving then towards the analysis of the most promising approaches for developing gluten-free bread with particular emphasis on sourdough technology and alternative ingredients.

Coeliac disease (CD)

CD, also called gluten enteropathy and coeliac sprue, is one of the most common food induced diseases in humans caused by the intolerance to wheat gluten and similar proteins of barley and rye in genetically-susceptible individuals. CD is an immune-mediated enteropathy causing inflammation in the small intestine and triggered by the ingestion of the storage protein gluten (Shan et al., 2002). In some humans, leukocyte antigen (HLA), DQ2 and DQ8-positive lead to a destruction of the villous structure in the small intestine. This is caused by an inflammatory reaction of the small intestine due to the exposure to gluten (Catassi and Fasano, 2011). Recent epidemiological studies verify that 1 in 100 people worldwide suffers from CD. Such a high rate makes CD one of the most widespread food intolerances (Gujral, Freeman, & Thomson, 2012). CD commonly appears in early childhood, with symptoms including chronic diarrhoea and failure to thrive. The symptoms can also develop later in life when the disease symptoms include diarrhoea, fatigue, and weight loss due to malabsorption and anaemia (Vilppula et al., 2011). CD is a lifelong disease, and untreated it is associated with raised morbidity and mortality. Recent research associate CD with coronary heart disease and cerebrovascular disease (Heikkilä et al., 2015). The only treatment of CD is a strict and lifelong adherence to a gluten-free diet. The re-exposure to gluten reactivates the disease, even after many years of avoidance (Koehler, Wieser, & Konitzer, 2014).

Other gluten-related disorders

CD is not the only disease that is caused by the ingestion of gluten. The intolerances which also fall in the category have the umbrella term "gluten-related disorders" (Sapone et al., 2012). The four main forms, besides CD are: non-celiac gluten sensitivity, dermatitis herpetiformis, wheat allergy and gluten ataxia (Table 2-1). Although the only current treatment for these disorders is the avoidance of gluten-containing products, they show different symptoms. Based on this reason, it is important to differentiate between the disorders in order to allow more efficient and generalizable advances in the treatment of patients with CD and other gluten-related disorders (Ludvigsson et al., 2013). Recent research focuses also on extraintestinal manifestations of coeliac disease and gathers research of the last three decades (Leffler, Green, & Fasano, 2015). An obstacle to the diagnosis of patients suffering from gluten-related disorders is irritable bowel syndrome (IBS), since its symptoms are similar to those typical for CD and NCGS (non-coeliac gluten sensitivity), such as abdominal pain, gas, bloating and by altered bowel habits (diarrhoea with or without constipation) (Whitehead et al., 1980). Despite these similarities, IBS is triggered by the consumption of the poorly-absorbed fermentable oligo-, di-, monosaccharides and polyols (FODMAPs) and insoluble fibre (Lovell & Ford, 2012). However, the restriction of wheat-based products, (i.e. gluten-free diet) may also lead to reduced intake of fibre such as arabinoxylan, which in turn has a positive effect on people with IBS. A review of the recent developments in the pathophysiology of IBS found compelling evidence that genetic factors, diet, the intestinal microbiota and mucosal low-grade inflammation play a major role (El-Salhy, 2015).

Non-coeliac gluten sensitivity (NCGS) is the least clearly defined and researched gluten-related disorder (Lammers, Vasagar, & Fasano, 2014). NCGS has been frequently termed "gluten sensitivity" and has been described as gluten-mediated disorder (Koehler et al., 2014). The prevalence of NCGS (3%–6%) is estimated to be much higher than CD (1%) (Cascella et al., 2011; Sapone et al., 2012). NCGS has similar symptoms to CD and wheat allergy (WA) and overlaps with irritable bowel syndrome (Koehler et al.,2014). However, recent research was able to narrow the spectrum of irritable bowel syndrome by diagnosing NCGS (Shahbazkhani et al., 2015). Also, a recent study critically reviewed the evidence of NCGS and focuses on diagnostic efforts

to rule out CD (Molina-Infante, Santolaria, Sanders, & Fernández-Bañares, 2015). Wheat allergy (WA) is defined as an IgE-mediated immunological response to proteins of wheat and related cereals that affects the gastrointestinal tract, the respiratory tract or the skin (Keet, Matsui, Dhillon, Lenehan, Paterakis & Wood, 2009; Tatham & Shewry, 2008). WA is also known as baker's asthma and is mainly caused by inhalation of cereal flours, particularly wheat flour (Salcedo, Quirce, & Diaz-Perales, 2011). The allergic response can be divided into the well-defined wheat-dependent exercise-induced anaphylaxis (WDEA) and the less understood immune reactions (Morita, Kunie, & Matsuo, 2007). Patients who suffer from WA display a range of clinical symptoms. WDEA is often diagnosed by first level diagnostics like skin prick test and the IgE-assays. Dermatitis Herpetiformis (DH) is also known as Duhring-Brocy disease and sometimes referred to as CD of the skin (Kárpáti, 2015). DH is characterised by urticarial plaques and blisters on the elbows, buttocks and knees. Other areas of the body can also be affected (Caproni, Bonciolini, D'Errico, Antiga, & Fabbri, 2012). Gluten Ataxia (GA) is one of the most common neurological manifestations attributed to CD. It is like the other gluten-related disorders, an immune- mediated disease triggered by the ingestion of gluten-containing products in individuals which are genetically susceptible to it (Marios Hadjivassiliou, Sanders, & Aeschlimann, 2015). GA is also defined as idiopathic sporadic ataxia in presence of circulating antigliadin antibodies (IgA/IgG types) (Hadjivassiliou, Sanders, Woodroofe, Williamson, & Grünewald, 2008). GA has no unique features that distinguish it from the other types of ataxia and, hence, is more difficult to identify than CD (Hadjivassiliou et al., 2013). The current identification is performed by a serological screening for the antibodies AGA, TGA and anti-TG6. A gluten-free diet is recommended for people who are suffering from GA. Depending on the duration of the disease prior to the treatment, it has been stated that a strict adherence to a gluten-free diet will stabilize or even improve the well-being of the patients (Hadjivassiliou et al., 2010).

Table 2-1 Summarizing the prevalence and symptoms of the gluten-related disorders.

Disease	Prevalence in the World (approx. values)	Symptoms	Reference
Coeliac Disease (CD)	1 %	Chronic diarrhoea, failure to thrive, fatigue, malabsorption, anaemia	(Gujral et al., 2012; Vilppula et al., 2011)
Non-celiac gluten sensitivity (NCGS)	3 – 6 %	Gastrointestinal complaints, weight loss, bloating, diarrhoea, muscular disturbances, bone pain, tiredness, neurological disorders	(Cascella et al., 2009; Newnham, 2011; Sapone et al., 2012)
Wheat allergy (WA)	0.5 – 9 %	Urticaria, angioedema, erythema, dyspnoea, oropharyngeal symptoms, urticaria, angioedema, atopic dermatitis flare, rhinitis, asthma, gastrointestinal symptoms, and anaphylaxis, pruritus, eczema	(Lammers et al., 2014; Matricardi et al., 2008; Morita et al., 2007; Scibilia et al., 2006; Zuidmeer et al., 2008)
Dermatitis-Herpetiformis (DH)	0.0001 - 0.05 %	Urticarial plaques, blisters on the elbows, buttocks and knees	(Borroni et al., 2013; Caproni et al., 2012; Kárpáti, 2015)
Gluten Ataxia (GA)	14 %	Insidious onset of predominantly gait ataxia, often associated with symptoms and signs suggestive of peripheral neuropathy.	(Hadjivassiliou et al., 2013; Hadjivassiliou et al., 2003; Hadjivassiliou et al., 2015; Hadjivassiliou et al., 2008)
Crohn's disease[a]	0.0007 – 0.0199 %	Fever, weight loss, diarrhoea, abdominal pain	(Kohn et al., 2010; Lennard-Jones et al., 1997; Loftus et al., 2007; Rizzi, 2010; Rubin et al., 2000; Shivananda et al., 1996)
Irritable bowel syndrome[a]	5 – 30 %	Similar to CD and NCGS, bloating, diarrhoea, gas and abdominal pain	(Hillilä and Färkkilä, 2004; Thompson et al., 2006; Thompson et al., 2000)

[a] Gluten-free diet often recommended but gluten is not the cause for the disease.

Treatment

The avoidance of gluten-containing products is currently the essential treatment of coeliac disease (CD). CD patients must strictly adhere to the gluten-free diet, as a re-exposure to gluten would reactivate the disease. In general, a strict gluten-free diet has been recommended for all gluten-related disorders such as coeliac disease, dermatitis herpetiformis, non-coeliac gluten sensitivity and gluten ataxia (Pietzak, 2012). The gluten-free market offers a lot of products, which can be consumed by patients. Nevertheless, patients have to follow rules and be aware of "hidden" sources of gluten as it is also used as a functional ingredient such as in sauces, meat and fish products (Kalin, 1979, Day, Augustin, Batey, & Wrigley, 2006). Further obstacles are products that do not contain gluten in the ingredient list but might have been contaminated during production. To avoid any type of contamination, gluten-containing ingredients have to be located and manipulated in areas strictly separated from the gluten-free ones.

Based on this, it is recommended that patients suffering from gluten-related diseases stick to gluten-free labelled products as they are certified and analytically checked on a regular basis for gluten residues (Thompson & Simpson, 2015). The treatment of gluten-related disorders with a gluten-free diet has positive effects on the mucosal histology as it will normalize and the clinical symptoms will ease. However, it has been reported that in some cases the natural balance of the microbiome in patients suffering from gluten sensitivity may not be completely restored (Nadal, Donant, Ribes-Koninckx, Calabuig, & Sanz, 2007). Furthermore, a gluten-free diet was reported to modify the composition and immune properties of a gut microbiota in adults (Marasco et al., 2016). It was speculated that the prebiotic action of gluten, which is absent in a gluten-free diet, can induce a different gut microbiota composition in comparison to a healthy person (Jackson, 2010). A literature review by Marasco et al. (2016) highlighted that a gluten-free diet allows only a partial recovery of the gut microbiota but leads to a reduction of potentially harmful bacteria.

Gluten-free bread: current situation of the quality and nutritional profile

Bread derived from wheat (*Triticum aestivum L.)* flour is one of the most common food items consumed all around the world. The comparison of the bread production with wheat flour and gluten-free flour is depicted in Figure 2-2. Gluten, the protein present in wheat, is a key component in flour for the breadmaking process. Mixing with water allows the gluten to develop a network, which gives a viscoelastic dough with gas retaining ability, mixing tolerance, resistance to stretch and extensibility (Gallagher, Gormley, & Arendt, 2004; Moore, Schober, Dockery, & Arendt, 2004). Thus, gluten is of fundamental importance for the overall appearance and textural properties of wheat-based baked products.

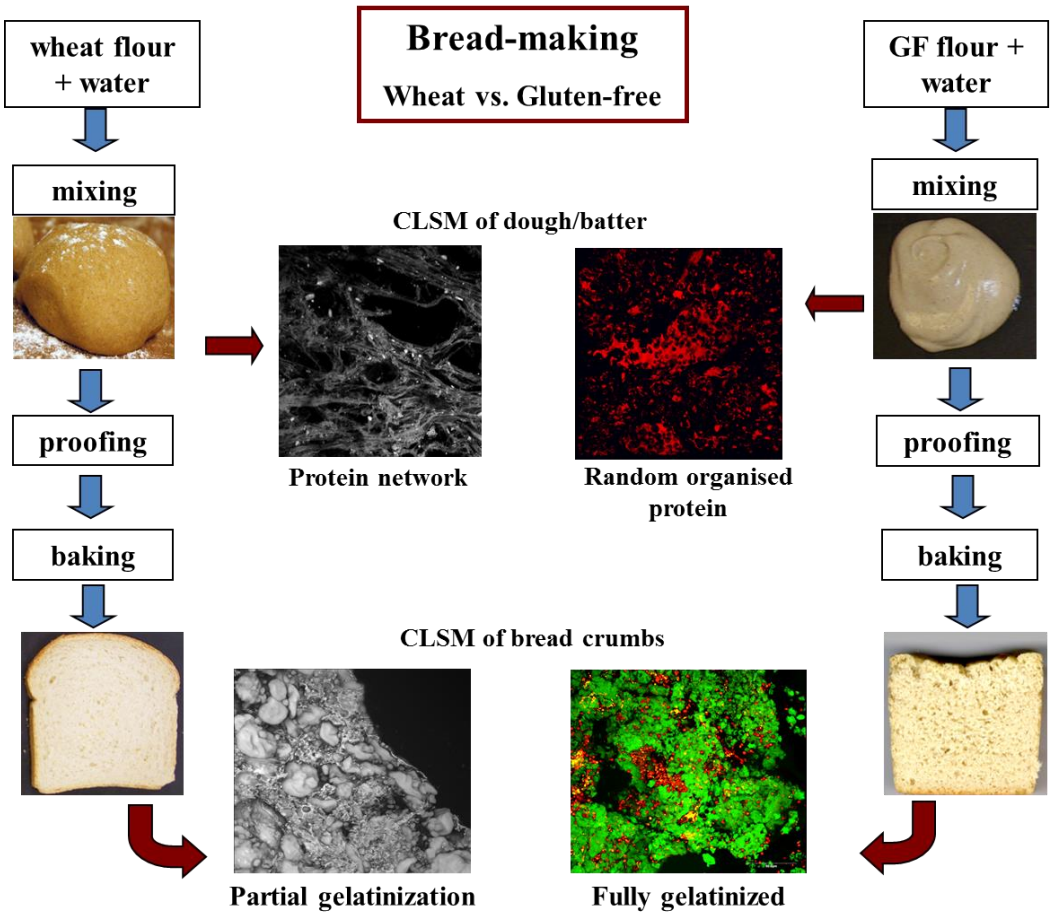

Figure 2-2 Comparison of the dough and crumb structure between wheat flour and gluten-free bread.

In wheat breads, the solid matrix of the crumbs consists of a continuous phase of gelatinized starch and a continuous gluten network, which encloses the starch granules and fibre fragments (Dürrenberger, Handschin, Conde-Petit, & Escher, 2001).

A lack of gluten in dough results in a less cohesive and elastic system than wheat dough. For this reason, gluten-free doughs are more like a liquid batter, highly smoothly and difficult to handle (Figure 2-2). As a consequence, the end product shows a crumbling texture, poor colour and many other quality defects, as shown in Figure 2-3 (Gallagher, Gormley, & Arendt, 2004). It can also be seen that there are major differences in the structure of the final bread. In wheat dough, the water is much more limited than in gluten-free bread formulations, which leads to only partial gelatinization of the starch molecules. In a gluten-free system, the water is added at a much higher level and therefore full gelatinization of the starch is observed in the final product (Figure 2-2) (Hager, Wolter, Jacob, Zannini, & Arendt, 2012). These differences in the gelatinization can partially explain why gluten-free formulations stale quickly and also have a crumbly texture.

Despite the quality improvement of these food products in the last years, most gluten-free breads on the market have been recently described as being a blend of refined or chemically-based food ingredients with unpalatable, frequently artificial flavours (Rosell & Matos, 2015). Although the quality of gluten-free breads is improving, they still lack in nutritional value. This is concluded by comparing a survey conducted by Thompson et al. (2005) and Mintel (2015), which both state that in the consumer's opinion the nutritional value of gluten-free products is poor. A further concern of consumers is weight gain due to a gluten-free diet. This concern is raised, as a result of the introduction of lipid-rich ingredients (Pellegrini & Agostoni, 2015). Some gluten-free breads contain twice the amount of fat in comparison to its gluten-containing counterpart (Miranda, Lasa, Bustamante, Churruca, & Simon, 2014). Among the several approaches for the development of gluten-free bread, the most suitable and promising are the utilization of highly nutritious gluten-free ingredients and sourdough technology, which will be discussed in the following chapters.

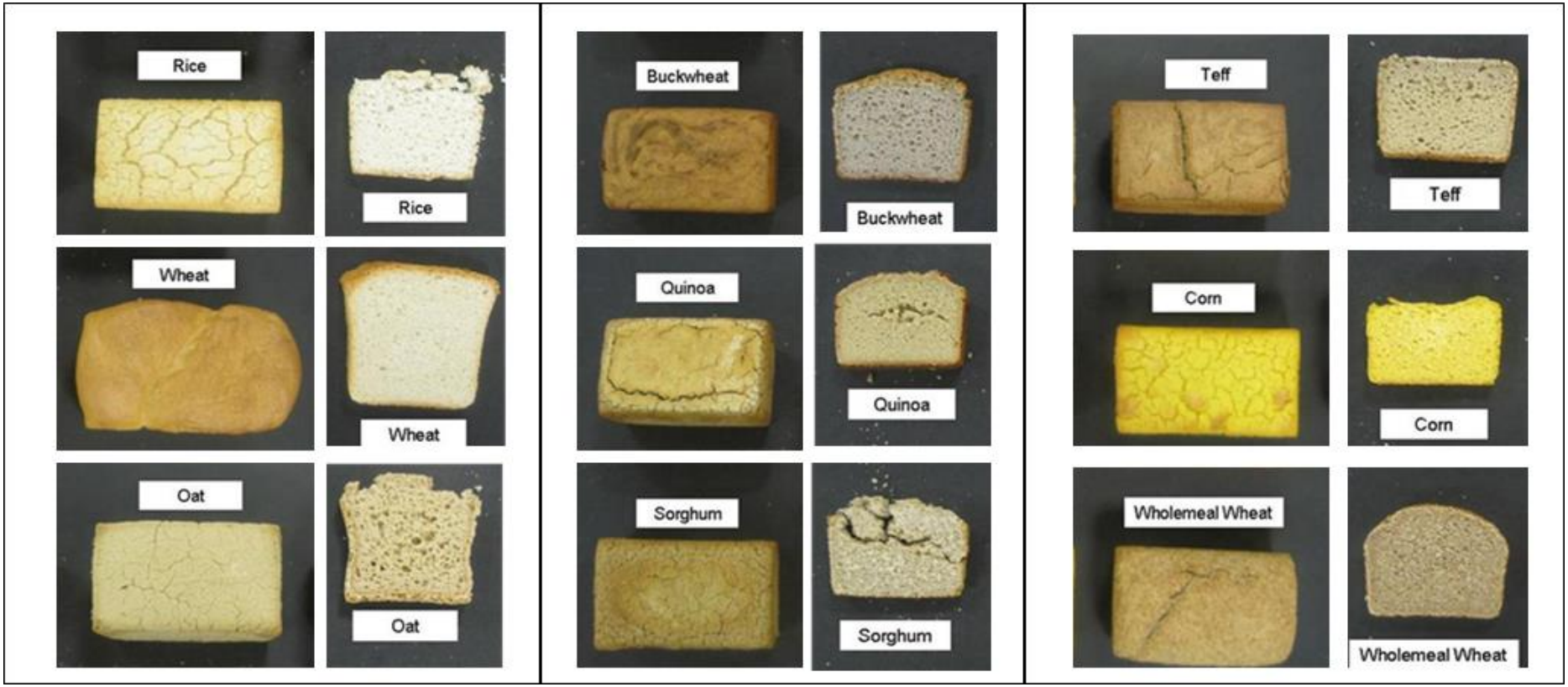

Figure 2-3 Photographs of crust surface and crumb of bread loaves prepared from 100% gluten-free flours and wheat flours (Hager, Wolter, Jacob, Zannini, & Arendt, 2012).

Gluten-free ingredients in commercial bread formulations

Currently, the most suitable cereal flours for preparing gluten-free commercial breads come from rice (*Oryza sativa*) and corn (*Zea mays L. ssp. mays*) (Table 2-2), due to their hypoallergenic properties, blend flavour and easy availability (Kadan, Robinson, Thibodeaux, & Pepperman, 2001). However, due to their hydrophobic nature, rice proteins are insoluble and are unable to form the viscoelastic dough necessary to hold the carbon dioxide produced during proofing of yeast-leavened bread-like products (He & Hoseney, 1991). As a consequence, the carbon dioxide produced during fermentation cannot be retained, thus leading to a product with low specific volume and very compact crumb (Figure 2-2), limiting the use of rice flour in bread making (Gujral & Rosell, 2004a; Marco & Rosell, 2008). Corn has a high energy value but exhibits proteins with a low biological value (low levels of the essential amino acids lysine and tryptophan) (Lošák et al., 2010). The corn kernel also lacks many essential minerals. In addition, it is generally poor in B vitamins and provides only negligible amounts of niacin (vitamin B3), which is essential for human health (Zeng, 2010).

The strategy in including a combination of ingredients has been the main choice for producers; with this approach, it is possible both mimic the viscoelastic properties and improve the nutritional profile (Dar, 2013). Gluten-free ingredients such as proteins, starches, hydrocolloids, fibres and sourdough are combined with gluten-free flours in order to improve the quality of the end product. As seen in Table 2-2, proteins (such as dairy, egg, soybean and pea) are used in the production of gluten-free bakery products. Indeed, they are able to improve the perceived quality by enhancing Maillard browning and flavour, improve texture, reduce the rate of staling and increase water absorption and therefore improve the handling of batters (Arendt, Morrissey, Moore, & Dal Bello, 2008). Moreover, the addition of starch in the gluten-free formulations could improve batter consistency during mixing, enhance the softness of the crumb, and control starch gelatinization during the baking process (Gallagher, 2009). Due to this, different starches from natural gluten-free sources such as corn, potato, rice and tapioca can be found in gluten-free commercial formulations (Table 2-2).

Table 2-2 List of ingredients commonly used in commercial gluten-free bread formulations and their occurrence (%).

Category	Ingredients	Use in commercial formulations [%][a]
Flours	Rice	59.3
	Corn	40.7
	Buckwheat	22.2
	Whole grain corn	18.5
	Tapioca	11.1
	Potato	7.4
	Millet	7.4
	Quinoa	3.7
Starches	Corn	88.9
	Potato	70.4
	Rice	59.3
	Tapioca	59.3
	Whole grain corn	18.5
	Wheat	3.0
Proteins	Egg white	63.0
	Pea	25.9
	Soya	18.5
	Whey	7.4
	Dried Skim Milk	7.4
	Milk	3.7
Hydrocolloids	HPMC	70.4
	Cellulose	40.7
	Xanthan	29.6
	Guar Gum	25.9
	SCMC	11.1
	Agar Agar	7.4
Fibres	Psyllium	74.1
	Rice Bran Extract	18.5
	Millet Flakes	11.1
	Rice Bran	7.4
	Soya Bran	3.7
	Apple fibre	3.7
	Flaxseeds	3.7
Sourdoughs	Rice Flour	22.2
	Corn Flour	11.1
	Fermented Quinoa	7.4

[a] Based on 27 bread samples of leading gluten-free companies in Ireland.

Hydrocolloids such as hydroxypropyl methylcellulose (HPMC), cellulose, xanthan and guar gum are added to improve the gas retaining properties of a gluten-free batter and also contribute to water binding (Casper, 2016).

Dietary fibres have been capturing the attention of gluten-free producers not only for their potential health benefits (cholesterol and fat binding, decrease in blood glucose levels, preventing constipation and facilitating good colonic health) (Kaczmarczyk, Miller, & Freund, 2012), but also for their physicochemical and functional properties such as their ability to enhance viscosity and modulate texture (Collar, Santos, & Rosell, 2007; Ronda, Gómez, Blanco, & Caballero, 2005). The safety of all these gluten-free ingredients might be compromised by the presence of contaminants such as mycotoxins, heavy metals and pesticides (Clarke, Connolly, Frizzell, & Elliott, 2015; Pussemier, Larondelle, Van Peteghem, & Huyghebaert, 2006). For instance, the consumption of corn-based foods, the most common ingredient in a gluten-free diet, could cause an exposure to higher levels of mycotoxins (Pellegrini & Agostoni, 2015). Indeed, corn is strongly contaminated by mycotoxins such as fumonisins, due to the strong fungal infection incidence in the field and during storage (Wild & Gong, 2009). As a consequence, it is essential for the suppliers of ingredients to know the critical control points during harvesting, drying and storage stages in the crop production chain; only in this way effective prevention strategies can be successfully developed. Scientific research on the influence of different ingredients and sourdough technology on gluten-free bread has also been conducted. The main findings of research from the last decade are discussed in the following sections.

Effect of the combination of gluten-free ingredients on dough characteristics and bread quality

In Table 2-3 the research work conducted on gluten-free bread is summarized. As seen for the commercial products, gluten-free breads present a complex formulation, where a wide range of gluten-free ingredients from different botanical sources are combined. For this reason, a proper comprehension and explanation about how every single component interacts in a gluten-free system is still not possible. Therefore, it is quite difficult to identify the best performing ingredients and their optimal process conditions for developing high-quality gluten-free bread. This would be overcome if more

fundamental studies were conducted. For example, it has still to be fully understood why the botanical origin and amount of starch affect differently the rheological and crumb quality of gluten-free bread (Onyango, Mutungi, Unbehend, & Lindhauer, 2011). However, based on Table 2-3, it can be stated that the utilization of flours in combination seems to be a good approach in order to obtain gluten-free breads with acceptable quality characteristics and nutritional profile. Indeed, some authors reported that the combination of soy flour with buckwheat, rice and corn flour resulted in breads of satisfactory quality (Moore, Schober, Dockery, & Arendt, 2004; Ribotta et al., 2004; Sciarini, Ribotta, León, & Pérez, 2010b). In particular, soy flour addition greatly affects batter and gluten-free bread characteristics, leading to an improvement in bread volume probably due to the increase in batter consistency. Additional positive effects were a good crumb appearance, a soft texture, and low staling rate. The addition of soy caused crumb softening and retarded bread staling as soy proteins had a high water-holding capacity and they could interfere in starch retrogradation (Sciarini et al., 2010b). These positive effects on gluten-free bread quality can also be due to the emulsifier lecithin, contained in the soy flour. It is able to retard the starch retrogradation by inhibiting the migration of water (Eduardo, Svanberg, & Ahrné, 2016; Stauffer, 2000) and increase the gas cell stabilization in the dough by forming liquid lamellar films surrounding the gas cells (Nunes, Moore, Ryan, & Arendt, 2009). Finally, Nunes et al. (2009) related the higher specific volume for gluten-free breads with the strength of the dough, which was linked to the presence of lecithin in the formulation. Another interesting combination of flours was reported by Elgeti et al. (2014), who evaluated the impact of quinoa white flour on gluten-free bread quality parameters, in particular volume. The partial replacement of rice flour and corn flour by quinoa white flour enhanced the specific volume of the product, which was related to the absence of bran components and the increased α-glucosidase activity. Moreover, the crumb featured homogeneous and finely distributed gas bubbles and the taste was not compromised. Thus, it was possible to improve the quality of gluten-free bread by using quinoa white flour. The majority of research in the literature has focused on the utilization of hydrocolloids in complex gluten-free bread formulations (Cato, Gan, Rafael, & Small, 2004; Gujral, Guardiola, Carbonell, & Rosell, 2003; Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007; López, Pereira, & Junqueira, 2004; Mariotti, Pagani, & Lucisano, 2013; Onyango,

Unbehend, & Lindhauer, 2009; Peressini, Pin, & Sensidoni, 2011; Sciarini, Ribotta, León, & Pérez, 2010a; Sivaramakrishnan, Senge, & Chattopadhyay, 2004) since they seem to be the most promising ingredients to mimic the viscoelastic properties of gluten. In particular, xanthan gum and hydroxypropyl methylcellulose (HPMC) seem to be able to improve bread making performance (Cato, Gan, Rafael, & Small, 2004; Mariotti, Pagani, & Lucisano, 2013; Peressini, Pin, & Sensidoni, 2011) such as well distributed gas cells, reduction in the diffusion and loss of water from breadcrumb. Furthermore, these hydrocolloids can limit the interactions between starch and proteins, leading to a softer crumb and slower staling kinetics during storage. Moreover, the combination of HPMC and carboxymethylcellulose (CMC) seems to be the best in regards to the viscoelastic properties of dough, thanks to which the dough was able to trap CO_2 and to develop a rigid but porous cell structure, as well as a good loaf volume (Cato et al., 2004). However, HPMC functionality can be suppressed by the presence of soy protein isolate or egg white protein in formulations contained rice flour and cassava starch, leading to a reduction of the dough stability. In addition, Andersson et al. (2011) and Schober et al. (2008) demonstrated, that HPMC is essential in a mixture of zein (protein fraction of corn) and starch for the development of gluten-free bread. In particular, the authors observed that HPMC positively affects the structural and rheological properties of zein, which yield dough similar to wheat dough and bread with increased volume (Andersson et al., 2011; Schober et al., 2008). Finally, the addition of dietary fibre such as psyllium fibre, β-glucan, corn fibre and locust bean gum, in gluten-free bread formulations represents an interesting strategy, which allows to obtain breads with significantly higher volume and softer crumb compared to the control gluten-free bread containing no fibre (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007; López, Pereira, & Junqueira, 2004; Mariotti, Lucisano, Pagani, & Ng, 2009; Martínez, Díaz, & Gómez, 2014; Pérez-Quirce, Collar, & Ronda, 2014; Ronda, Perez-Quirce, Lazaridou, & Biliaderis, 2015; Sabanis, Lebesi, & Tzia, 2009; Sciarini, Ribotta, León, & Pérez, 2010a, 2010b; Torbica, Hadnađev, & Dapčević, 2010; K Tsatsaragkou, Gounaropoulos, & Mandala, 2014). Besides the positive effect on the product quality, this category of ingredients and in particular psyllium fibre, β-glucan, corn fibre and locust bean gum, are able to improve the overall nutritional profile of the gluten-free bread due to its ability to prevent nutrition-related diseases and enhance the health of

consumers (cholesterol and fat binding, decrease in blood glucose levels, preventing constipation and facilitating good colonic health) (Kaczmarczyk, Miller, & Freund, 2012). In general, the presence of dietary fibre causes an increase in dough viscosity and consistency likely induced by the presence of insoluble matters. The consistency of the dough is a very important physical property since it influences the gas holding capacity during the mixing time (Turabi, Sumnu, & Sahin, 2008). If the apparent viscosity of the dough is as high as has been reported for samples containing fibres (Sabanis, Lebesi, & Tzia, 2009), the bubbles in the dough can rise the surface and remain in the bread during baking. For this reason, the gluten-free breads containing fibres exhibited the highest loaf volume and porosity values than the control. Moreover, Sabanis et al. (2009) suggested that the fibre interact synergistically with starch to promote the formation of a more stable structure. In addition to these positive effects of fibres on gluten-free bread characteristics, resistant starch showed to be able to promote crumb elasticity (Tsatsaragkou, Gounaropoulos, & Mandala, 2014). All the research studies focusing on the utilization of dietary fibre concluded that it is important to optimize the amount of water in order to maximize the gluten-free bread quality. Basically, dietary fibre in a low water content system is unable to establish cross-links or entanglements in the dough, probably due to the high water-binding capacity of fibres, which restrict the available water needed for the development of a network (Cavallero, Empilli, Brighenti, & Stanca, 2002; Gill, Vasanthan, Ooraikul, & Rossnagel, 2002). In conclusion, comparing the gluten-free formulations found in the market and in literature (Table 2-2 and Table 2-3) it emerges, that even though a lot of research is conducted on alternative gluten-free flours such as amaranth (*Amaranthus spp.*), buckwheat (*Fagopyrum esculentum, Moench*) and quinoa (*Chenopodium quinoa, Willd.*), the knowledge transfer from the science perspective towards the production by industries seems to be slow. In fact, it can be observed from Table 2-2 that less than 10% of the available gluten-free breads contain flours high in nutritional value, such as quinoa, amaranth, buckwheat and teff. These types of flours should be considered by industry since they would significantly improve the nutritional properties of gluten-free bread products (Alvarez-Jubete, Arendt, & Gallagher, 2009; Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010; Arendt & Zannini, 2013; Costantini et al., 2014; Flander, Salmenkallio Marttila, Suortti, & Autio, 2007; Hager, Wolter, Jacob, Zannini, & Arendt, 2012; Pulido et al., 2009).

Table 2-3 Selected studies on the effects of different ingredients on the quality of gluten-free bread.

References	Ingredients	Main outcomes
Andersson et al. (2011)	Zein, corn starch, HPMC, β-glucan oat bran	Gluten-free dough containing zein-starch (with and without hydrocolloids) showed similar extensional rheological properties, such as high extensional viscosity and strain hardening, to wheat dough. Bread products showed an open foam-like structure and increased volume. Hydrocolloids stabilized zein-starch dough, resulting in prolonged dough softness
Cato et al. (2004)	Rice flour, potato starch, HPMC, guar gum and carboxymethylcellulose (CMC)	HPMC had the most favourable effect on bread qualities; guar gum had no effect. The best solution: combination of HPMC and CMC, which gave a dough with the viscosity necessary to trap fermented gases, and also to develop a rigid but porous cell structure and good loaf volume
Crockett et al. (2011)	Rice flour, cassava starch, Methocel© E15(a type of HPMC), soy protein isolate, dried egg white powder	Soy protein isolate and egg white solids reduced dough stability by suppressing HPMC functionality, reducing available water, weakening HPMC interactions with the starch matrix and reducing foam stability. At 15% addition, egg white solids overcame negative interactions with HPMC, improving the loaf volume. Low consumer acceptability.
Elgeti et al. (2014)	Ground whole grain rice flour, ground corn flour without sperm, corn starch, quinoa white flour, HPMC	Replacement of rice and corn flour with quinoa white flour enhanced the specific volume and the crumb resulted homogeneous with finely distributed gas bubbles without compromising the taste
Gujral et al. (2003)	Rice flour, HPMC, cyclodextrin glycosyl transferase (CGTase)	Addition of CGTase produced a reduction in the dough consistency and elastic modulus and an improvement in bread quality (specific volume, shape index and crumb texture) were detected.
Lopez et al. (2004)	Cassava starch, corn starch, rice flour, corn flour, dried milk, dried egg	Rice flour-based bread: softer product, better consistency with small alveoli homogeneously distributed, higher sensory properties. Corn starch bread: larger alveoli. Cassava starch bread: expandable and gummy crumb, with granulation without alveoli, and undesirable sensorial characteristics

Table 2-3 continued

References	Ingredients	Main outcomes
Lazaridou et al. (2007)	Rice flour, corn starch, sodium caseinate. pectin, sodium, CMC, agarose, xanthan and oat β-glucan	Xanthan: strengthened doughs, farinograph curve typical of wheat flour doughs, low loaf volumes, high crumb firmness, higher elasticity and lightness crumb. β-glucan: increase in bread loaf volume, crumb firmness and porosity and lightness values of crust. Agarose and pectin: favourable effect on loaf volume. At increasing level of hydrocolloids, the loaf volume decreased except for pectin. CMC and pectin seem to be the best hydrocolloid improvers of gluten-free breads
Martinez et al. (2014)	Rice flour, corn starch and HPMC Insoluble fibres: oat, bamboo, potato and pea Soluble fibres: Nutriose® and polydextrose	Soluble fibres effects: decrease of dough consistency, increase of volume during fermentation, higher specific volumes, lower hardness, lower luminosity and greater cell density than control breads. Fine insoluble fibres effects: higher specific volumes and lower hardness than controls. Coarser insoluble fibres effects: lower specific volumes and greater hardness. Combination of soluble fibres with HPMC: favoured the creation of a film that coated the starch granules and flour particles, giving more stability to the structure. Combination of insoluble fibres with HPMC: disruption of the structure
Mariotti et al. (2009)	Corn starch, amaranth flour, pea isolate, Psyllium fiber	*Psyllium* fiber generally enhanced the physical properties of the doughs due to the film-like structure that it was able to form
Mariotti et al. (2013)	Recipe 1: corn starch, skimmed milk powder, sugar, *Psyllium* fiber, guar gum, maltodextrins Recipe 2: corn starch, tapioca starch, potato starch, rice flour, salt, sugar	These recipes were compared with those of the same mixtures added with HPMC and different buckwheat flours. Inclusion of dehulled buckwheat improved the baking performances of the commercial GF mixtures. The presence of a small amount of puffed buckwheat flour and HPMC limited both the diffusion and the loss of water from the bread crumb and the interactions between starch and protein macromolecules, resulting in a softer GF bread crumb and reduced staling kinetics during storage

Table 2-3 continued

References	Ingredients	Main outcomes
Onyango et al. (2011)	Sorghum flour and starch from cassava, corn, potato or rice starch, egg white powder	Increasing starch content the batters resulted more liquid-like, crumb firmness and chewiness decreased and cohesiveness, springiness and resilience increased in all breads. Cassava-sorghum and rice-sorghum breads had better crumb properties than corn-sorghum or potato-sorghum breads. The best overall texture: formulation containing 50% of cassava starch
Minarro et al. (2010)	Corn starch, rice flour, soya flour, granulated buckwheat flour, white skim milk powder, ovalbumin, xanthan gum, unicellular protein from *Saccharomyces cerevisiae*	Gluten-free starch-based breads with ovoalbumin: good baking characteristics. Addition of unicellular protein: darker crumb colour and low consumer acceptability. Starch based formulations without unicellular protein and dairy proteins were the most preferred by consumers
Moore et al. (2004)	Recipe 1: corn starch, brown rice, soya, and buckwheat flour. Recipe 2: brown rice flour, skim milk powder, whole egg, potato and corn starch, soya flour.	Hydrocolloids used were xanthan gum and/plus konjac gum. Better keeping quality dairy-based gluten-free bread. Dairy-based gluten-free bread crumb contained network-like structures resembling the gluten network in wheat bread crumb
Peressini et al. (2011)	Buckwheat flour, rice flour, xanthan gum (XG) and propylene glycol alginate (PGA)	The addition of both hydrocolloids significantly enhanced the elastic behaviour of batter and xanthan gum exerted greater effect than propylene glycol alginate. PGA breads showed higher improvement in terms of increased specific volume, decreased crumb firmness and crumb structure than XG breads
Sciarini et al., (2010b)	Rice flour, corn flour, inactive, micronized and defatted soy	Effects of soy flour addition: higher batter firmness and specific volume, reduction of the staling rate. Soy flour addition had a higher effect on rice than on corn starch

Table 2-3 continued

References	Ingredients	Main outcomes
Onyango et al. (2009)	Cassava starch, red sorghum, microcrystalline cellulose (MCC), CMC, methyl cellulose (MC), (HPMC), hydroxypropylcellulose (HPC), egg white powder, glycerol monostarate, sodium stearoyl-2-lactylate, diacetyl tartaric acid esters of mono- and diglycerides, calcium stearoyl-2-lactylate	The effect of cellulose-derivatives on dough strength was influenced by the type, concentration and ionic character. In general, cellulose treated doughs had lower resistances to deformation than emulsifier-treated doughs. Emulsifiers decreased crumb firmness and staling rate when compared to the control, while cellulose-derivatives did not. Addition of egg white powder eliminated several textural defects associated with gluten-free bread.
Ribotta et al. (2004)	Rice flour, cassava flour, soybean flour*, dried milk, gelatine *Full-fat enzyme-active, semiactive and inactive	Bread quality affected by particle size and concentration of the soybean flours. Active soybean flour: the best gluten-free bread quality. Semiactive and inactive soybean flours: neither well-aerated bread structure nor good loaf volume
Ronda et al. (2015)	Rice flour, HPMC, barley (1 → 3) (1 → 4)-β-D-glucan (BBG), oat (1 → 3) (1 → 4)-β-D-glucan concentrate (OBG)	Optimization of dough hydration is indeed of primary importance on improving GF bread quality. Low molecular weight barley β-glucans develop a gel network structure at higher concentrations, whereas the high molecular weight oat β-glucan exhibited a more viscous-like rheological response
Sivaramakrishnan et al., (2004)	Rice flour and HPMC	Rice flour supplemented with HPMC reached a consistency of 500 BU at a later time than the standard wheat dough. Rice flour dough with HPMC had similar rheological properties to wheat flour dough

Table 2-3 continued

References	Ingredients	Main outcomes
Perez-Quirce et al. (2014)	Rice flour, HPMC (semi-frim and weak gel forming), barley β-glucan (BBG)	Single BBG addition fails to mimic gluten visco-elasticity properly, but simultaneous incorporation of both HPMC-types contributed to bread improvement in terms of bigger volume and smoother crumb. HPMC weak gel-forming led to harder and lower volume breads than semi-firm gel forming.
Sciarini et al., (2010a)	Rice flour, corn flour, micronised and defatted soy flour.	Five different hydrocolloids were used: xanthan gum (XG), gelatine (Gel), carrageenan (C), alginate (Al) and carboxymethylcellulose (CMC). Hydrocolloids increased batter consistencies with following extent: XG > CMC > Al > Gel > C. Breads with hydrocolloid presented higher specific volume and lighter crust than control. Crumbs with Gel, XG and CMC presented higher cell average size. XG and CMC crumbs looked spongier. Crumb firmness was decreased by XG and CMC addition, and staling rate was slower. XG was the best performing hydrocolloid.
Sabanis et al. (2009)	Corn starch, rice flour, HPMC Fibres: wheat, corn, oat and barley	Corn and oat fibre: crumb showed the continuous matrix between starch and corn and/or oat fibre obtaining a more aerated structure; positive impact on bread nutritional and sensory properties. Barley fibre: loaves with more intense colour and volume comparable to the control. During storage of breads a reduction in crumb moisture content and an increase in firmness were observed.
Torbica et al., (2010)	Rice flour, unhusked buckwheat flour husked buckwheat flour, amaranth flour, soybean flour	Unhusked buckwheat flour effects: higher water absorption values, weaker protein network structure, lower stability, peak viscosity and sensory properties; a content of unhusked buckwheat flour up to 20% caused a decrease of the dough elastic behaviour. Husked buckwheat flour content up to 30% resulted in an increase of the elastic behaviour of the dough. Hardness of the final products increased with the amount of both types of buckwheat flour

Table 2-3 continued

References	Ingredients	Main outcomes
Tsatsaragkou et al., (2014)	Rice flour, carob flour, resistant starch, egg white powder, whey protein concentrate, locust bean gum (LBG), enzyme of alpha-amylase with additional transglutaminase and hemicellulase activity	RS addition did not influence crumb firmness, but it acted as an elastifying agent. Model gluten-free bread was further improved by adding carob flour, even if a higher amount of water was required. Water amount increase diminished crumb firmness and contributed to the development of an open crumb cell structure.

Sourdough technology

Sourdough, a mixture of flour and water fermented with lactic acid bacteria (LAB) and yeasts (Hammes & Gänzle, 1998), has a well-established role in improving flavour and structure of bread (Arendt, Ryan, & Dal Bello, 2007). The use of sourdough for gluten-free bread has been comprehensively reviewed (Arendt & Dal Bello, 2011; Moroni, Dal Bello, & Arendt, 2009; O'Shea, Arendt, & Gallagher, 2014; Zannini, Jones, Renzetti, & Arendt, 2012). Since sourdough is a very heterogeneous material and its manufacturing conditions vary significantly, the range of sourdough effects on the textural and sensory properties of gluten-free bread is extensive. Several functions/quality attributes of sourdough technology may be employed in a gluten-free system providing a useful tool for improving the low-quality of gluten-free bread generally characterised by a quick staling and poor flavour. For wheat bread those disadvantages could be improved and prevented with the incorporation of sourdough (Clarke, Schober, & Arendt, 2002; Crowley et al., 2002). When used in optimized proportions to produce bakery products, sourdough can enhance (i) gas retention, (ii) textural quality, (iii) flavour, (iv) nutritional value in terms of mineral bioavailability, starch digestibility and concentration of bioactive compounds, (v) shelf life by retarding the staling process and by protecting bread from mould and bacterial spoilage (Luc De Vuyst & Vancanneyt, 2007; Gobbetti, 1998; Kaisa Poutanen, Flander, & Katina, 2009). These positive effects are associated with the metabolic activities of sourdough LAB and yeasts, such as lactic acid fermentation, proteolysis, exopolysaccharides (EPS) production and synthesis of volatile and antimicrobial compounds (Arendt, Ryan, & Dal Bello, 2007; Corsetti & Settanni, 2007; RŘhmkorf, Jungkunz, Wagner, & Vogel, 2012). Consequently, the exploitation of sourdough for the development of new gluten-free products seems appealing.

Table 2-4 Impact of different gluten-free flours and starter cultures on sourdough and dough, batter and bread properties[a].

Gluten-free flour/starter culture	Sourdough properties	Effect on batter/dough/bread properties	Reference
Sorghum flour (*L. reuteri* Y2, *L. reuteri* VIP and *W. cibaria* MG1)	Formation of EPS(0.6 to 8.0 g/kg sourdough) and oligosaccharides	EPS formation during fermentation decreased dough strength and elasticity	(Galle et al., 2012)
Amaranth (*Amaranthus hypochondriacus*, *Lactobacillus plantarum* AL30 and *Lactobacillus paralimentarius* AL28)	*L. plantarum* AL30 strain had the best rheological characteristics	Dough with viscosity and elasticity similar to that found in pure wheat flours	(Houben et al., 2010)
Buckwheat flour (*Lactobacillus plantarum* AB 26, *Lactobacillus brevis* AB 27, *Lactobacillus paralimentarius* AB 28 and *Weissella cibaria* AB 25)	Hydrolysis of proteins starch decreased elasticity	Significant increase in the dough network strength	(Moroni et al., 2011)
Buckwheat, quinoa, sorghum and teff flour (*Weissella cibaria* MG1, *Lactobacillus plantarum* FST1.7)	Resistant starch was significantly decreased in buckwheat and teff sourdough breads	Predicted glycemic index was not reduced upon sourdough addition in most gluten-free breads with the exception of sorghum and teff sourdough breads	(Wolter, Hager, Zannini and Arendt, 2014)
Buckwheat, quinoa, sorghum, teff flour (*Weissella cibaria* MG1)	Quinoa sourdough showed the higest acidification	Sourdough addition decreased dough strength in buckwheat, and sorghum bread dough. Reduced crumb hardness and staling rate; increased crumb porosity. Aroma of most breads not improved by sourdough addition	(Wolter, Hager, et al., 2014b)

Table 2-4 continued

Buckwheat, oat, quinoa, sorghum, teff flour (*Lactobacillus plantarum* FST1.7)	Changes in protein profiles as were observed in all sourdoughs	Sourdough addition led to decreased dough strength resulting in softer dough. No influences on specific volume. Crumb porosity was significantly increased in all gluten-free sourdough breads. The inferior aroma of breads prepared from the gluten-free flours was not improved by sourdough addition.	(Wolter, Hager, et al., 2014a)
Refined corn meal (*L. plantarum*)	Starch granule modification	Corn dough softer and less elastic improving the resulting bread volume and texture.	(Falade et al., 2014)
Buckwheat flour, Buckwheat core, quinoa, rice (*L. animalis* TMW 1.971, *L. reuteri* TMW 1.106 and *L. curvatus* TMW 1.624	All the LAB strains were found to produce technological relevant amounts of EPS in sourdoughs not until several parameters were varied	In situ produced EPS can replace added hydrocolloids in gluten-free baked goods.	(Ruehmkorf et al., 2012)
Teff and buckwheat flour (*Lactobacillus, Pediococcus, Leuconostoc, Kazachstania* and *Candida*)	*Lactobacillus plantarum* and *Lactobacillus pontisis* were dominant species for buckwheat and teff flour, respectively	pH values of buckwheat sourdoughs were higher than those measured in teff sourdoughs	(Moroni et al., 2011)
Oat batter (*Leuconostoc argentinum, Pedicoccus pentosaceus* and *Weissella cibaria, Lactobacillus coryniformisdominated* SD 37)	Hydrolysis of proteins	Decreased starch viscosity of oat batter	(Hüttner et al., 2009)
Fanio dough	Changes in starch characteristics	Reduction in starch gel firmness, increased pasting viscosity	(Edema et al., 2013)
Sorghum batter (*L. plantarum*)	Acidification	Formation of strong starch gel in sorghum batter	(Schober et al., 2007)

[a]Extended from Deora et al. (2014).

Sourdough application on gluten-free baked goods

In the last 20 years, several studies have shown that sourdough fermentation can be positively applied for enhancing the gluten-free dough and bread quality. Table 2-4 summarizes the recent findings in the application of sourdough technology in improving gluten-free dough and bread properties. However, a clear framework of the microbiological interactions between LAB/yeast and gluten-free ingredients/raw materials during sourdough fermentation is essential for controlling the fermentation and maintaining consistent quality parameters of the resulting gluten-free bread. In particular, the effect on bread volume can be positive or negative depending on the ingredients combination. For improving aeration through sourdough LAB/yeast microorganisms, all essential nutrients have to be accessible and the optimum pH and temperature range should be guaranteed. For the growth of sourdough LAB/yeast culture in the gluten-free dough, sugar composition (mono- and di-saccharides) of the gluten-free flour is of key interest (Elgeti, Jekle, & Becker, 2015). For instance, a lack of maltose at the beginning of the fermentation in the sorghum sourdough can lead to the failure of the starter strain, *Lactobacillus sanfranciscensis*, to grow (Galle, Schwab, Arendt, & Gänzle, 2010). Elgeti et al. (2014) found that quinoa white flour improved the volume of gluten-free bread by significantly elevated α-glucosidase activity in comparison with rice and corn flour. Similarly, the addition of 10% quinoa bran improved aeration by providing substrates for yeast (Föste et al., 2014).

Biodiversity of sourdough lactic acid bacteria

Beside the presence of all nutrients in the gluten-free dough, a selection of gluten-free sourdough LAB and yeast, able to dominate the fermentation and inhibit the growth of contaminants is also a key condition (De Vuyst, Vrancken, Ravyts, Rimaux, & Weckx, 2009; Minervini et al., 2010). To this regard, recent investigations indicate that commercial starters are not suitable as such for the fermentation of gluten-free materials and specific starters should be developed for such fermentations (Moroni, Arendt, Morrissey, & Dal Bello, 2010; Vogelmann, Seitter, Singer, Brandt, & Hertel, 2009). Ecological studies on gluten-free sourdoughs, either developed by starters or by spontaneous fermentation, indicate that gluten-free materials harbour novel and competitive LAB and yeasts strains which are not commonly isolated in traditional

sourdoughs and which could serve as suitable candidates for starters development (Edema & Sanni, 2008; Meroth, Hammes, & Hertel, 2004; Moroni et al., 2010; RŘhmkorf, Jungkunz, Wagner, & Vogel, 2012; Sterr, Weiss, & Schmidt, 2009; Vogelmann et al., 2009; Wolter, Hager, Zannini, Czerny, & Arendt, 2014a, 2014b; Wolter, Hager, Zannini, Galle, et al., 2014). *Lactobacillus fermentum, Lactobacillus plantarum* and also *Lactobacillus Paralimentarius* are frequently isolated from gluten-free sourdoughs from rice, corn, buckwheat, teff and amaranth. Furthermore, species such as *Lactobacillus gallinarum, Lactobacillus graminis, Lactobacillus sakei* and *Pediococcus pentosaceus*, which are not commonly associated with conventional sourdoughs, were part of the dominant microbiota of various gluten-free sourdoughs (Moroni, Dal Bello, & Arendt, 2009). Since those strains are especially adapted to gluten-free systems, they could serve as promising cell-factories to produce biomolecules and nutrients in gluten-free bread (Arendt, Moroni, & Zannini, 2011).

Sourdough lactic acid bacteria as a tool to improve gluten-free bread structure

Sourdough fermentation has a positive effect on crumb structure of gluten-free sorghum bread (Schober, Bean, & Boyle, 2007). Later, Moore et al. (2008) obtained softer gluten-free bread when using *L. plantarum* FST 1.7 as sourdough starter culture which also inhibited mould growth. Furthermore, Hüttner and Arendt (2010) found that sourdough *Leuconostoc argentinum, Pediococcus pentosaceus, Weissella cibaria* and *Lactobacillus coryniformis* bacteria isolated from oats have the potential to increase loaf-specific volume as well as to improve crumb structure enhancing oat bread quality. Some LAB can produce a wide variety of long-chain sugar polymers called EPS, which are varied in their chemical composition, structure and physical properties (De Vuyst & Degeest, 1999). These polysaccharides are synthesised extracellularly from sucrose by glucansucrases, or intracellularly by glycosyltransferases from sugar nucleotide precursors. Those polysaccharides produce from sucrose can improve the technological as well as the nutritional properties of gluten-free breads acting as hydrocolloids and prebiotics, respectively (Lacaze, Wick, & Cappelle, 2007; Waldherr & Vogel, 2009).

The application of the EPS-producing strains *Lactobacillus reuteri* LTH5448 and *Weissella cibaria* 10Min quinoa and sorghum sourdoughs showed that both strains were suitable as

sourdough starters and able to produce a fructo-oligosaccharide, levan, and a gluco-oligosaccharide (GlcOS), dextran, respectively (Schwab, Mastrangelo, Corsetti, & Gänzle, 2008). Gluten-free breads containing sourdough fermented by *W. cibaria* were softer than the ones without EPS-containing sourdough (Schwab et al., 2008). And GlcOS produced by *W. cibaria* were not digested by baker's yeast and therefore still present in the bread. Thus, the consumption of 300 g of sorghum bread prepared with *W. cibaria* 10M would allow for a significant intake of prebiotic GlcOS (Schwab et al., 2008).

EPS-forming *Weissella* strains can serve as starter strains in sorghum and wheat sourdoughs. Independently of which strain is used, higher amounts of EPS were formed in sorghum sourdough than in wheat, due to the higher concentration of glucose in the gluten-free flour. In particular, the strains *Weissella kimchi* and *W. cibaria* MG1 produced dextrans in concentrations high enough to be used as potential replacers of non-bacteria hydrocolloids, such as guar gum and HPMC in gluten-free sourdoughs bread (Galle, Schwab, Arendt, & Gänzle, 2010; Galle et al., 2012). Recently, Ruehmkorf et al. (2012) investigated structurally different bacterial homoexopolysaccharides for their ability to enhance the structure and the quality of gluten-free dough and bread. The aforementioned authors investigated 4 different LAB strains (*Lactobacillus sanfranciscensis* TMW 1.392, *Lactobacillus curvatus* TMW 1.624, *L. reuteri* 1.106 and *Lactobacillus animalis* 1.971) which are known to produce exopolysaccharides. Based on the study, a structure– function relationship for different EPS was proposed. The dextran of *L. curvatus* TMW 1.624 was suggested to be the most promising candidate for application in gluten-free formulations by the group. The authors concluded that when choosing an EPS source, the linkage type, molecular weight, degree of branching, and conformations have to be taken into consideration to achieve optimal baking results.

Results obtained so far suggest that gluten-free flours represent a suitable substrate for the production of sourdough and that gluten-free sourdough can be successfully applied for improving the quality of gluten-free bread (Galle et al., 2010; M. Moore, Juga, Schober, & Arendt, 2007; Moroni, Arendt, Morrissey, & Dal Bello, 2010; RŘhmkorf et al., 2012; Sterr, Weiss, & Schmidt, 2009; Vogelmann, Seitter, Singer, Brandt, & Hertel, 2009; Wolter, Hager, Zannini, Czerny, & Arendt, 2014a, 2014b; Wolter, Hager, Zannini,

Galle, et al., 2014). Sourdough fermentations require a specific knowledge on the effects of process parameters, raw materials and microorganisms in order to obtain a specific, reproducible sourdough and bread quality (Brandt, 2007). As sourdough fermentation is a labour-intensive and a time-consuming process, a growing demand for convenient products has arisen in the last two decades. Dried sourdough has added advantages over fresh sourdough since the quality is consistent and there are no longer end-product variations due to the freshly produced sourdough. Companies which produce such ready-to-use sourdough, claim for a convenient, direct production of baked goods in constant quality, in combination with all advantages of biological sourdough fermentation, e.g. aroma and taste, fresh keeping and extended microbial shelf-life (Brandt, 2007). A variety of shelf stable dried sourdough with different fermented gluten-free cereals for the improvement of gluten-free dough and products are currently available on the market.

Conclusion

The importance of simultaneously improving both the quality and the nutritional profile of gluten-free products has been stimulating researchers to investigate new ingredients and technologies to be applied in gluten-free bread-making. Development of gluten-free bread remains a technological challenge due to the key role of gluten in the bread-making process and in the bread rheology, appearance and shelf-life.

The present review is clearly showing that there is no raw material, ingredient, or additive that can fully replace gluten, but complex formulations are necessary to obtain gluten-free bread with good quality. Several studies highlighted the importance of including hydrocolloids in the formulations. The most commonly hydrocolloids used are HPMC and xanthan gum. These hydrocolloids seem to be the most promising ingredients due to their ability to mimic the viscoelastic properties of gluten. They are also known to reduce staling, improve water binding and the overall structure of the bread. In addition, it has been demonstrated that soluble fibres combined with HPMC favour the creation of a film that coated the starch granules and flour particles, giving more stability to the structure.

Numerous alternative flours, starches and proteins from different sources have been included in gluten-free bread formulations. Based on the literature, gluten-free flours such as amaranth, quinoa, buckwheat flour and fibres should be present in gluten-free product formulation due to their high nutritional value. However, the analysis of the commercial gluten-free products revealed that this suggestion has not been adapted by the industry. The review of literature also revealed that the addition of fibres such as Psyllium fibre, β-glucan, corn fibre and locust bean gum can have beneficial effects on the bread quality both from a textural as well as nutritional point of view. One of the most promising technologies to be applied during the production of gluten-free bread is sourdough. Sourdough can improve not only the textural and sensory properties, but it also has the potential to increase the nutritional value of these products. It is important that cultures, which are used in the process, are selected specifically for the raw materials, which are applied in the specific formulations. This review shows clearly that even if there is an increase in research in the area of gluten-free products, no definite conclusion can be reached on the optimal raw material characteristics for the production of good quality gluten-free bread. For this reason, a series of fundamental studies are needed to get a better understanding of gluten-free system.

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Chapter 3 Correlation analysis of protein quality characteristics to gluten-free bread properties

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Abstract

The interest in gluten-free cereal products has increased significantly over the last number of years and there is still a significant demand for high-quality products. This study aims to establish possible connections between protein properties, dough and bread quality which could advance the knowledge for gluten-free product development. The objective of the present study was to correlate protein properties with bread characteristics. Therefore, a wide range of tests (solubility, emulsifying, foaming, water hydration properties) was performed to characterize a range of food proteins (potato, pea, carob, lupin and soy). Furthermore, the performance of these proteins in a dough matrix (pasting, rheology) and bread formulation (volume, structure, and texture) was analysed. Statistical analysis showed significant ($p < 0.05$) correlations between protein properties, dough properties and final bread characteristics. The addition of the proteins to the gluten-free bread formulation affected pasting rheological and bread characteristics such as crumb density, crumb hardness and specific volume. The addition of potato and soy protein resulted in the lowest volume with a dense crumb structure and a low consumer acceptance score. However, lupin, pea and carob containing gluten-free breads had a higher specific volume and softer and less dense crumb structure. The protein solubility (r, 0.89; $p < 0.01$) and its foaming properties (r, 0.97; $p < 0.05$) were found to be the most important protein properties with correlations significantly with dough properties and bread quality.

Introduction

Coeliac disease, also called coeliac sprue or gluten enteropathy, is caused by the ingestion of gluten-containing products. Ingestion of gluten can cause an inflammation of the small intestine in susceptible individuals (Shan et al., 2002). Strict adherence to a gluten-free diet is mandatory. This diet has to be life-long since the re-exposure to gluten reactivates the disease (Koehler, Wieser, & Konitzer, 2014). Gluten is the major texture and structure-forming ingredient in most yeast leaved products, based on its ability to build a viscoelastic network, which can entrap gas cells. Recent research has mainly focused on the development of good quality gluten-free bread, which resulted in products with very complex formulations. Although the quality of gluten-free breads has improved, the nutritional value of these has been neglected. A review by Foschia et al. (2016) compared surveys conducted by Thompson, Dennis, Higgins, Lee, and Sharrett (2005) and Mintel (2015), which both showed that, over the past decade, consumers have remained unsatisfied with the nutritional value of gluten-free products.

The application of proteins, in particular, legume proteins, is promising due to their functional properties and high nutritional value and has been under investigation in recent years (Waglay, Karboune, & Alli, 2014). The literature states that proteins, based on their properties like foaming and emulsifying, which, in turn, have an influence on gas retention and gas cell expansion, have an effect on the final bread product (Schoenlechner, Mandala, Kiskini, Kostaropoulos, & Berghofer, 2010; Ziobro, Juszczak, Witczak, & Korus, 2016; Ziobro, Witczak, Juszczak, & Korus, 2013). Some of these proteins such as lupin, pea and soy have already been utilized in complex gluten-free bread formulations (Ziobro et al., 2016; Ziobro et al., 2013). The use of legume proteins in cereal products has been reviewed and recommended for use in gluten-free formulations, where they have a key role to play in terms of techno-functional properties and the improvement of the nutritional profile of gluten-free products (Foschia, Horstmann, Arendt, & Zannini, 2017). In this paper a range of legume proteins and plant proteins were studied. Pea protein is an extract of the pea seed, which has become more common as an ingredient in food applications (Dijkink & Langelaan, 2002; McCarthy et al., 2016). It can contribute to an improved nutritional value based on its well-balanced amino acid profile with high amounts of the essential amino acid lysine (M. Nunes, Raymundo, & Sousa, 2006). Lupin protein is extracted

from the lupin seed. This protein has been comprehensively reviewed regarding its many health benefits (Arnoldi, Boschin, Zanoni, & Lammi, 2015). Based on its functional properties it was reported to improve the sensorial properties of gluten-free bread (Ziobro et al., 2016). Carob protein, which is extracted from the carob pod, contains high amounts of dietary fibre and nutrients (Tsatsaragkou, Gounaropoulos, & Mandala, 2014). Research conducted on its application in gluten-free bread has reported its 'gluten-like' properties (Smith et al., 2010). Soy protein is extracted from the soybean and has found use in many food products. It adds improved biological value to food products based on the high amounts of the essential amino acid's lysine and methionine (Iqbal, Khalil, Ateeq, & Khan, 2006). Potato protein is extracted from the side stream, which is left after the removal of starch (Waglay, Karboune, & Alli, 2014). It is of great interest to the food industry based on its high nutritional quality (Bártová & Bárta, 2009).

The aim of this work was to link protein techno-functional properties with gluten-free bread quality characteristics. To accomplish this, extensive characterisation of five commercially available vegetable proteins (pea, potato, soy, lupin and carob) and their addition to a model bread system were conducted. Commercially purchased proteins commonly differ in composition regarding their total protein content. The authors decided to keep the ingredient content in the various recipes the same to be more approachable for research and development purposes in the gluten-free area.

Experimental

Materials

Five commercially available gluten-free proteins were used in this study. Potato protein (201P) was obtained from Solanic, the Netherlands; soy protein isolate (Clarisoy) from ADM, Ireland; pea protein (NUTRALYS PEA BF) from Roquette, France; lupin protein from Lup Ingredients, France; and carob germ protein (Grinsted Veg Pro) from Danisco, UK. Potato starch was supplied by Emsland, Germany; dry yeast by Puratos, Belgium; sugar by Siucra Nordzucker, Ireland; salt by Glacia British Salt Limited, UK; and HPMC (hydroxypropyl methylcellulose) by J. Rettenmaier, Germany.

Compositional analysis

The total nitrogen content of the protein samples was determined according to the Kjeldahl method (MEBAK 1.5.2.1). To convert the nitrogen content into the protein content the factor of 6.25 was used. The air oven method (AACC Method 44-15A) was applied to determine the moisture content of the samples. The determination of the lipid content was performed according to the Soxlet-method (AACC Method 30-25.01) with a pre-digestion of the samples in HCl, to release bound lipids.

Microscopy

Samples of protein were dried in an air-oven for 1 h at 103 °C. Double-sided carbon tape was used to mount the samples on an aluminium stub. Samples were coated with a layer of 25 nm of sputtered palladium–gold. Hereupon, samples were examined under high vacuum in a field emission scanning electron microscope (SEM) with a working distance of 8 mm. Secondary electron images were acquired at an accelerating voltage of 5 kV. SEM Control User Interface software, Version 5.21 (JEOL Technics Ltd, Japan) was used for processing the images.

Solubility as a function of pH

The solubility of the proteins was determined in water by adjusting 2% protein solutions to a pH of 5.5, which was found to be the pH in allthe gluten-free dough formulations. Samples were equilibrated at 4 °C overnight. The pH was readjusted if necessary and the samples were centrifuged at 10 000g for 15 min (4 °C). The Bradford assay was used to analyse the protein content of the supernatant. Results are expressed as % of the protein content (analysed using the Kjeldahl method), taken from the supernatant of the solution.

Water hydration capacity

The measurement of water hydration capacity (WHC) of proteins was determined according to AACC method 56-30.01 with some modifications: samples (1.000g ± 0.005g) were mixed with 30 ml of distilled water using an Ultra-Turrax equipped with a S10N-5G dispersing element (Ika-Labortechnik, Janke and Kunkel GmbH, Staufen, Germany) for 15 s and then shaken for 30 min at 1000 rpm using a platform shaker

(UNI MAX 1010, Heidolph, Schwabach, Germany). Subsequently, the mixture was centrifuged at 2000 g for 10 min. WHC was expressed as grams of water retained per gram of solid:

WHC [g water/g ingredient] = (W2-W1)/(W0)

Where W2 is the weight of the tube plus the sediment, W1 is the weight of the tube plus the sample and W0 is sample weight.

Foaming Properties

The dispersions of proteins (20 ml, 2% w/v) in distilled water were homogenized by using an Ultra-Turrax equipped with S10N-10G dispersing element (Ika-Labortechnik, Janke and Kunkel GmbH, Staufen) at high speed for 30 s in graduated cylinders. For the analysis of the foaming properties over time, every 15 min over 3 hours the level of foam and the water, with respect to height, were measured. The foaming capacity was elaborated by the foam height immediately after stirring and after 15 min. The results for the foaming capacity are given in %, which indicates the ratio between foam and water (un-foamed material). The foam stability was calculated by the decrease in the foaming capacity over time [%/s].

Emulsifying properties

Emulsifying solutions were obtained by adding 50 ml sunflower oil to 500 ml of distilled water of 1% (w/v) protein solution. Samples were pre-homogenized using an Ultra Turrax® T25 equipped with a S25N-10G dispersing head (IKA-Werke, Staufen, Germany) rotating at max speed for 1 min. After that, a 2-step homogenization (210/40 bar) was carried out using the Homogenizer APV / Sebe group product from Denmark. The capacity and stability of the emulsions were immediately measured by the LUMiSizer (L.U.M. GmbH, Germany), an instrument employing centrifugal sedimentation to accelerate the occurrence of instability phenomena such as sedimentation, flocculation, or creaming. Emulsion samples were subjected to centrifugal force, while near-infrared light illuminated the entire sample cell to measure the intensity of transmitted light as a function of time and position over the entire sample length simultaneously. The instrumental parameters used for the measurement

were as follows: volume, 1.8 mL of dispersion; 4,000g, timeExp, 7,650 s; time interval, 30 s, temperature, 25 °C.

Bread production

Bread samples were prepared according to Horstmann, Belz, Heitmann, Zannini, and Arendt (2016). The formulation for the breads was as followed: 80% water, 2% protein, 2% HPMC, 2% salt, 4% sugar, 2% dried yeast, based on starch weight. Dry ingredients where mixed and yeast was suspended in warm water (25 °C) and regenerated for a period of 10 min. Mixing was carried out with a k-beater (Kenwood, Havant, UK) at low disk speed (level 1 of 6) for 1 minute in a Kenwood Major Titanium kmm 020 Mixer (Kenwood, Havant, UK). After the first mixing, the dough was scraped down from the bowl walls. A second mixing step of 2 minutes at higher disk speed (level 2 of 6) was applied. The batter was weighed (300 g) into baking tins of 16,5 cm x 11 cm x 7 cm and placed in a proofer (KOMA, Netherlands) for 45 min at 30 °C and 85% relative humidity (RH). The proofed samples were then baked for 55 min at 220 °C top and 220 °C bottom heat in a deck oven (MIWE, Germany), previously steamed with 0.7 L of water. The breads were cooled for 2 hours prior to analysis.

Rapid visco analysis

The pasting behaviours of the bread formulation (dry mix, excluding yeast) were measured using a Rapid Visco Analyzer (RVA Super 3 Rapid Visco Analyser Newport Scientific, Warriewood, Australia). Each blend (3.0 g) was mixed with adjusted amounts of distilled water in a canister, heated at a rate of 0.2 °C/sec from 50 °C to 95 °C, maintained at 95 °C for 162 s, cooled at the rate of 0.2 °C/sec to 50 °C, and held for 120 s at 50 °C before the test ended.. Chosen parameters for the evaluation of the pasting properties were peak viscosity, breakdown viscossity, final viscosity and peak temperature.

Viscoelastic properties of the dough

Rheological measurements of dough samples were carried out by using a Rheometer Physica MCR 301 (Anton Paar GmbH, Germany) equipped with serrated parallel plate geometry (diameter 50 mm, gap 1 mm). Dough samples were placed between the plates of the rheometer. Each sample was left to rest for 5 min after loading. A frequency sweep test was performed at 25 °C from 0.1 Hz to 100 Hz within a linear viscoelastic range. Data obtained were storage modulus (*G'*), loss modulus (*G"*) and tan δ (*G"/G'*).

Bread analysis

The specific volume of the bread was determined by the use of a Vol-scan apparatus (Stable Micro System, UK). The specific volume is calculated on the basis of loaf volume and weight. An image analysis system (Calibre Control International Ltd., UK) was used to analyse the breadcrumb structure. Analysed parameters were the course/ fin cluster and the net cell-elongation. Crumb hardness, cohesiveness, springiness and resilience were analysed using a Texture Profile Analyser (TA-XT2i, Stable Micro Systems, Godalming, England) with a 25 kg load cell, which compresses the breadcrumb with a 35 mm aluminium cylindrical probe. Bread samples were sliced in 20 mm slices and analysed with a test speed of 5 mm/s and a trigger force of 25 g, compressing the middle of the bread crumb to 10 mm. The measurement with the various parameters was conducted on the baking day, 2 days after baking and 5 days after baking to monitor the staling process. For the determination of crumb moisture content, a gravimetric method was used according to AACC Method 44-15A. Baked breads were stored in polythene bags (polystyrol-ethylene vinyl alcohol-polyethylene) at 25 °C.

Statistical analysis

All measurements were performed at least in triplicate. One-way analysis of variance and Tukey's test were used to establish the significance of differences among the mean values at a 0.05 significance level (R version 3.0.1). The level of significance was determined at $p<0.05$. In addition, Pearson correlation analysis (R version 3.0.1) was applied to find correlations between protein properties and the quality parameters of the baked products.

Results and Discussion

Compositional and functional properties of proteins

Five plant proteins, namely potato, pea, carob, lupin and soy protein, were selected and their functional properties determined, these were then correlated to their performance in a gluten-free bread model system. Therefore, a comprehensive analysis of the protein samples was carried out (Table 3-1, Figure 3-1). The analysis included morphological, chemical and physicochemical properties.

Figure 3-1 illustrates the morphology of the various protein powders. It can be seen that potato and soy protein show damaged, broken protein particles. Lupin, carob and pea protein, however showed bigger, clustered, intact protein particles. It is hypothesised that the disrupted structure of soy and potato protein was caused by the isolation procedure. It is known that the application of heat, acid and pressure is a common way to isolate proteins and that these affect the structure of the proteins. It is hypothesised by the authors that these differences in morphology have further influences on protein properties.

The compositional analysis of the proteins in terms of protein, fat and moisture content are reported in Table 3-1. The protein content of the different plant protein powders ranged from 39% to 92.4%. Soy protein had the highest protein content (92.4%), while lupin and carob showed the lowest protein content of 39.0% and 55.4% respectively. Differences in protein purity are based on the production of the protein powders and their desired functionalities. Significant differences between the proteins were also found regarding their fat content. Lupin and pea protein showed high-fat contents of 10.3% and 7.5%, respectively. The remaining proteins showed fat contents below 0.1%. Such high differences in the protein and fat content are considered to influence the individual protein powder properties. The moisture content of the proteins was in most cases similar: potato and pea proteins had the highest moisture content of 7.4%, while carob and soy showed lower moisture values of 4.3% and 4.6%, respectively. The water hydration capacity (WHC) determines the amount of water (in grams) bound per gram of protein in an aqueous dispersion.

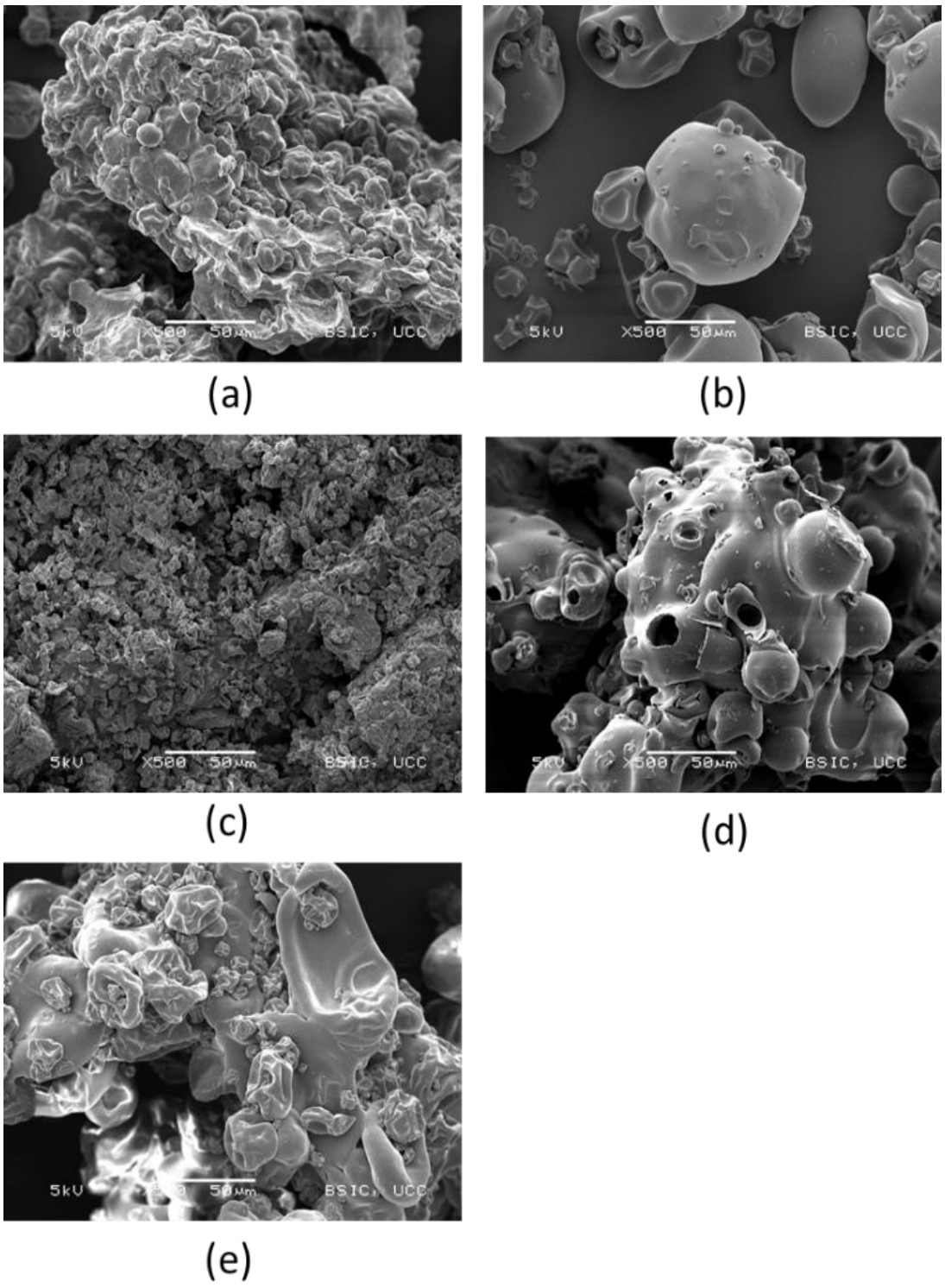


Figure 3-1 SEM images of the various proteins. Magnification ×500. (a) lupin protein; (b) soy protein; (c) carob protein; (d) potato protein; and (e) pea protein.

Table 3-1 Compositional properties of the selected proteins (potato, pea, carob, lupin, soy) and their effect on pasting properties of dough.

	Potato	Pea	Carob	Lupin	Soy
Composition					
Protein [g/100g]	84.6±1.1^{d}	76.2±0.7^{c}	55.4±0.2^{b}	39.0±0.2^{a}	92.4±0.1^{e}
Fat [g/100g]	0.03±0.00^{a}	7.50±0.36^{b}	0.06±0.02^{a}	10.26±0.24^{c}	0.02±0.01^{a}
Moisture [g/100g]	7.4±0.0^{a}	7.4±0.0^{b}	4.3±0.0^{a}	6.1±0.1^{ab}	4.6±0.3^{a}
Solubility at pH 5.5 [%]	39.6±5.7^{a}	0.7±0.5^{e}	19.2±2.4^{b}	6.1±1.8^{d}	12.2±5.6^{c}
Foaming					
Capacity [%]	36.9±2.0^{c}	10.6±1.4^{a}	17.2±2.4^{b}	13.9±0.6^{ab}	36.4±0.5^{c}
Stability [%/min]	0.36±0.07^{b}	0.52±0.00^{c}	0.61±0.00^{a}	0.33±0.04^{b}	0.15±0.01^{a}
Emulsion					
Capacity [%]	93.4±0.0^{b}	89.2±0.1^{a}	90.0±0.8^{a}	92.7±0.1^{b}	93.5±0.4^{b}
Stability [%/min]	0.37±0.00^{c}	0.41±0.00^{d}	0.38±0.01^{c}	0.27±0.00^{a}	0.29±0.00^{b}
WHC [g water / g protein]	n.d.^{a}	2.66±0.26^{c}	1.78±0.10^{b}	1.50±0.16^{b}	n.d.^{a}
Rapid visco Analyser					
Peak Viscosity [Pa s]	6.50±0.38^{c}	4.20±0.10^{a}	4.74±0.05^{ab}	4.85±0.12^{ab}	5.435±0.19^{b}
Breakdown [Pa s]	3.64±0.27^{c}	1.57±0.07^{a}	1.99±0.04^{a}	2.02±0.10^{a}	2.95±0.18^{b}
Peak Temp [°C]	80.4±0.6^{a}	84.4±0.7^{a}	83.98±0.03^{a}	84.5±0.6^{a}	82.2±1.1^{a}
Final Viscosity [Pa s]	3.72±0.02^{d}	3.54±0.02^{b}	3.54±0.0^{c}	3.62±0.02^{b}	3.31±0.01^{a}

Means in the same row with different letters are significantly different (≥3 = One-way ANOVA; ≥2 0 =t-Test, $p < 0.05$). n.d. = not detected

In general, the WHC of ingredients used in food formulations plays an important role, since it influences functional and sensory properties (Zayas, 1997b). Significant differences between the WHC of the different proteins were detected. The results ranged from 0.0 g/g to 2.66 g/g, where the highest value was obtained for pea protein and the lowest for potato and soy proteins. The WHC of potato and soy proteins is explained by the high solubility of potato and soy proteins, resulting in a full removal of the sample, leaving no sediment behind. Influencing factors regarding the WHC of proteins are the number of hydrophilic amino acids present in the structure of the protein (Zayas, 1997b). Furthermore, the amount of charged amino acids has an impact on how much water is bound to the protein. However, the WHC in this study is assumed to be related to the different purity grades of the protein powders. The protein powders showing a high WHC have low protein concentrations compared to the protein powders with a low WHC. Based on this, it is hypothesised that the other constituents of the protein powder (starch, sugar, fat, fibre) will also have an effect on the WHC. It is additionally suggested by the authors that the fragile morphology of the proteins leads to an easier disintegration, in comparison to the other proteins, contributing to the high solubility.

The foaming properties of proteins have been reported to be important factors in the bread making process since they affect gas retention and gas cell expansion during kneading and proofing (Schoenlechner, Mandala, Kiskini, Kostaropoulos, & Berghofer, 2010; Ziobro, Juszczak, Witczak, & Korus, 2016; Ziobro, Witczak, Juszczak, & Korus, 2013). The foaming properties of proteins are influenced by their source, and the method and thermal condition of their production and isolation. They can further be influenced by factors related to the methodology applied to generate a foam, such as pH, temperature, protein concentration and the mixing time (Zayas, 1997b). The results of the foaming properties revealed that the soy and potato protein isolates had the highest foaming capacities, of 36.38% and 36.68%, respectively (Table 3-1). Soy protein was also able to keep this foam significantly stable for longer when compared to the other proteins (0.15%/min). The lowest foam stability was determined for carob protein, with a foam decreasing rate four times higher than that of soy protein (0.61%/min).

Emulsifiers are used in the production of bread, to stabilise the structure and to reduce the staling rate. Based on this, it is assumed that the emulsifying properties of a protein can also influence the properties of gluten-free breads. Soy (93.53%), potato (93.41%) and lupin (92.72%) showed significantly higher emulsion capacity than pea (89.17%) and carob (89.96%) (Table 3-1). Emulsifying stability is represented as the separation rate, where lower values indicate a higher stability. Amongst all the evaluated proteins, lupin showed the highest emulsion stability with a separation rate of 0.27%/min, followed by soy protein with a separation rate of 0.29%/min. The lowest stability was found for the pea protein sample with a separation rate of 0.41%/min. The differences in emulsifying properties can be explained by the protein composition, solubility and hydrophobicity (Liang & Tang, 2014). A high solubility leads to a faster distribution of the proteins between the water/oil interface. In addition, a proportion of hydrophilic and hydrophobic groups realigns around the oil droplets and contributes to a lower interfacial tension (Lam & Nickerson, 2013). Factors such as pH, ionic strength, protein concentration and the oil fraction of the sample also affect the emulsifying properties of proteins (Ettoumi, Chibane, & Romero, 2016; Liang & Tang, 2014).

Viscosity properties of dough formulations

The analysis of viscosity/pasting properties by the use of the Rapid Visco-Analyser is recommended for gluten-free batters, since it measures larger deformations, which are considered to be important parameters to explain the baking performance (Schober, 2009). However, the measurement analyses the effect of the protein in an excess of water and is hence not directly comparable to the limited water-dough system. The rapid visco analysis of the gluten-free bread formulation was performed to examine possible correlations between the pasting properties and the bread characteristics. Parameters considered were the peak viscosity (PV), breakdown viscosity (BV), peak temperature (PT) and final viscosity (FV). The results obtained for the RVA-analysis showed significant differences between the different dough formulations (Table 3-1). Potato protein reached the highest peak viscosity (PV) (6.50 Pa s), while the lowest PV was found in the pea protein formulation (4.20 Pa s).

PV is known as the point where starch swells to its maximum capacity. In addition, the denaturation of proteins and the gelling of HPMC are also known to have an impact on

the PV (BeMiller, 2008; Marcoa & Rosell, 2008). The BV is an indicator of the stability of a product and the ability to withstand heat and shear stress. Potato protein had the highest BV (3.64 Pa s), followed by soy protein (2.95 Pa s). No significant differences between pea (1.57 Pa s), carob (1.99 Pa s) and lupin (2.02 Pa s) were found.

The PT is the temperature where the formulation reached its maximum water uptake and viscosity. No significant differences between pea, carob and lupin were found. In contrast, potato and soy proteins were significantly different from each other and the other tested proteins, having lower PT.

Significant differences between the final viscosity results, which indicate the stability of the formulation paste or gel after cooking, were determined. The potato protein formulation resulted in the most viscous paste compared to the other remaining formulations. The lowest viscosity was determined for the soy protein formulation, even though it showed the second highest PV. In this study, only the differences between the protein powders were compared and discussed, while other authors analysed the influence of proteins on gluten-free formulations (Marco & Rosell, 2008; Shevkani, Kaur, Kumar, & Singh, 2015). These authors found that the addition of higher concentrations of protein lowered the viscosity profile. They explained that the higher protein concentration had a diluting effect on the starch, leading to lower pasting properties. In this study, the addition of protein powders was kept constant; however, the purity of the proteins was different. The difference in protein purity and composition (fat, moisture, ash, and carbohydrates) can further explain the differences in the pasting properties of the formulations. The rheological properties of the various bread formulations (excluding yeast) were determined to obtain information about the viscoelastic properties. A visco-elastic dough is needed to entrap air and gases produced during fermentation, to form a good crumb structure. The analysed samples showed a higher elastic than viscous behaviour ($G' > G''$) (data not shown), with a decrease in the damping factor ($\tan \delta = G''/G'$) (Figure 3-2). The results obtained showed significant differences for the damping factor, where the addition of potato protein resulted in the lowest damping factor at each frequency, indicating the highest elastic behaviour.

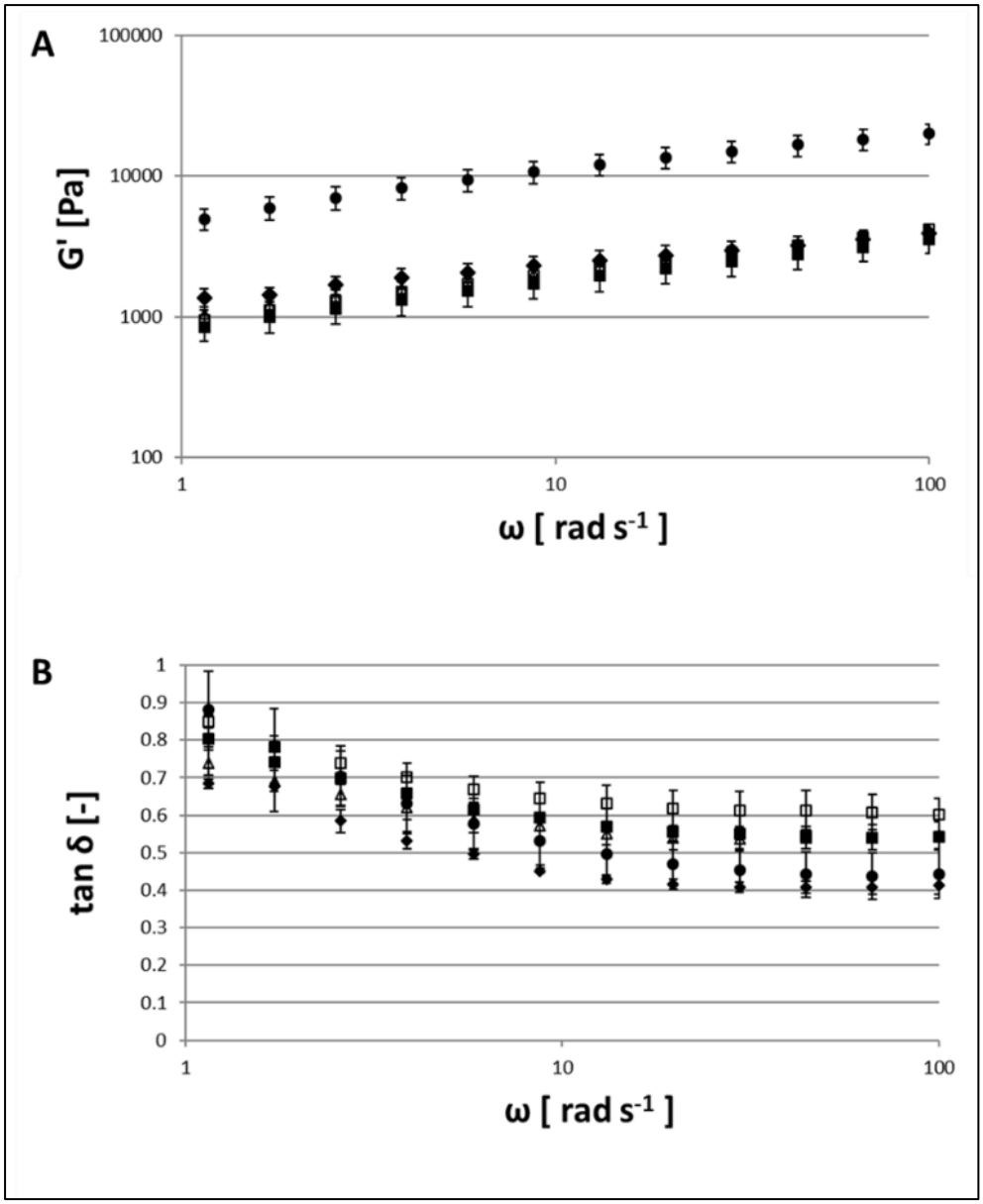


Figure 3-2 Rheology profile of the various protein dough formulations: values represent the mean of triplicates. Graph A: Storage modulus profile. Graph B: Damping factor. ■ Lupin, ● soy, □ pea, Δ carob, ◆ potato.

The highest decrease in damping factor, however, was observed in the formulation with added soy protein. This shift was also reported by Crockett, Ie, and Vodovotz (2011a) and recently by Ziobro, Juszczak, Witczak, and Korus (2016), who also analysed the addition of soy protein in dough formulations. Pea protein showed no significant differences for the storage modules compared to the other proteins (except soy). Nevertheless, pea protein had the highest damping factor compared to all proteins, indicating a higher viscous behaviour. High storage module values for pea protein were also reported by Ziobro et al. (2016); however, only a low damping factor was found. Overall, the different dough formulations showed significant differences during the rheological analysis. All formulations resulted in a damping factor of $0.1 < \tan \delta < 1$, which is in agreement with different studies (Pruska-Kędzior et al., 2008; Witczak, Juszczak, Ziobro, & Korus, 2012; Ziobro et al., 2016).

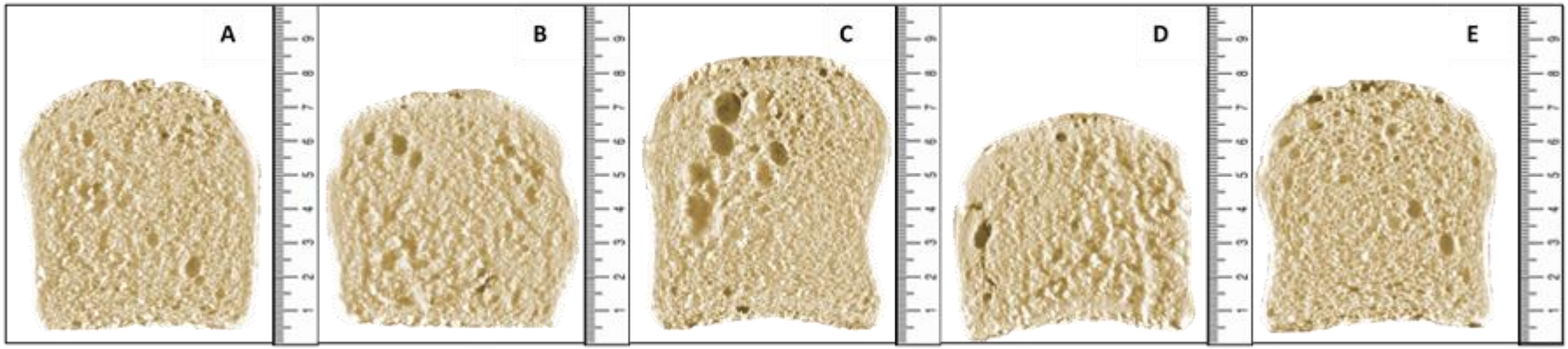
Baked bread properties

Cross sections of baked breads containing the different proteins are shown in Figure 3-3. Pictures were taken with the C-cell apparatus, guaranteeing the same image quality of the bread slices. The illustrated pictures provide a good overview of the various breads, showing differences in slice height, cell pore size and arrangement. Breads containing potato and soy proteins in the formulation showed slices of a small volume with a high cell density, whereas breads based on carob, pea and lupin have a higher volume with a lower density of cells. The results gained from instrumental analysis are shown in Table 3-2.

The ability of dough to entrap gas, which is produced during the fermentation, has the highest impact on the crumb structure and the volume of bread (Ziobro et al., 2016). In gluten-free breads the use of starch and hydrocolloids such as HPMC (hydroxypropyl methylcellulose) alone can create a bread like product (Horstmann, Belz, Heitmann, Zannini, & Arendt, 2016). Nevertheless, the addition of proteins affects the crumb structure and specific volume of bread. Table 3-2 highlights significant differences in the bread volume depending on the proteins used. The differences found in the specific volume of breads based on different proteins can be caused by individual differences in foaming properties (Ziobro, Juszczak, Witczak, & Korus, 2016), denaturation temperature (Ziobro et al., 2016), the specific content of amino acids and the

emulsifying properties (Ribotta et al., 2004). Ribotta et al. (2004) and Ziobro et al. (2016) reported inferior baking performance when using pea protein in comparison to a control not containing pea protein, whereas a study by Miñarro et al. (2012) reported a positive influence, similar to the findings in this study. Differences in findings can be caused by different processing steps and differences in bread formulations.

Figure 3-3 Images of gluten-free bread slices baked with different proteins. A: lupin protein; B: soy protein; C: carob protein; D: potato protein; E: pea protein.



As mentioned earlier, a bread-like structure can already be created, solely by the use of starch and HPMC (Horstmann, Belz, Heitmann, Zannini, & Arendt, 2016). Based on this, it is assumed that higher foaming properties in conjunction with a linear aggregation result in a stronger developed network and rather restrict the expansion of air/gas cells. This is in agreement with the literature, which states that high foaming properties have the ability to decrease the surface tension, thereby increasing the stability of a multiphase system, which in turn increases the gas retention (Ziobro, Juszczak, Witczak, & Korus, 2016; Ziobro, Witczak, Juszczak, & Korus, 2013). However, the authors believe that if the multiphase system becomes too strong, gas cell expansion is restrained. The authors share the opinion that a more elastic behaviour, as demonstrated by soy and potato proteins, causes the main limitation in gas cell expansion.

A difference in viscosity is considered a further important factor, but not as important as the viscoelastic properties of the dough itself. This would explain the findings for potato and soy proteins, having high foaming properties and linear aggregation, but a dense crumb cell structure. A previous review also reported that proteins can suppress the functionality of HPMC (Foschia, Horstmann, Arendt, & Zannini, 2017). This behaviour can be explained by the competition between surface-active compounds found in HPMC and some proteins which could alter the distribution of water in the dough. It is hypothesised that a synergetic effect between the linear aggregation of the protein and the functional properties of HPMC can create a strong network, limiting the expandability of the gas cells in the bread. The bake loss of bread is the weight it loses during baking and cooling. It gives information mainly about the amount of evaporated water, but also lost the organic material (fermented sugars, released as CO_2) (Alvarez-Jubete, Auty, Arendt, & Gallagher, 2010). The bake loss determined amongst the protein-containing bread samples revealed significant differences (Table 3-2). The highest bake loss was found in bread containing lupin protein; the significantly lowest bake loss was in potato protein-based breads. The authors hypothesise that the purity of the protein and bread characteristics such as the specific volume, where a higher volume provides a greater surface area to evaporate, influence the results of the bake loss. The crumb moisture is the water bound to the breadcrumb which was not evaporated during baking or cooling.

Table 3-2 Effect of different proteins on the quality of gluten-free model breads

	Potato protein	Pea protein	Carob protein	Lupin protein	Soy protein
Specific volume [ml/g]	2.68 ± 0.03^{a}	3.46 ± 0.16^{bc}	3.59 ± 0.10^{c}	3.66 ± 0.14^{c}	3.27 ± 0.05^{b}
Crumb moisture [g/100g]	51.55 ± 0.18^{a}	56.77 ± 2.35^{b}	53.06 ± 0.19^{a}	53.24 ± 1.98^{ab}	50.90 ± 0.12^{a}
Bake loss [g/100g]	17.11± 0.95^{a}	23.18± 2.66^{bc}	17.99 ± 1.30^{ab}	24.44 ± 1.69^{c}	19.86 ± 2.66^{ab}
Course / Fine Clustering [-]	0.19 ± 0.05^{b}	0.07 ± 0.01^{a}	0.08 ± 0.02^{a}	0.09 ± 0.02^{a}	0.16 ± 0.05^{b}
Net Cell Elongation [-]	1.07±0.02^{b}	1.03± 0.02^{a}	1.07± 0.02^{b}	1.03 ± 0.01^{a}	1.03 ± 0.02^{a}
Hardness [N]	17.79 ± 1.61^{d}	8.69 ± 0.39^{b}	11.88 ± 1.09^{c}	7.12 ± 0.79^{a}	9.78 ± 0.21^{b}
Staling rate [N/day]	2.89 ± 0.37^{b}	2.69 ± 0.26^{ab}	2.70 ± 0.22^{ab}	2.02 ± 0.21^{a}	2.62 ± 0.47^{ab}
Springiness rate [%/day]	-0.010 ± 0.003^{c}	-0.429 ± 0.047^{a}	-0.016 ± 0.006^{b}	-0.230 ± 0.087^{a}	-0.008 ± 0.001^{c}
Cohesiveness rate [%/day]	-0.023 ± 0.001^{c}	-0.060 ± 0.002^{a}	-0.038± 0.002^{b}	-0.057 ± 0.001^{a}	-0.020 ± 0.004^{d}
Resilience rate [%/day]	-0.016 ± 0.000^{c}	-0.052 ± 0.002^{a}	-0.034 ± 0.001^{c}	-0.049 ± 0.000^{b}	-0.010 ± 0.002^{c}

Means in the same row with different letters are significantly different (≥3 = One-way ANOVA; ≥2 0 =t-Test, $p < 0.05$).

The highest content of moisture in the breadcrumb was found for lupin protein breads, while potato protein breads had the lowest moisture content. A higher moisture content is desirable, as it gives the bread a softer crumb and slows down the staling process (Fadda, Sanguinetti, Del Caro, Collar, & Piga, 2014). The parameters analysed relating to the cell-structure were course/fine clustering and cell elongation (Table 3-2).

Course/ fine clustering defines the ratio between course and fine cells which gives information about the uniformity of the cell distribution in the breadcrumb. Cell elongation is a measure of the circularity of the cell pores. High values indicate a higher deviation of the pore shape from a circle. Only potato and soy breads revealed significant differences regarding their course/ fine clustering. The results found for potato and soy proteins were twice as high as the results for the other protein breads, indicating a lower uniformity in cell distribution. The authors assume that there is a direct link between the structure of the foam and the breadcrumb structure. Foaming of proteins showed differences not only in the foaming capacity and stability but also in the foam cell size (data not shown). Foams prepared by potato and soy showed a high capacity of foam build up by densely packed small gas cells, while the other proteins showed greater cells with lower capacity, indicating a lower denaturation of proteins and less surface activity. The described density of foam structure can also be observed in the bread pictures (Figure 3-3). The elongation of the crumb cells for carob and potato proteins showed significantly higher values compared to the remaining proteins. This indicates a higher diversity of circularity. It is hypothesised that interactions between the protein and HPMC could occur. The literature reported that the solubility of a protein affects the functionality of HPMC (Foschia, Horstmann, Arendt, & Zannini, 2017). Thus, soluble proteins, which have high surface activity, may have a synergetic effect with HPMC. As described earlier, this synergetic effect would lead to a stronger network, which is less elastic and hence leads to a denser crumb texture.

The texture is one of the most important quality characteristics in bread. The change in texture due to staling also affects the flavour. The staling process is a chain reaction of several physical–chemical changes occurring during storage (Gray & Bemiller, 2003). The migration of water from the crumb to crust and the recrystallization of starch are the two main factors causing changes to the bread structure (Gray & Bemiller, 2003). These processes, however, can be influenced by the addition of protein. Ziobro et al.

(2016) recently reported a significant influence of proteins and their source on gluten-free bread crumb hardness. Similar observations were made in this study, where significant differences between the protein bread formulations and hardness values (Table 3-2) were found. On the day of baking, the highest hardness value was measured in breads containing potato protein. These breads were approx. 40% harder than the formulation containing carob protein, which had the second highest crumb hardness value. The addition of lupin protein resulted in the softest bread crumb (60% softer than potato protein). Significant differences in the staling rate were found amongst the breads. The staling rate of the bread crumb is the increase in the hardness of the crumb over a period of time. The potato protein breads had the highest staling rate (2.89 N/ day) in comparison with the breads containing the other tested proteins. The slowest staling rate was found for lupin protein (2.02 N/ day). A further parameter measured by the texture profile analysis is the cohesiveness rate, which is an indicator for the loss of the bread crumbs ability to withstand a second deformation. This deformation is relative to its resistance under the first deformation. Potato protein and soy protein showed the smallest rate for the cohesiveness loss while pea protein showed the highest. The springiness rate describes the loss of springiness per day. Springiness itself is a measure of the breadcrumb structural integrity. Potato (0.01 %/day) and soy proteins (0.008 %/day) showed the smallest rate for the loss in integrity, the highest rate being found in breads baked with pea protein (0.429 %/day). Like the springiness, resilience is also a parameter that measures the regain of the original height. Nevertheless, in contrast to springiness which is measured in distance at the two compressions, resilience is measured based on energy up and down stroke, during the first compression. The resilience rate thus describes the loss of this attribute. Again, potato and soy had the smallest resilience rate and pea was found to have the highest.

Relationship between protein properties, dough properties and gluten-free bread-like products

The global interest in gluten-free products led to an increase in research and published literature in this area (Chapter 1). However, only a very few research papers have addressed the relationship between ingredient properties and the final product. Knowledge of the relationship between ingredient properties and the final product

quality could provide a better understanding of gluten-free systems. Therefore, the investigation of possible connections between protein properties, gluten-free dough and gluten-free bread quality could advance this knowledge. To investigate such correlations the data generated (physical–chemical properties of proteins, dough properties and final bread product characteristics) were subjected to Pearson correlation analysis. Table 3-3, however, illustrates only the significant correlations between the ingredient properties and the bread quality. In general, no correlations were found between the chemical composition of the proteins and the techno-functional properties analysed. Nevertheless, strong correlations were found between protein techno-functional properties and dough properties. The foaming capacity of the protein powders had a significantly positive correlation with the viscosity parameters measured by the RVA ($p < 0.01$), but also with the damping factor from the rheometer analysis ($p < 0.01$). This relationship suggests that a high foaming capacity leads to a higher peak viscosity at a lower temperature at an earlier stage. It further results in more elastic dough. However, there is no direct link between these parameters and it is not clear whether it is a correlation or causation. Further studies on this possible relationship could contribute to clarify this. The authors hypothesise that the amount of denatured proteins could influence these parameters. The method used for the foaming capacity included a whipping step which denatures the proteins to a certain extent. The denatured proteins would realign around the incorporated air cells and create a foam (Jones & Lyttleton, 1972). It is hypothesised that the amount of denatured proteins is proportional to the increase in the viscosity of the dough formulation in the RVA. In addition, the solubility of the proteins was found to have a similar or synergetic effect on the peak viscosity ($p < 0.05$), time and temperature. It is suggested that soluble proteins distribute more evenly in the liquid phase, creating a stronger network by linear aggregation when they denature, compared to the random aggregations formed by insoluble proteins (Zayas, 1997b). Significant correlations between dough properties and bread characteristics were also observed ($p < 0.05$). In particular, the breakdown viscosity measured with the Rapid Visco Analyser showed high correlations with many bread characteristics. A higher breakdown viscosity (BV) indicates a lower stability as more granules are disrupted or have a lower tendency to resist shear force during heating (Thirathumthavorn & Charoenrein, 2006).

Table 3-3 Correlation matrix (correlation coefficients and p value) between protein properties, dough properties and indicative parameters of the gluten-free bread-like products

		RVA-Parameter				Rheometer-Parameter
		Peak Viscosity	**Breakdown**	**Peak Time**	**Peak Temp**	**tan.Delta**
Protein Properties	**Foaming capacity**	0.97**	0.97**	-0.97**	-0.97**	-0.96**
	Solubility at pH 5.5	0.89*		-0.89*	-0.88*	-0.92*
Gluten-free Bread	**Cohesiveness Rate**		0.88*			
	Resilience Rate		0.88*			
	Coarse / Fine Clustering	0.94*	0.98**			-0.91*
	Average Cell Elongation		-0.90*			
	Volume	-0.89*	-0.88*	0.99**	0.99***	

*p< 0.05; ** p< 0.01; *** p< 0.001

This characteristic is hypothesised to be the cause of the correlation between the breakdown viscosity and most of the bread structural parameters observed. In particular, the cohesiveness rate and resilience rate as part of the crumb texture measurement showed a relationship with the BV (r. 0.88; $p < 0.05$). The cohesiveness rate is the indicator of how well a product withstands a second deformation relative to its resistance under the first deformation. The resilience rate of the breadcrumb is an indicator of the withdrawal of the first penetration, before the waiting period starts. The observed correlation indicates that a high breakdown viscosity leads to a decreased rate of the cohesiveness and the development of resilience over time. Peak viscosity showed significant correlations with cell structure and the final loaf volume ($p < 0.05$).

The positive correlation relation associated with the coarse/fine clustering and the negative correlation with the bread loaf volume can be explained as follows. A higher dough viscosity restrains the cell expansion, leading to smaller, finer cells, which further leads to a smaller bread volume. The coarse/fine clustering was also found to be negatively correlated with the damping factor (r. −0.91; $p < 0.05$), which is an indicator of viscoelastic behaviour. This result indicates that a lower damping factor (more elastic dough) leads to smaller cells, based on increased viscosity.

The correlation matrix allows a further conclusion to be drawn that gluten-free doughs with lower peak viscosity and higher gelling temperature lead to an increased loaf volume.

Conclusion

In this study, the application of plant proteins (potato, pea, carob, lupin, and soy) in a gluten-free bread formulation was compared. The analysed proteins were found to be significantly different in their composition and their properties, which is mainly based on their source or origin. Significant differences were further observed in the baked breads. The addition of potato protein and soy protein to the bread formulation resulted in smaller breads with a denser crumb structure in comparison with the other proteins. The addition of carob, lupin and pea, however, resulted in a high volume with greater cell pores and a softer bread crumb. Based on the correlation analysis of the data it was possible to link certain protein properties to the bread characteristics. Foaming functionalities and the solubility of the proteins in the dough significantly correlated with dough properties, which in turn affected the final bread quality. Proteins with high foaming properties lead to a higher dough viscosity (r. 0.97; $p < 0.01$). Furthermore, the higher viscosity had a negative effect on the specific volume of the bread (r. −0.89; $p < 0.05$).

The knowledge gained in this study enables the prediction of the impact of a plant protein in a gluten-free bread formulation. This could help industry to improve the gluten-free bread quality, and lead to improvement of the nutritional value. Further studies are suggested with regard to the differences in the composition, in particular the protein and fat content could help to eliminate further influencing factors which need to be considered for the analysis of complex formulations. This could be achieved by using the defatted protein powders i.e. for pea and lupin.

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Chapter 4 Water absorption as a prediction tool for the application of hydrocolloids in potato starch-based bread

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Abstract

To create visco-elastic networks in gluten-free doughs, hydrocolloids have been used most commonly to compensate for the lack of gluten. This study applies a prediction tool in the form of an equation, which considers the right water absorption level to obtain optimised conditions for the use of six different hydrocolloids (guar gum, hydroxypropyl methylcellulose, locust bean gum, pectin, sodium alginate, xanthan gum). For this purpose, the water holding capacity of each hydrocolloid was determined and the water amount in the formulation was adjusted accordingly. The hydrocolloids were analysed in five concentrations (0.25%, 0.5%, 1%, 1.5%, 2.0%). Analysis of water adjusted doughs included rheological properties, pasting properties and the baking performance. With the aid of the prediction tool, it was possible to obtain bread-like products for each hydrocolloid. However, the various hydrocolloids showed different concentration levels, where they performed best. In this study, the main influencing factors on bread quality were linked to the charge and the molecular weight of the various hydrocolloids. The negative charge of some hydrocolloids was hypothesised to created repelling forces between them and the negatively charged phosphate groups of potato starches. Bread baked with sodium alginate reached the highest specific volume at a concentration level of 1% and 2% xanthan gum had the softest breadcrumb. Based on the hydrocolloid used, the analysis of the rheological and pasting properties revealed connections between dough properties and bread quality parameters.

Introduction

The production of high quality leavened baked gluten-free goods remains a technological challenge. The absence of gluten, with its unique viscoelastic properties, results in reduced gas retention and structure formation (Hager & Arendt, 2013). A lot of research has been conducted to tackle this problem by the addition of hydrocolloids. These are water-soluble polysaccharides with varied chemical structures that have a wide range of functional properties that make them suitable for different applications particularly in the area of gluten-free bread products (Li & Nie, 2016). Previously published literature related to gluten-free bread formulations state that xanthan gum and hydroxypropyl methylcellulose (HPMC) as the most used hydrocolloids (Cato, Gan, Rafael, & Small, 2004; Hager & Arendt, 2013; Lee & Lee, 2006; Mancebo, San Miguel, Martínez, & Gómez, 2015; Sciarini, Ribotta, León, & Pérez, 2010; Sivaramakrishnan, Senge, & Chattopadhyay, 2004). The gluten-free market reflects this research showing that 40-70% of gluten-free breads contain xanthan gum and/or HPMC in their formulation, respectively (Foschia, Horstmann, Arendt, & Zannini, 2016).

Hydrocolloids have now become a vital ingredient in the formulation of gluten-free breads. However, consumer demands are focused more and more on ingredient declaration. It is known that ingredient names like "xanthan gum" or "hydroxypropyl methylcellulose" and their production background do not appeal to consumers. Hydrocolloids like guar gum, locust bean gum, pectin and sodium alginate could have the potential to replace xanthan gum and HPMC by keeping the quality of the product or even improve it. Locust bean gum and guar gum both belong to the family of galactomannans and are found in the carob and guar bean, respectively. Both galactomannans have a linear structure and a neutral charge. In comparison to other hydrocolloids, they have a wide range in size up to high molecular weights categorized from 50 kDa to 8000 kDa and 50 kDa to 3000 kDa, respectively (FAO 2017). Literature on the effect of locust bean gum in gluten-free bread formulations is scarce (Masure, Fierens, & Delcour, 2016). Nevertheless, it was reported that a blend of locust bean gum and xanthan gum was more effective in improving dough structure and bread quality parameters, than locust bean gum on its own (Demirkesen, Mert, Sumnu, & Sahin, 2010). Also, a recent study on the effect of xanthan gum and guar gum on gluten-free pan bread reported increases in quality parameters when the hydrocolloids

were blended (Gadallah, Mahmoud, Yousif, & Alawneh, 2016). On the other hand, the application of guar gum on its own has recently been reported to improve quality and storage stability of gluten-free frozen dough (Asghar & Zia, 2016). Differences in the effect of hydrocolloids are assumed to be greatly influenced by the differences in formulation and occurring interactions with other components. Pectin is mainly extracted from citrus peel. It consists of a linear chain with a molecular weight between 110 kDa and 150 kDa. It has been demonstrated to contribute to volume and structure in a gluten-free bread formulation (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007). Sodium alginate, a linear hydrocolloid (10 kDa - 600 kDa) with a negative charge is a structural component in marine brown algae. So far, it has only been incorporated in wheat-bread formulations where it was reported to have negative effects on volume and crumb hardness (Guarda, Rosell, Benedito, & Galotto, 2004; Rosell, Rojas, & De Barber, 2001). Guarda et al. (2004) stated that the properties of sodium alginate very much depend on the extraction method and the source of algae. This study provides a prediction tool in the form of an equation. It considers the water holding capacity (WHC), to obtain optimised conditions for the use of six different hydrocolloids (guar gum, HPMC, locust bean gum, pectin, sodium alginate, xanthan gum) in gluten-free dough formulations. Table 4-1 gives a general overview of the important characteristics like their sources, molecular weights and charges.

The objectives of this study were to compare these hydrocolloids and to test the tool in gluten-free bread formulations based on potato starch. For this purpose, the WHC of each hydrocolloid and potato starch was determined and the water amount in the dough formulation was adjusted accordingly. The hydrocolloids were analysed in 5 concentrations (0.25%, 0.5%, 1%, 1.5%, 2.0%). The obtained knowledge from this work is thought to contribute to the gluten-free product production and help to improve the knowledge and quality of gluten-free products.

Table 4-1 Summarizing the important characteristics of the hydrocolloids used in this study.

Sample	Origin*	Structure*	Charge*	Chain length / Molecular mass*
Guar gum [E412]	Guar seed	Linear	Neutral	50 - 8,000 kDa
Hydroxypropyl-methyl cellulose [E464]	Modified cellulose	Linear	Neutral	13 – 200 kDa
Locust bean gum [E410]	Carob pod	Linear	Neutral	50 - 3,000 kDa
Pectin [E440]	Citrus peel	Linear	Negative	~100 kDa
Sodium Alginate [E401]	Brown algae	Linear	Negative	10- 600 kDa
Xanthan gum [E415]	Xanthomonas campestris	Linear	Negative	~ 1,000 kDa

*Data sourced from fao.org [Accessed 15.8.2017] (FAO 2017)

Experimental

Materials

Six commercially available hydrocolloids were used in this study. Guar gum and locust bean gum were obtained from Cargill, France; pectin and xanthan gum from Kelco, Germany; sodium alginate from Chemcolloids Ltd, Congleton, UK and HPMC by J. Rettenmaier & Söhne GmbH + Co. KG, Germany. Potato starch was supplied by Emsland, Germany; dry yeast by Puratos, Belgium; sugar by Siucra Nordzucker, Ireland; salt by Glacia British Salt Limited, UK.

Microscopy

Sample preparation of the doughs with the various hydrocolloids included the preparation of the dough (excluding yeast) and a freeze-drying process for 48 h. The dough samples at 2% level of hydrocolloids were then cut and mortared. Samples were then mounted on aluminium stubs, with the use of double-sided carbon tape. Samples were coated with a layer of 25 nm of sputtered palladium-gold. Hereupon, samples were examined under high vacuum in a field emission scanning electron microscope (JSM-5510 Scanning Electron Microscope, JEOL, München, Germany) with a working distance of 8 mm. Secondary electron images were acquired at an accelerating voltage of 5 kV. SEM Control User Interface software, Version 5.21 (JEOL Technics Ltd., Japan) was used for processing the images.

Water holding capacity and water adjustment

The measurement of WHC of the hydrocolloids was determined according to AACC method 56–30.01 with some modifications: samples (1.000 g ± 0.005 g) were mixed with 30 ml of distilled water using an Ultra-Turrax equipped with a S10N-5G dispersing element (Ika-Labortechnik, Janke and Kunkel GmbH, Staufen, Germany) for 15 s and then shaken for 30 min at 1000 rpm using a platform shaker (UNI MAX 1010, Heidolph, Schwabach, Germany). Subsequently, the mixture was centrifuged at 2000 g for 10 min. WHC was expressed as ml of water retained per gram of solid:

WHC [ml water / g ingredient] = (W2-W1)/ W0 [Eq. 1]

Where W2 is the weight of the tube plus the sediment, W1 is the weight of the tube plus the sample and W0 is the sample weight.

The generated values were used in Eq. 2 to calculate and adjust the water content accordingly based on the hydrocolloid and its concentration.

Water content [%] = (((a/100*c1) + (b/100*c2))*d)/e [Eq. 2]

Where:

a = WHC of potato starch (= 0.590 ml/g)

b = WHC of Hydrocolloid

c1 = percentage of starch used in the formulation based on a dry base (98.00–99.75)

c2 = percentage of hydrocolloid used in the formulation based on a dry base (2.00–0.25)

d = 80% (based on starch) - optimal amount of water added to the base formulation (control)

e = 0.786 ml/g - combined WHC of the base formulation (potato starch 98%and HPMC 2%; control).

The control values d and e were generated and calculated from previous research conducted on the impact of different starches on gluten-free formulations, here named as base formulation or control which contained 98% potato starch and 2% HPMC as solid base (Horstmann, Belz, Heitmann, Zannini, & Arendt, 2016). Using Equation 2, the calculated percentages of water were then applied in the various dough formulations throughout the study (Table 4-2).

Bread production

Bread samples were prepared according to (Horstmann et al., 2016). The formulation of the breads was as followed: 0.25–2% hydrocolloid, 2% salt, 4% sugar, 2% yeast, based on a dry base. The water addition depended on the used hydrocolloid and its concentration. Amounts were calculated as described as mentioned above. Dry ingredients where mixed and yeast was suspended in warm water (25°C) and regenerated for a period of 10 min. Mixing was carried out with a k-beater (Kenwood, Havant, UK) at low disk speed (level 1 of 3) for 1 min in a Kenwood Major Titanium kmm 020 Mixer (Kenwood, Havant, UK). After the first mixing, the dough was scraped down from the bowl walls. A second mixing step of 2 min at higher disk speed (level 2 of 3) was applied. The batter was weighed (300 g) into baking tins of 16,5 cm × 11 cm x 7 cm and placed in a proofer (KOMA, Netherlands) for 45 min at 30°C and 85% relative humidity (RH). The proofed samples were then baked for 55 min at 220°C top and 220°C bottom heat in a deck oven (MIWE, Germany), previously steamed with 0.7 L of water. The breads were cooled for 2 h prior to analysis.

Rapid visco analysis

The pasting behaviours of the bread formulation (dry mix, excluding yeast) were measured according to the Newport Scientific Method 6, Version 4, December 1997, using a Rapid Visco Analyzer (RVA Super 3 Rapid Visco Analyser Newport Scientific, Warriewood, Australia). Samples were heated at a rate of 0.2 °C/sec from 50 °C to 95 °C, maintained at 95 °C for 162 s, cooled at the rate of 0.2 °C/sec to 50 °C, and held for 120 s at 50 °C before the test ended.

Viscoelastic properties of the dough

Oscillation measurements of dough samples (excluding yeast) were carried out by using a Rheometer Physica MCR 301 (Anton Paar GmbH, Ostfildern, Germany). Parallel serrated plates to prevent slippery, were used. The temperature of the lower plate was set to 30 °C and used in conjunction with a 50 mm diameter upper plate. Frequency sweeps were conducted using a target strain of 0.01% and a frequency range from 100

to 0.1 Hz at 30 °C. Before each test, the sample rested for 5 min to allow equilibration. Data obtained were complex modulus *G** and the damping factor tan δ (*G"/G'*).

Bread analysis

Bread analysis was performed according to a previous work (Horstmann, Foschia, & Arendt, 2017). The specific volume of the bread was determined by a Vol-scan apparatus (Stable Micro System, UK. An image analysis system (Calibre Control International Ltd., UK) was used to analyse the breadcrumb structure. Crumb texture was analysed using a Texture Profile Analyser (TA-XT2i, Stable Micro Systems, Godalming, UK) with a 25 kg load cell. Bread samples were sliced into 20 mm slices and analysed with a test speed of 5 mm/s and a trigger force of 25 g, compressing the middle of the breadcrumb to 10 mm. Baked breads were stored in polyethene bags (polystyrol-ethylene vinyl alcohol-polyethylene).

Statistical analysis

Results are reported as averages with standard deviation. Statistical analyses were performed with Minitab18 Software. A one-way ANOVA was conducted on the water holding capacity. Two-way ANOVA was conducted on the pasting properties and baking results using multiple comparison of the two experimental factors concentration (with levels "0.25%"; "0.5%", "1.0%", "1.5%" and "2.0%") and hydrocolloid type (with levels "Locust bean gum", "Guar gum", "Sodium alginate", "Pectin", "HPMC" and "Xanthan"). Depending on the parameter measured different contribution levels of the concentration or the type of hydrocolloid were found. The contribution and significance levels of the various parameters are discussed in each individual paragraph. Correlation analysis was conducted to investigate correlations between the viscosity measurements and baking results.

Results and Discussion

In wheat dough formulations, the water is generally adjusted using the farinograph-method (AACC 54–21.02). This method allows determining the exact amount of water, which is necessary to hydrate the dough and reach a set value measured in Brabender-Units (BU). The most commonly used value is 500 BU (Faubion & Hoseney, 1990). However, this method was also used for the prediction of water absorption in gluten-free bread formulations (Gujral & Rosell, 2004a, 2004b; Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007; Sivaramakrishnan, Senge, & Chattopadhyay, 2004).

Table 4-2 Percentages of water added to various formulation of different hydrocolloids at different concentrations

	Water holding capacity [g/g sample weight]	**Water addition based on solid (starch and hydrocolloid [%])**				
Hydrocolloid Concentration		0.25%	0.50%	1.0%	1.5%	2.0%
Guar gum [E412]	21.05 ± 0.63[a]	65.25	70.46	80.87	91.28	101.69
Hydroxypropyl-methyl cellulose [E464]	10.39 ± 0.63[c]	62.54	65.04	70.02	75.01	80.00*
Locust bean gum [E410]	15.02 ± 1.46[b]	63.72	67.39	74.73	82.07	89.41
Pectin [E440]	4.65 ± 1.55[d]	61.30	62.55	65.50	67.57	70.15
Sodium Alginate [E401]	4.63 ± 0.30[d]	61.07	62.10	64.16	66.21	68.27
Xanthan gum [E415]	18.72 ± 0.23[a]	64.66	69.27	78.50	87.73	96.95

Means in the same column for each individual hydrocolloid with different letters are significantly different (≥3 = One-way ANOVA; ≥2 0 =t-Test, $p < 0.05$).

These studies used flours and proteins in their formulations providing protein network and hydration. In this study, the farinograph showed limitations when the water additions were applied to the analysis of starch-based gluten-free formulations containing hydrocolloids. These limitations are believed to be caused by the lack of protein and their network forming properties. A study by Hager and Arendt (2013) adjusted the optimal water content with the aid of response surface methodology. However, prior to the use of this tool preliminary trial-and-error baking test had to be

conducted. None of the above methods are ideal and very often are time-consuming. Therefore, a need exists to develop a simple method to predict the water level in gluten-free formulations.

Water hydration capacity and water adjustment

The WHC determines the amount of water (in grams) bound per gram of hydrocolloids in an aqueous dispersion. In general, the WHC of ingredients used in food formulations plays an important role since it influences functional and sensorial properties. The WHC showed significantly different results between the various hydrocolloids (Table 4-1). Xanthan gum and guar gum showed the highest WHC indicating cold swelling properties, while sodium alginate and pectin had almost no swelling power demonstrating a high solubility and hot swelling properties. These characteristics are linked to the source of origin, chain length, molecular weight and distribution as well as the polar charge of the hydrocolloid (Table 4-1) (Anton & Artfield, 2008; Capriles & Arêas, 2014). It is generally known that the polar charge has a high impact on the water affinity. Negatively charged hydrocolloids are more prone to build intermolecular hydrogen bonds with water, while uncharged hydrocolloids have intramolecular hydrogen bonds, which reduce the interactions with water. As stated in the literature also the chain length and the molecular weight affect the WHC of hydrocolloids. A study by Funami et al. (2005) correlated the molecular weight with the radius of gyrations, which is a measure for the distribution of components of an object around an axis, which in this study refers to water around the hydrocolloid. The study showed that the higher the molecular weight the higher the radius of gyration indicating a higher water holding capacity for hydrocolloids with a higher molecular weight. This can explain the low WHC for pectin and sodium alginate based on their low molecular weight. Furthermore, it justifies that xanthan gum despite its negative charge leads to a high WHC. These findings are in agreement with the earlier stated influencing factors on WHC in the literature. Additionally, it has been reported that a high number of branches increase the interactions with water. However, in this study, only linear hydrocolloids were chosen and hence the factor of branching is neglected.

Scanning electron microscopy analysis

The microstructure investigation of the bread dough formulation (excluding yeast) is depicted in Figure 4-1. The images show the network formation of the hydrocolloids at a concentration of 2%. HPMC (b), locust bean gum (c) and to a certain extend guar gum (a) show thick strands expanding over the starch granules, forming a network. On the contrary, the dough formulation including sodium alginate shows a thin film coating the starch granules. Pectin (d) and xanthan gum (f) show a mixture of film coating and particle strands covering the surface of the starch granules. The arrangement and thickness of strands are believed to have an influence on the dough properties regarding pasting and viscosity. This is in agreement with observations of Chaisawang and Suphantharika (2006). The authors found that xanthan gum molecules in contrast to guar gum coated the starch granules. This difference is thought to inhibit the granule swelling and reduce peak viscosity (Song, Kim, & Chang, 2006). The effect of hydrocolloids on starch was comprehensively reviewed by Bemiller (2011) and showed that a combination of hydrocolloid and starch could suppress the starch granule swelling and lower the viscosity. One of the explanations was the limited availability/accessibility of free water for the granules to swell.

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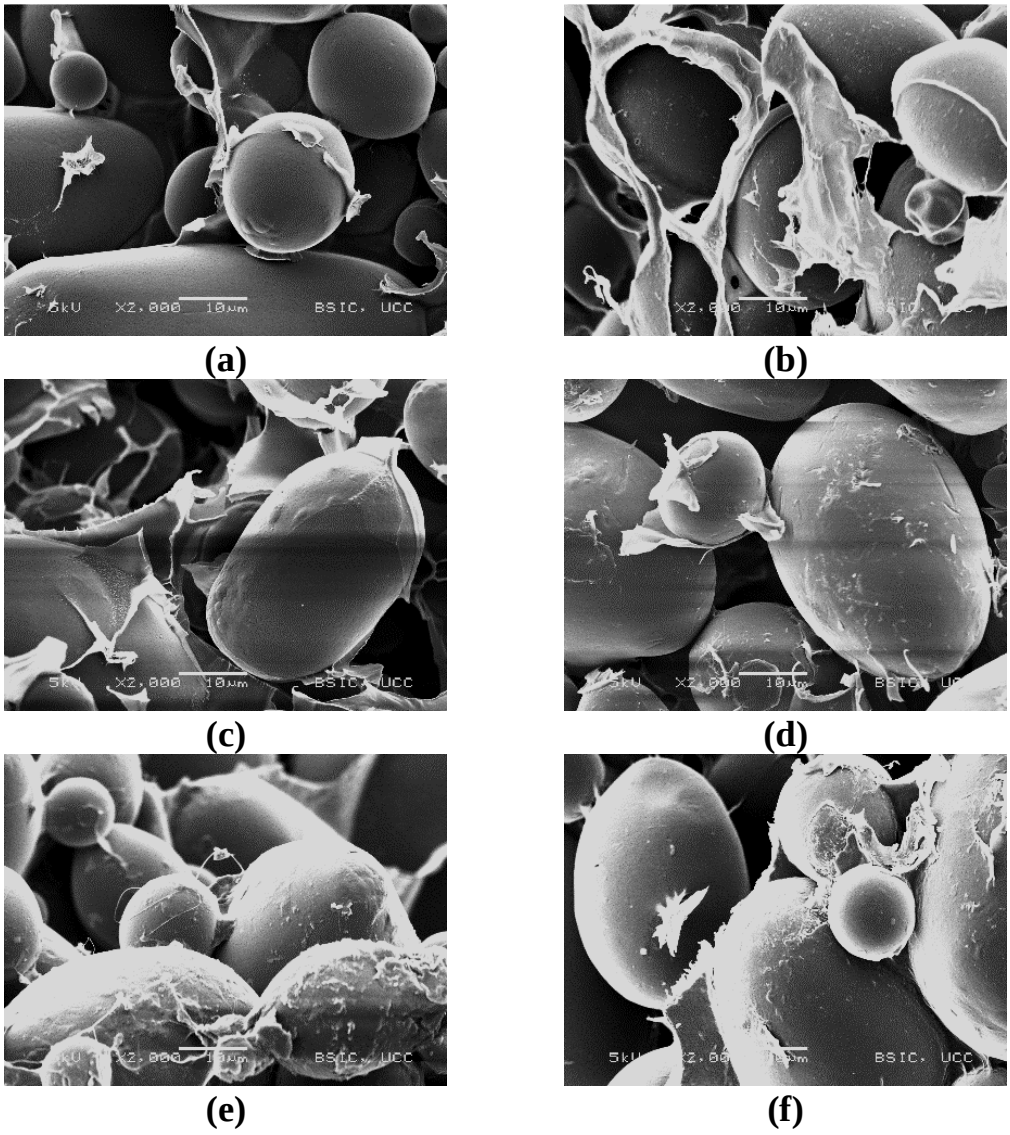


Figure 4-1 SEM images of the various dough formulations (excluding yeast; 2% hydrocolloid). Magnification x2000. (a) guar gum; (b) HPMC; (c) locust bean gum; (d) pectin; (e) sodium alginate; (f) xanthan gum.

Pasting properties of dough formulations

The characteristics of starch granule swelling, breakdown and retrogradation during processing and storage determine the textures and stabilities of high moisture starch-based foods. These properties are modified and or controlled by the addition of hydrocolloids (BeMiller, 2011). Starch is the main constituent in gluten-free products. Hence, its functional properties like pasting play a key role in the production of those.

Pasting properties (peak viscosity, breakdown viscosity and the peak time) of the various bread formulations are summarized in Table 4-3. Significant differences between the various hydrocolloids were observed. The different formulations exhibit a range of properties like degrees of associations with other molecules of the same hydrocolloid and other molecules like water (BeMiller, 2011). Shi and BeMiller (2002) found that the molecules of the applied gums (CMC, carrageenan, alginate, xanthan) interact with leached amylose molecules, producing a viscosity increase via synergetic effects and prevent retrogradation. This increase in viscosity can be caused by hydrogen bonds created between the hydrocolloid and the leached amylose (Morris et al., 2008). Also, significant differences between the concentration level were expected as a higher concentration would strengthen the above-mentioned interactions. The peak viscosity is the point where starch granules swell to their maximum before they burst. Two-way ANOVA indicated that the type of hydrocolloid is the main affecting parameter (79.03%, $p < 0.05$). The significant highest peak viscosities were reached by formulations containing locust bean gum and guar gum. The significant lowest viscosity was found in formulations containing sodium alginate.

Overall it showed, that higher concentration of locust bean gum and guar gum, led to an increase in viscosity, whereas sodium alginate and pectin revealed a decrease in the viscosity with increasing levels. A similar effect was also observed by Kaur, Singh, Singh, and Mccarthy (2008), who suggested that the decrease in viscosity in potato starch pastes was due to reduced granule swelling caused by the addition of cassia gum. In this study, lower viscosities by increasing levels of sodium alginate and pectin could be attributed to their negative charge. This negative charge can create repelling forces with the negatively charged phosphate groups on potato starch.

Table 4-3 Pasting properties of various bread formulations

Properties	Peak 1 [RVU]	Breakdown [RVU]	Final Viscosity [RVU]	Peak Time [min]
Locust bean gum 2 %	2964.0 ± 2.0^{eA}	1097.7 ± 10.7^{eA}	2600.3 ± 22.1^{bA}	6.6 ± 0.1^{aD}
Locust bean gum 1.5 %	2716.7 ± 23.2^{dA}	912.3 ± 41.9^{dA}	2427.3 ± 33.5^{abA}	6.8 ± 0.1^{abD}
Locust bean gum 1.0 %	2477.0 ± 1.0^{cA}	777.3 ± 2.5^{cA}	2393.3 ± 35.4^{aA}	6.9 ± 0.0^{bcD}
Locust bean gum 0.5 %	2273.3 ± 30.6^{bA}	611.3 ± 68.3^{bA}	2328.0 ± 98.0^{aA}	7.0 ± 0.0^{bcD}
Locust bean gum 0.25 %	2141.7 ± 30.2^{aA}	492.3 ± 31.9^{aA}	2361.7 ± 100.9^{aA}	7.1 ± 0.1^{cD}
Guar gum 2%	2535.0 ± 136.8^{dB}	785.0 ± 44.0^{dB}	2424.7 ± 153.2^{aAB}	7.1 ± 0.0^{aD}
Guar gum 1.5 %	2473.0 ± 94.6^{cdB}	705.7 ± 46.1^{cdB}	2445.7 ± 86.5^{aAB}	7.1 ± 0.1^{aD}
Guar gum 1.0 %	2328.7 ± 20.2^{bcB}	661.0 ± 5.2^{cB}	2410.0 ± 22.3^{aAB}	7.0 ± 0.1^{aD}
Guar gum 0.5 %	2132.0 ± 30.8^{abB}	555.0 ± 42.7^{bB}	2245.0 ± 116.2^{aAB}	7.1 ± 0.1^{aD}
Guar gum 0.25 %	2059.7 ± 13.6^{aB}	459.0 ± 11.5^{aB}	2331.3 ± 16.9^{aAB}	7.1 ± 0.1^{aD}
Sodium alginate 2.0%	958 ± 2.6^{aE}	155.3 ± 5.5^{bF}	2035.7 ± 46.5^{aC}	8.9 ± 0.1^{cA}
Sodium alginate 1.5%	1049.3 ± 15.3^{abE}	142.7 ± 3.1^{abF}	2048.3 ± 5.5^{aC}	8.5 ± 0.2^{bcA}
Sodium alginate 1.0%	1215.3 ± 29.1^{bE}	124. 7 ± 3.1^{abF}	2110.7 ± 27.5^{aC}	8.9 ± 0.9^{bcA}
Sodium alginate 0.5%	1565.3 ± 102.8^{cE}	115.3 ± 4.6^{aF}	2229.3 ± 21.0^{bC}	7.7 ± 0.2^{abA}
Sodium alginate 0.25%	1717.3 ± 41.2^{cE}	210.0 ± 27.2^{cF}	2314.0 ± 35.4^{bC}	7.4 ± 0.0^{aA}
Pectin 2 %	1524.3 ± 16.1^{aD}	203.3 ± 7.7^{aE}	2060.0 ± 48.9^{aC}	7.6 ± 0.1^{bC}
Pectin 1.5 %	1520.0 ± 28.2^{aD}	190.3 ± 4.5^{aE}	2066.3 ± 17.2^{aC}	7.6 ± 0.1^{bC}
Pectin 1.0 %	1683.3 ± 7.2^{bD}	199.3 ± 4.0^{aE}	2191.0 ± 18.1^{bC}	7.5 ± 0.1^{abC}
Pectin 0.5 %	1867.7 ± 11.8^{cD}	311.7 ± 37.9^{bE}	2349.7 ± 22.7^{cC}	7.2 ± 0.1^{aC}
Pectin 0.25 %	1938.3 ± 37.1^{dD}	322.0 ± 21.1^{bE}	2351.3 ± 15.0^{cC}	7.2 ± 0.2^{aC}
HPMC 2%	1996.3 ± 8.6^{aC}	263.7 ± 45.5^{aD}	2419.0 ± 42.6^{aA}	7.9 ± 0.2^{bB}
HPMC 1.5 %	2024.0 ± 11.8^{abC}	283.3 ± 46.0^{abD}	2427.0 ± 39.5^{aA}	7.8 ± 0.2^{abB}
HPMC 1.0 %	1990.3 ± 8.4^{aC}	312.7 ± 10.0^{acD}	2368.7 ± 26.4^{aA}	7.8 ± 0.1^{abB}
HPMC 0.5 %	2021.3 ± 5.1^{aC}	360.7 ± 6.8^{bcD}	2384.3 ± 3.5^{aA}	7.6 ± 0.0^{abB}
HPMC 0.25 %	2060.3 ± 26.3^{bC}	387.7 ± 35.4^{cD}	2421.3 ± 35.1^{aA}	7.5 ± 0.1^{bB}
Xanthan 2%	2044 ± 4^{aC}	420.3 ± 49.6 ^{aC}	2279.0 ± 5.3 ^{aB}	6.7 ± 0.2 ^{aE}
Xanthan 1.5 %	1990.7 ± 18.6 ^{aC}	455.3 ± 23.7 ^{aC}	2278.7 ± 12.2 ^{aB}	6.5 ± 0.3 ^{aE}
Xanthan 1.0 %	1996.7 ± 45.2 ^{aC}	455 ± 11.3 ^{aC}	2342.0 ± 54.7 ^{aB}	6.3 ± 0.1 ^{aE}
Xanthan 0.5 %	2010.3 ± 40.8 ^{aC}	408.3 ± 46.7 ^{aC}	2373.0 ± 77.2 ^{aB}	6.3 ± 0.1 ^{aE}
Xanthan 0.25 %	1992.3 ± 28.3 ^{aC}	442 ± 15.4 ^{aC}	2320.0 ± 59.5 ^{aB}	6.5 ± 0.1 ^{aE}

Means in the same column for each individual hydrocolloid with different letters are significantly different (≥3 = One-way ANOVA; ≥2 0 =t-Test, $p < 0.05$). Results with different numbers are significantly different and grouped by two-way ANOVA. (A-F) type of hydrocolloid as main contributing factor; (G-K) concentration of applied hydrocolloid as main contributing factor.

Antagonistic forces restrict the pasting and gelatinization of starch granules, hence lowering the viscosity and delaying the pasting (Shi & BeMiller, 2002). In contrast to the other hydrocolloids, HPMC and Xanthan at different concentrations did not affect the potato starch formulations viscosity. Song, Kim, and Chang (2006), reported that xanthan gum reduced the peak viscosity in potato starch, but found an increased viscosity in wheat starch. In this study, potato starch was used in combination with various hydrocolloids. Hence, it is believed that different interactions in comparison to wheat starch will occur. The starches of different origin leach different types of amylose, which in turn cause stronger or weaker interactions with applied hydrocolloids (Shi & BeMiller, 2002). In addition, it can be assumed that the coating of the starch granules, observed in the SEM micrographs (Figure 4-1), restrict the swelling leading to a decreased or maintained viscosity.

The breakdown viscosity (BV), considered as an indicator for product stability to withstand heat and shear, also showed significant differences with the type of hydrocolloid as the main contributing factor (80.44%, $p < 0.05$). The significant highest BV was found in formulations containing locust bean gum, while formulations with sodium alginate had the lowest. The data also showed a trend, where higher values for BV of locust bean gum and guar gum were measured with increasing hydrocolloid concentration, while sodium alginate, pectin and HPMC recorded a decrease in BV. Repeatedly, different concentration of xanthan gum did not change BV. The final viscosity (FV) is where recrystallization of the starch occurs and hence can be considered as an indicator for staling of cereal products. The applied two-way ANOVA test on the pasting properties revealed that the final viscosity was mainly influenced by the type of hydrocolloid (52.41%, $p < 0.05$). Even though the contribution is not as high in comparison to the other parameters it can be seen that formulations with locust bean gum and HPMC showed the highest FV. The peak time (PT), which is the time to reach the peak viscosity, was delayed by the application and increasing concentration of sodium alginate, pectin and HPMC. Locust bean gum, xanthan gum and guar gum did not affect gelatinisation time. The main contributing factor affecting the peak time was also found to be the type of hydrocolloid applied (75.6%, $p < 0.05$). It is hypothesised that a higher peak time, hence a delayed peak viscosity leads to a longer development of

the bread structure before the setting occurs. In general, formulations including locust bean gum and guar gum showed the significant highest viscosity values followed by HPMC and xanthan gum. The lowest viscosity was found for sodium alginate and pectin. The effect of hydrocolloids on starch pastes and pasting behaviour has been intensively studied and been summarized in a literature review by Bemiller (2011). The literature review cites over 250 studies, which conducted work on this topic and indicates that there is no general rule, which applies when combining hydrocolloids with starches. Each combination of hydrocolloid and starch has different interactions.

Rheological studies

Dynamic oscillatory measurements have been described to be non-destructive tests that measure the elastic (*G'*) and viscous (*G''*) moduli by applying sinusoidal oscillating shear stress or strain over time, temperature, strain and frequency (Dobraszczyk & Morgenstern, 2003). Viscoelastic behaviour is an important characteristic of dough in order to facilitate gas/air cell expansion. Hydrocolloids have been reported to improve dough development and gas retention through an increase in viscosity, which in turn allowed the production of improved gluten-free breads (Capriles & Arêas, 2014). Figure 4-2 A and B display the effect of the chosen hydrocolloids at various concentrations on the viscoelastic properties of the bread dough (excluding yeast) over angular frequency. For all the doughs, it was observed that the increasing concentration of the hydrocolloid resulted in decreasing viscosity values. The major influencing factor for this is the higher amount of water added (Table 4-2) to the formulation. However, since the viscosity decrease was not proportional for all the hydrocolloids (e.g. xanthan gum), further factors such as the replacement of starch by hydrocolloids can have an influence on the lowered viscosity with increasing amounts of the hydrocolloids. Additionally, it is assumed that since the rheological measurements, different to the RVA measurements, which were conducted at low temperatures, the starches did not gelatinise dependent on the hydrocolloid and hence did not increase the viscosity. This effect is also described by Bemiller (2011) when preparing starch/hydrocolloid composite pastes or gels. Furthermore, a decrease in viscosity with higher frequency was observed, indicating a shear thinning effect. This shear thinning effect was also reported by other authors when hydrocolloids were added to a bread formulation (Demirkesen, Mert, Sumnu, &

Sahin, 2010; Gadallah, Mahmoud, Yousif, & Alawneh, 2016; Kim, Patel, & BeMiller, 2013; Sivaramakrishnan, Senge, & Chattopadhyay, 2004). The behaviour of shear thinning is caused by the alignment of microstructure with the flow direction (Song, Kim, & Chang, 2006). Demirkesen et al. (2010) stated, that the viscosity decreases, due to increasing shear, which leads to a break down molecular interaction.

The analysis of the damping factor is an indication of the visco-elastic behaviour. The dough formulations demonstrated more an elastic behaviour than viscous behaviour ($G'>G''$). Nevertheless, an increase in viscous behaviour was detected with increasing concentration of the hydrocolloids (except xanthan gum, Figure 4-2B). Repeatedly, this is mainly caused by the adjusted water content of the formulation. However, as also mentioned above further factors have to be taken into consideration. The exception of xanthan gum could be related to its higher molecular weight which is at least twice as high in comparison to pectin and sodium alginate. They showed the significant highest viscous behaviour values over the frequency of 8.73 1/s ($p < 0.05$). It is hypothesised that the starch granules are restrained from swelling and hence do not develop elastic but rather viscous networks. It was observed that the increasing concentration levels of guar gum and xanthan gum did not affect the viscosity curve significantly. Due to the higher molecular weight of guar gum, it is assumed, that the highest viscosity level was already reached with the lowest concentration, therefore no viscosity changes were observed when the hydrocolloid concentration was increased. Xanthan gum is believed to have no effect on the viscosity profile with increasing concentration, this can be explained by the capability to coat starch granules (Figure 4-1). Even the lowest concentration of xanthan gum seems to be sufficient enough to retard the starch granule swelling.

A higher molecular weight, the distribution and the spatial arrangement would be able to form more complex aggregates through hydrogen bonds and polymer entanglements and therefore affecting the viscosity of the dough (Sciarini, Ribotta, León, & Pérez, 2010).

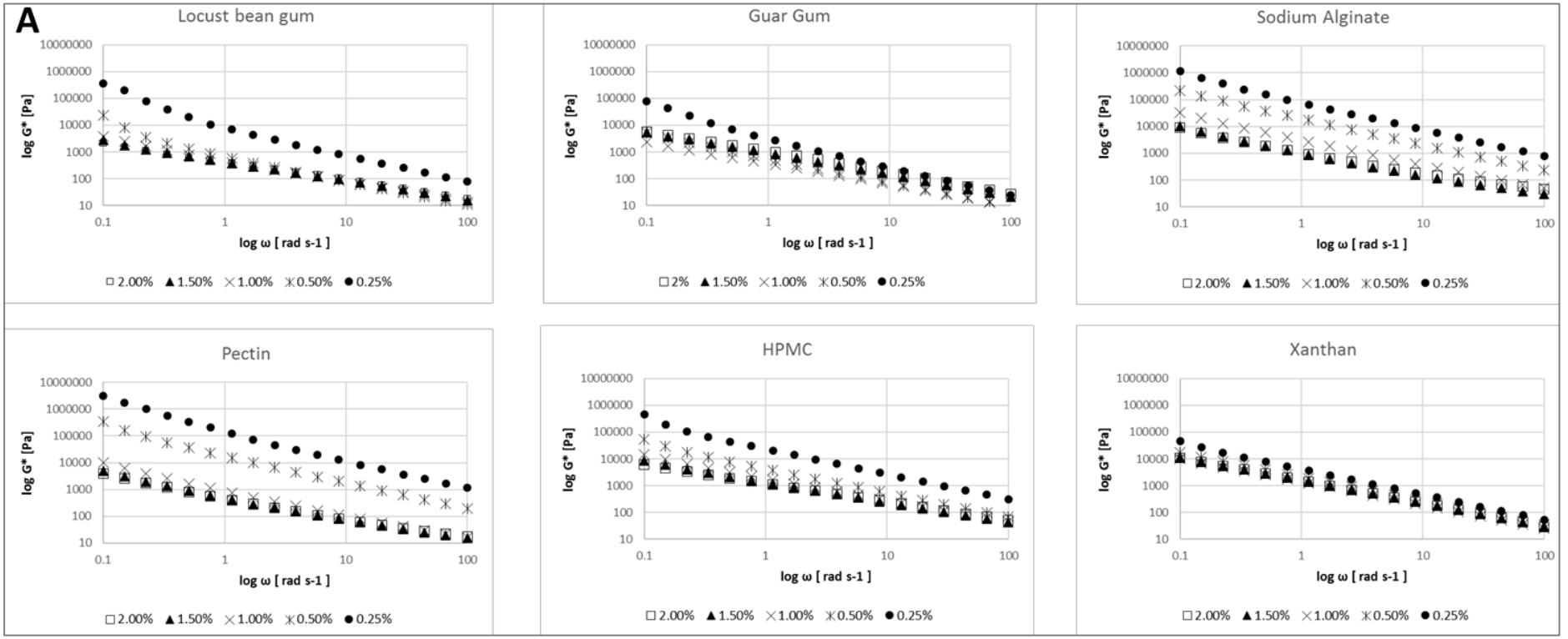


Figure 4-2 Oscillation measurements on doughs prepared with the various hydrocolloids at different concentrations. A: Complex modulus over frequency; B: tan ∂ (damping factor) over frequency.

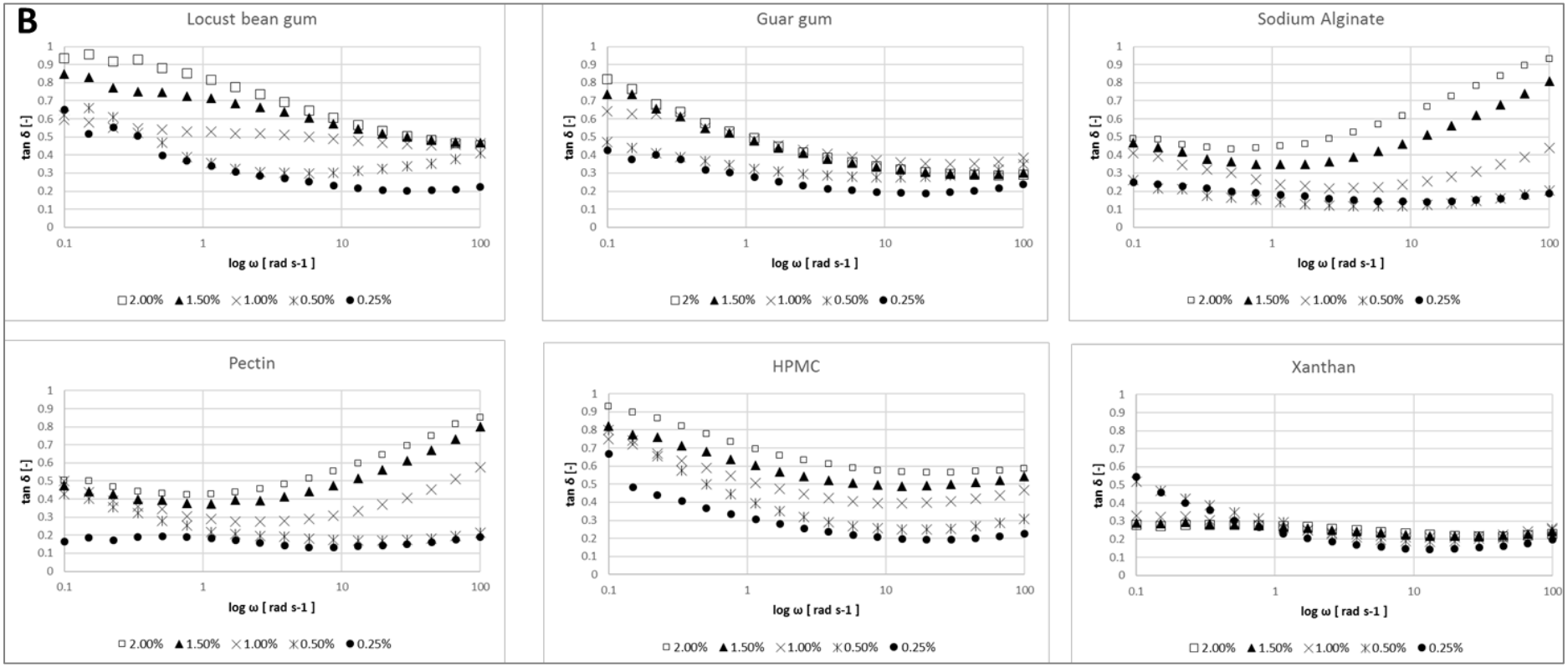


Figure 4-2 continued Oscillation measurements on doughs prepared with the various hydrocolloids at different concentrations. A: Complex modulus over frequency; B: tan ∂ (damping factor) over frequency.

Baking performance of hydrocolloid-containing formulations

Cross sections of the baked breads with the different hydrocolloids at various concentrations are depicted in Figure 4-3. The illustrated bread slices allow a quick and broad overview of the differences in volume and cell structure. Overall, it can be seen that all the formulations revealed bread-like products. This indicates that the calculation for the water adjustment was successfully applied as a prediction tool for hydrocolloids in this dough formulation. A more detailed description of the quality parameters is provided in Table 4-4. Despite the water adjustment, the bread quality parameters show significant differences. This was already expected after the significant differences in the pasting and rheological properties of the dough formulations were measured. The two-way ANOVA revealed the type of hydrocolloid as the main contributor to the results of the specific volume (65.5%, $p < 0.05$). It showed breads baked with sodium alginate reached the significant highest bread volume, while breads baked with locust bean gum reached the smallest volume. The one-way ANOVA in the individual hydrocolloid groups showed that an increasing concentration of hydrocolloid showed no significant effect on the specific volume of the formulations containing pectin, HPMC or xanthan gum. Whereas, locust bean gum, guar gum and sodium alginate showed significant differences in specific volume depending on the hydrocolloid concentration applied. It is worthwhile noting that an increased hydrocolloid concentration did not necessarily result in a higher bread volume. Guar gum and locust bean gum showed the opposite effect, reaching the highest loaf volume with the lowest concentration. Lazaridou, Duta, Papageorgiou, Belc, and Biliaderis (2007) showed that an increased concentration of xanthan gum, carboxyl methylcellulose, agarose and beta-glucan in gluten-free bread formulations based on rice flour, corn starch and sodium caseinate reduced the loaf volume. It is hypothesised that the effect as described by Lazaridou et al. (2007) is caused by the high molecular weights of the hydrocolloids applied.

Based on the results presented in Table 4-4 the lowest concentration of guar gum and locust bean gum reached the ideal level of hydration and hydrogen bonding with the potato starch and the leached amylose. An increase in any higher concentration seems to create too strong interactions, possibly due to the discussed insufficient effect of the

water addition. Especially the decreasing effect of higher xanthan gum concentration on bread volume has been reported before (Crockett, Ie, & Vodovotz, 2011; Hager & Arendt, 2013; Sabanis & Tzia, 2011; Sciarini, Ribotta, León, & Pérez, 2010). Based on the significant differences in bread volume it was assumed that the baking loss would be also significantly different, due to variations in the surface area of the different breads. However, the baking loss of the various formulations did not show any significant effect across the entire range (data not shown). Generated data only revealed relations between viscosity measured by the RVA and bread volume (r. -0.89, $p < 0.05$). A higher viscosity of the dough suppresses the gas cell expansion, hence leading to a smaller bread volume. The increasing concentration of hydrocolloids such as locust bean gum and guar gum increased the viscosity, while the increasing concentration of sodium alginate and pectin reduced it (Table 4-3). Additionally, it was found that doughs with a more viscous behaviour than elastic behaviour facilitated the gas cell expansion, leading to an increased specific volume. The differences in viscosity indicated some limitations of the applied method in relation to the analysis of the swelling properties of the various hydrocolloids and to use the generated data in the equation 1-2. The applied method does not take the effect of the hydrocolloids when heated into consideration. Generated data on this effect could give more information about the performance of hydrocolloids during the baking process. The factors; type of hydrocolloid (28.94%, $p < 0.05$), concentration (45.46%, $p < 0.05$) and interaction (19.89%, $p < 0.05$) were indicated to contribute to the hardness values. However, the concentration was used as the main affecting factor. The post-comparison with the Holm-Sidak test resulting in groupings was performed on this basis. The grouping revealed that concentration levels of 2% resulted in the softest breads while the 0.25% resulted in the significantly hardest breads. The authors assume that the higher amount of water added for the increased concentrations of hydrocolloid and the replacement of the starch by more hydrocolloids lead to this trend. This would lower interactions between starch and hydrocolloids, reducing the retrogradation and recrystallization (Funami et al., 2005). The significant lowest hardness was found in bread containing xanthan gum and the highest hardness values were found in bread containing locust bean gum.

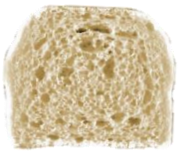
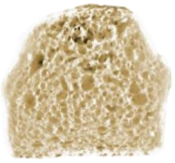
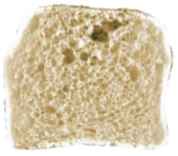
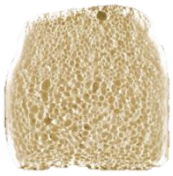
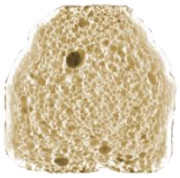
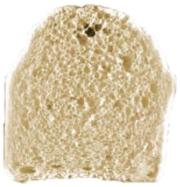
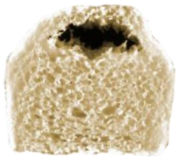
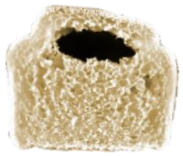
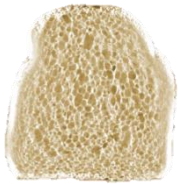
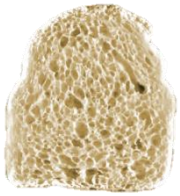
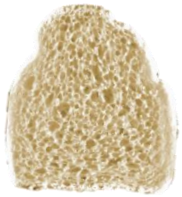
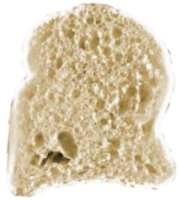
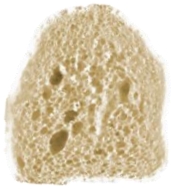
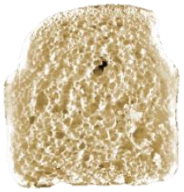
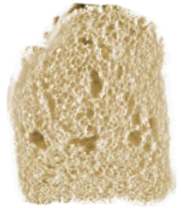
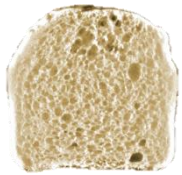
Sample / Concentration	0.25%	0.5%	1%	1.5%	2.0%
Guar gum					
HPMC					
Locust bean gum					

Figure 4-3 Cross sections of the baked breads with various hydrocolloids at different concentrations

Figure 4-3 continued

Sample / Concentration	0.25%	0.5%	1%	1.5%	2.0%
Pectin					
Sodium alginate					
Xanthan					

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The low hardness for xanthan gum breads is believed to be caused by the coating effect linked to its negative charge creating repelling forces and hindering the granules to swell and further retard the leaching of amylose. A reduced amount of leached amylose results in a reduced amount of retrograded amylose in the bread, which in turn leads to a softer crumb. Two-way ANOVA on the C-Cell parameters revealed low contribution levels for the type of hydrocolloid, the concentration and their interaction of the both, but high errors (data not shown). Hence it was not possible to draw clear conclusion on these parameters. The crumb structure parameters showed no significant differences for most of the hydrocolloids with increasing concentrations, except for locust bean gum. It showed a decrease in the number of cells with increasing concentration. This is assumed to be linked to the small loaf volume, leading to fewer cells than a higher bread volume.

In general, it is known that different hydrocolloids affect gluten-free formulations to a different extent, based on their chemical structure, the amount used and interactions with other ingredients but also by process conditions (Hager & Arendt, 2013; Houben, Höchstötter, & Becker, 2012). By applying a two-way ANOVA test to the set of data, the authors found as well that most of the parameters were influenced by the type of hydrocolloid used. Only for the hardness of the breadcrumb, the concentration of the various applied hydrocolloids was found to be the main contributing factor.

Table 4-4 Baking results of various hydrocolloid formulations

Baking properties	Specific Volume [g/L]	Hardness (baking day) [N]	Number of cells [-]	Number of cells/slice area [%]
Locust bean gum 2 %	2.7 ± 0.1^{aE}	11.33 ± 1.13^{aK}	2556.5 ± 121.0^{a}	0.53 ± 0.03^{a}
Locust bean gum 1.5 %	2.9 ± 0.1^{bE}	16.40 ± 0.82^{bI}	2829.1 ± 117.6^{ab}	0.56 ± 0.02^{a}
Locust bean gum 1.0 %	3.0 ± 0.1^{bcE}	15.33 ± 0.82^{bH}	2954.6 ± 171.4^{b}	0.57 ± 0.02^{a}
Locust bean gum 0.5 %	3.1 ± 0.0^{cE}	10.33 ± 0.68^{aJ}	2912.7 ± 89.6^{b}	0.53 ± 0.02^{a}
Locust bean gum 0.25 %	3.1 ± 0.0^{cE}	12.42 ± 0.93^{aG}	2988.1 ± 57.0^{b}	0.55 ± 0.01^{a}
Guar gum 2%	2.8 ± 0.0^{aDE}	5.40 ± 0.61^{aK}	2845.0 ± 92.8^{a}	0.58 ± 0.01^{b}
Guar gum 1.5 %	2.9 ± 0.0^{aDE}	9.74 ± 0.70^{bI}	2988.3 ± 95.7^{a}	0.59 ± 0.02^{b}
Guar gum 1.0 %	2.9 ± 0.0^{aDE}	13.32 ± 0.94^{dH}	2832.6 ± 158.3^{a}	0.55 ± 0.03^{ab}
Guar gum 0.5 %	3.2 ± 0.1^{bDE}	11.12 ± 0.69^{bcJ}	2962.1 ± 131.0^{a}	0.53 ± 0.03^{ab}
Guar gum 0.25 %	3.2 ± 0.1^{bDE}	12.70 ± 1.04 ^{cdG}	2916.7 ± 94.1^{a}	0.51 ± 0.02^{a}
Sodium alginate 2.0%	3.4 ± 0.1^{abA}	9.53 ± 0.61^{aK}	3021.7 ± 142.1^{a}	0.51 ± 0.02^{a}
Sodium alginate 1.5%	3.5 ± 0.1^{bA}	12.03 ± 0.67^{bcI}	3225.0 ± 248.6^{a}	0.52 ± 0.02^{a}
Sodium alginate 1.0%	3.6 ± 0.1^{bA}	12.95 ± 1.20^{bcH}	3078.5 ± 173.0^{a}	0.48 ± 0.02^{a}
Sodium alginate 0.5%	3.4 ± 0.0^{abA}	9.99 ± 0.76^{abJ}	2987.1 ± 253.9^{a}	0.48 ± 0.03^{a}
Sodium alginate 0.25%	3.3 ± 0.1^{aA}	14.50 ± 1.36^{cG}	3052.1 ± 178.38^{a}	0.52 ± 0.03^{a}
Pectin 2 %	3.4 ± 0.1^{aB}	7.22 ± 0.66^{aK}	3325.2 ± 543.47^{a}	0.54 ± 0.07^{a}
P**ectin** 1.5 %	3.3 ± 0.1^{aB}	9.92 ± 0.61^{abI}	2806.4 ± 107.51^{a}	0.48 ± 0.02^{a}
Pectin 1.0 %	3.4 ± 0.1^{aB}	11.76 ± 1.03^{bH}	2799.5 ± 109.82^{a}	0.48 ± 0.01^{a}
Pectin 0.5 %	3.4 ± 0.1^{aB}	10.76 ± 0.64^{bJ}	3080.7 ± 94.03^{a}	0.53 ± 0.02^{a}
Pectin 0.25 %	3.2 ± 0.1^{aB}	17.35 ± 1.96^{cG}	3036.3 ± 177.16^{a}	0.54 ± 0.02^{a}
HPMC 2%	3.1 ± 0.1^{aC}	8.39 ± 1.07^{aK}	2992.5 ± 190.76^{a}	0.55 ± 0.04^{a}
HPMC 1.5 %	3.3 ± 0.1^{aC}	11.57 ± 0.42^{bI}	2963.6 ± 102.70^{a}	0.53 ± 0.02^{a}
HPMC 1.0 %	3.2 ± 0.1^{aC}	14.94 ± 1.06^{cH}	2773.6 ± 112.16^{a}	0.50 ± 0.02^{a}
HPMC 0.5 %	3.2 ± 0.1^{aC}	10.31 ± 1.05^{abJ}	2760.3 ± 226.47^{a}	0.49 ± 0.03^{a}
HPMC 0.25 %	3.2 ± 0.1^{aC}	15.16 ± 1.67^{cG}	2758.4 ± 105.5^{a}	0.49 ± 0.03^{a}
Xanthan 2%	3.0 ± 0.1^{aD}	4.3 ± 0.43^{aK}	3039.4 ± 140.42^{a}	0.59 ± 0.03^{a}
Xanthan 1.5 %	3.0 ± 0.2^{aD}	6.58 ± 0.20^{bI}	3080.0 ± 128.87^{a}	0.58 ± 0.01^{a}
Xanthan 1.0 %	3.1 ± 0.1^{aD}	8.17 ± 0.57^{bH}	3052.7 ± 91.95^{a}	0.55 ± 0.01^{a}
Xanthan 0.5 %	3.1 ± 0.1^{aD}	7.97 ± 0.67^{bJ}	3081.2 ± 122.73^{a}	0.55 ± 0.02^{a}
Xanthan 0.25 %	3.1 ± 0.1^{aD}	11.43 ± 0.97^{cG}	3015.2 ± 141.53^{a}	0.55 ± 0.02^{a}

Means in the same column for each individual hydrocolloid with different letters are significantly different (≥3 = One-way ANOVA; ≥2 0 =t-Test, $p < 0.05$). Results with different numbers are significantly different and grouped by two-way ANOVA. (A-F) type of hydrocolloid as main contributing factor; (G-K) concentration of applied hydrocolloid as main contributing factor.

Conclusion

In this study, the application of hydrocolloids (guar gum, HPMC, locust bean gum, pectin, sodium alginate, xanthan gum) at different concentrations (0.25%, 0.5%, 1.0%, 1.5%, 2.0%) in a gluten-free bread formulation based on potato starch was analysed. To facilitate this, a tool was developed to add the optimal water amount to the formulation, based on different water absorption properties of the hydrocolloids. All the hydrocolloid formulations resulted in bread like products. However, even though the different WHC of the hydrocolloids were considered and the water was accordingly adjusted, the breads showed significant differences and revealed different optimal hydrocolloid concentrations.

In this study, the main influencing factor on bread quality was found to be the type of hydrocolloid used. This might be linked to the charge and the molecular weight of the various specific hydrocolloid. It is hypothesised, that sodium alginate and pectin due to their negative charge create repelling forces with the negatively charged phosphate groups of potato starch. These antagonistic forces have a negative impact on the granule swelling, lower the viscosity and therefore allow gas cell expansion which results in higher bread volumes. In contrast to this, hydrocolloids like guar gum and locust bean gum do not create such repelling forces. Based on their high molecular weight and their neutral charge, it is hypothesised that many hydrogen bonds with leached amylose were created leading to high viscosity values. These high viscosity values lower the elasticity hence allowing only limited gas cell expansion and ultimately lead to a smaller bread volume. This shows that the molecular weight had a stronger effect than the water. Hence, future research focusing on water absorption according to the molecular weight of the hydrocolloids is suggested. Also, the application of the prediction tool in a more complex system could give more insights of its applicability. The authors are confident to contribute to the knowledge in the gluten-free area, providing a new possibility to adjust the water content in a simple recipe containing hydrocolloids. In addition to this, the two-way ANOVA evaluation allowed to state that pectin was the significantly best performing hydrocolloid in improving the bread quality parameters. It reached its maximum potential at a concentration level of 2%.

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Chapter 5 Fundamental study on the impact of different *S. cerevisiae* yeast strains on gluten-free dough and bread quality parameters

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Abstract

Yeasts have been used for centuries for the leavening of bread. The main emphasis on the selection of yeast strains has been in relation to wheat products. This study is the first evaluation of different yeasts coming from the baking and brewing industry in a gluten-free system. Recent market studies revealed that gluten-free breads are still lacking in flavour and structure. Five different yeast strains (US-05, WB-06, T-58, S-23, Baker's yeast) of the species *Saccharomyces cerevisiae* were evaluated for their suitability to leaven gluten-free dough. A wide range of dough quality characteristics such as the time and temperature-dependent rising behaviour, the chemical composition of the dough and the pH were determined. In addition to this, bread quality attributes like, volume, texture, structure, aroma and flavour were evaluated. The results indicated different activity levels of the five yeast strains. Doughs prepared with US-05 showed a slower dough rise during proofing and a decreased height, in comparison to the Baker's yeast control. The application of WB-06 and T-58 however, resulted in a faster dough rise and increased dough height with greater gas cells. These observations were also found in the baked breads, where these two yeasts reached a higher specific volume and a softer breadcrumb than the Baker's yeast bread. In conclusion, significant differences both in the dough as well as in the bread characteristic were found. WB-06 and T-58 which originated from the brewing industry performed better than the traditional Baker's yeast in bread quality parameters, such as volume and hardness.

Introduction

The preparation of bread by yeast fermentation is one of the oldest biochemical processes in the world (Linko, Javanainen, & Linko, 1997). *Saccharomyces cerevisiae* (or Baker's yeast) is the commonly used yeastin bread products (Fleet, 2007). The fermentation plays a key role in the bread making process, as it can improve texture, structure, taste and flavour in the final product (Fleet, 2007). In recent years the effect of yeast modification and replacement by alternative yeast strains in the bread baking process has become a topic of interest. Studies focused on the harvesting time of Baker's yeast at different physiological phases (Rezaei et al., 2014) or the replacement of Baker's yeast by beer yeasts (Heitmann, Zannini, & Arendt, 2015). Beer yeast strains are known to have an optimized metabolism for beer making in terms of flavour compounds and alcohol production. On the other hand, Baker's yeast has a fast fermentation and uniform dough leavening due to carbon dioxide production (Amendola & Rees, 2003). Studies by Heitmann et al. (2015; 2017) demonstrated that the use of different *Saccharomyces cerevisiae* strains showed significant differences to the commonly applied Baker's yeast in wheat bread. It also was found that brewer's yeast can improve quality parameters like the texture, structure and the aroma profile of bread.

In a wheat bread, the gluten controls the gas cell expansion due to its network formation. However, in gluten-free bread products, this must be achieved by different ingredients such as hydrocolloids, which one of the major challenges for the gluten-free bread processing (Foschia, Horstmann, Arendt, & Zannini, 2016; Matos & Rosell, 2015). The demand for gluten-free bread products is based on the raising diagnosis of people who suffer from coeliac disease or other gluten-related disorders. For these individuals, a gluten-free diet is currently the only treatment for these disorders (Koehler, Wieser, & Konitzer, 2014). A recent study by Tsatsaragkou, Kara, Ritzoulis, Mandala, and Rosell (2017), stated that the gluten-free bread market still faces the main challenges of improving technological quality parameters bread technology quality, an extension of shelf life and a balanced nutritional value. The application of different yeast strains from the brewing and baking industry in gluten-free breads is a novel approach. It is believed that the different strains influence the final gluten-free bread properties due to different gas cell expansion and interactions.

Not only the influence on the dough and bread parameters but also aroma and flavour profile of breads can be influenced by the application of different yeasts and their individual fermentation process (Lai & Lin, 2006). Birch, Petersen, Arneborg, and Hansen (2013) identified a wide range of aroma active volatiles within the yeast metabolism. The change of this flavour and aroma profiles, using different yeasts has become a further topic of commercial interest. Since some of the aroma profiles are considered as quality parameters for bread products (Birch et al., 2013; Birch, Petersen, & Hansen, 2014; Pico, Bernal, & Gómez, 2015). Especially, the aroma and flavour profiles of gluten-free breads are still considered as improvable by the consumers. Hence, the modification of these profiles by the application of different yeasts could improve the perception and acceptance of gluten-free products. For this purpose, four different commercial beer yeasts (*Saccharomyces cerevisiae*) were compared to a commercial Baker's yeast (control) in relation to their effects on gluten-free model bread quality parameters. It comprehensively determines the effect of yeast on dough, bread texture, bread structure and the bread aroma and flavour profile in combination with descriptive sensory analysis using a trained panel. This study will broaden the understanding of the yeast on gluten-free dough characteristics, bread quality parameters and sensory attributes.

Experimental

Materials

Potato starch was supplied by Emsland, Germany; pea protein by Roquette, France; pectin by Cp Kelco, Germany; sugar by Siucra Nordzucker, Ireland; salt by Glacia British Salt Limited, UK. Instant active dry Baker's yeast was obtained from Puratos, Belgium; Dry yeast s-23, T-58, us-05 and wb-06 were supplied by Fermentis Division of S. I. Lesaffre, France. All the yeasts applied in this study belonged to the species *S. cerevisiae*. All chemicals were supplied by Sigma-Aldrich, Arklow, Ireland.

Compositional analysis

The total nitrogen content of the protein samples was determined according to the Kjeldahl method (MEBAK 1.5.2.1). To convert the nitrogen content into the protein content the factor of 6.25 was used. The air oven method (AACC Method 44-15A) was applied to determine the moisture content of the samples. The determination of the lipid content was performed according to the Soxlet-method (AACC Method 30-25.01) with a pre-digestion of the samples in HCl, to release bound lipids.

Cell count

Cell viability (cfu/g) of the yeast powders, was analysed by suspending 1 g freeze-dried yeast in 10 mL distilled water. From this stock solution, serial dilutions were prepared with ringer solution and spread on malt extract agar (Merck, Germany) plates and incubated aerobically for 2 days at 25°C. Plates with 30 to 300 colonies were selected for yeast cell counts.

Total available carbohydrates

The total available carbohydrate level from freeze-dried dough and breadcrumb samples was determined spectrophotometrically by using an enzyme kit (K-TSTA) supplied by Megazyme, Ireland.

Sugars and Acids

Sugar levels of dough and breadcrumb were analysed for glucose and fructose by an Agilent 1260 high-performance liquid chromatography system (HPLC) with a Hi-Plex H+ column (Agilent, Cork, Ireland) coupled to a refractive index detector (RID) at 35 °C. The sugars were extracted with distilled water for 20 min under shaking and then centrifuged at 3000g for 10 minutes. The HPLC analysis was performed at 30 °C column temperature with water (HPLC-grade) at a flow rate of 0.6 mL/min. The analysis of citric acid, succinic acid and acetic acid were analysed with the same system but small modifications. A Diode-Array Detection (DAD) and the HiPlex H+ Column at 65 °C were used to detect the acids. Samples were eluted with 0.005 M H_2O at a flow rate of 0.5 mL/min.

Dough and bread crumb pH measurement

Dough pH before and after proofing was measured according to the AACC method 02-52.

Time- and temperature-dependent rising behaviour of dough

The measurements were conducted using an Anton Paar MCR rheometer with the TruStrain™ option. A confined measuring system (CMS) was placed on the inset plate (I-PP25) of a plate-plate system (Figure 5-1). The CMS is a stainless-steel cylinder with the height of 33 mm and the inner diameter of 25 mm. A Peltier temperature device (PTD) was used as well as a convection temperature device (CTD) for temperature control (Figure 5-1). To mimic the proofing properties the PTD was set at 30 °C for 45 min with a constant normal force (FN) was set to 0.0 to ensure permanent contact between sample and upper plate. For determination of the oven spring and the determination of yeast activity during the baking process the temperature was increased to 90 °C with a heat rate of 4°C /min. Recorded and calculated parameters were the max height [mm], which is the maximum height the dough reached during the measurement. Further the slope during the fermentation process (Slope FP) and then during the baking process (Slope BP) for determination of yeast activity was calculated. Also, the max height temperature (TMH) [°C] was recorded and used as an indicator for the heat tolerance of the various yeasts.

Bread production

Bread samples were prepared according to S. Horstmann, Foschia, and Arendt (2017). The formulation of the various breads included: 2% pectin, 2% pea protein, 2% salt, 4% sugar, 75% water based on starch weight. Amounts of yeasts were added according to their cell viability (Table 5-2). Dry ingredients where mixed and yeast was suspended in warm water (27 °C) and regenerated for a period of 10 min. Mixing was carried out with a k-beater (Kenwood, Havant, UK) at low disk speed (level 1 of 6) for 1 minute in a Kenwood Major Titanium kmm 020 Mixer (Kenwood, Havant, UK). After the first mixing, the dough was scraped down from the bowl walls. A second mixing step of 2 minutes at higher disk speed (level 2 of 6) was applied. 300g of batter were weighed into baking tins of 16,5 cm x 11 cm x 7 cm and placed in a proofer (KOMA, Netherlands)

for 45 min at 30 °C and 85% relatively humidity (RH). The proofed samples were then baked for 45 min at 220 °C top and bottom heat in a deck oven (MIWE, Germany), previously steamed with 0.4 L of water. The breads were cooled for 2 hours prior to analysis.

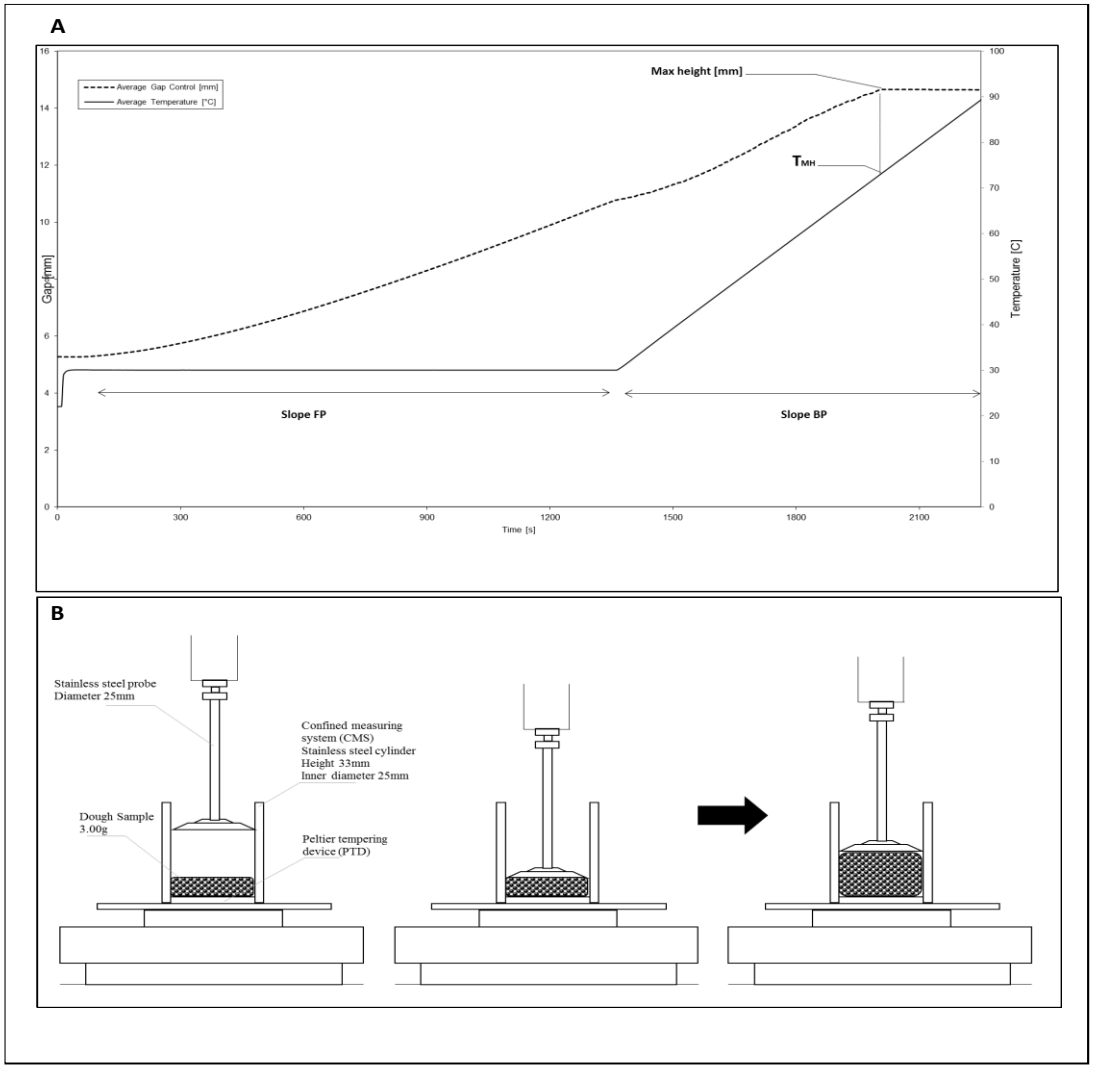

Figure 5-1 A: Example diagram for Time- and temperature-dependent rising behaviour of dough. B: Flow chart of methodology

Bread analysis

The specific volume of the bread was determined by use of a Vol-scan apparatus (Stable Micro System, UK). The specific volume is calculated on the basis of loaf volume and weight. An image analysis system (Calibre Control International Ltd., UK) was used to analyse the breadcrumb structure chosen parameters were the cell diameter and the number of cells per slice area. Crumb firmness was analysed using a Texture Profile Analyser (TA-XT2i, Stable Micro Systems, Godalming, England) with a 25 kg load cell, which compresses the breadcrumb with a 20 mm aluminium cylindrical probe. Bread samples were sliced into 20 mm slices and analysed with a test speed of 5 mm/s and a trigger force of 20 g, compressing the middle of the breadcrumb to 10 mm. The measurement with the various parameters was conducted on the baking day and 24h after baking to monitor the staling process. Baked breads were stored in polythene bags (polystyrol-ethylene veniyl alcohol-polyethylene).

Extraction of Volatile Aroma Compounds by Thermal Desorption (TD) and Quantification

VOC analysis was performed by Scientific Analysis Laboratories Ltd. To extract volatile aroma compounds, samples were prepared by weighing 0.1g of bread crumb into a clean glass thermal desorption (TD) tube to concentrate the volatile aroma compounds in a gas stream prior to injection (Perkin Elmer Turbomatrix 650). Subsequently, the aroma compounds were absorbed at 90°C for 10 min. For the quantification of the aroma-active volatiles, a gas chromatography-mass spectrometer (GC-MS, Agilent 5977B MSD) with a Rxi 624-Sil 20m column and helium as a carrier gas was used. The details for the temperature profile are: start temperature: 35°C (4 min) with an increase of 15°C/min to 220°C (hold 1 minute). The total run time was 17.3 min. For the detected compounds a database search was conducted. The aroma compounds detected and analysed in this study by GC-MS TD were ethanol and acetic acid.

Sensory Analysis

Aroma profile analysis on bread samples was performed by a trained panel consisting of 10 panellists. Training began by generating a consensus vocabulary for attributes and descriptors based on the control sample. The sensory evaluation was performed by each panellist individually in an isolated booth. All trainings and sensory analyses were performed in a sensory panel room at 21 +/- 1°C. Agreed descriptors were (Table 5-1). For the descriptive aroma profile, each breadcrumb sample was cut into slices (thickness 2cm) and presented to panellists 90 minutes after baking. The sensory scale was based on an unstructured line scale to describe the intensity of rated sensory attributes.

Table 5-1 Sensory descriptors

Smell (Odour)	Description
Whey	Aroma typical of Whey powder
Eggy	Aromatic characteristics of boiled eggs (sulphuric)
Nutty	Aromatic characteristics of mixed nuts, e.g. walnuts, hazelnuts, brazil nuts and pine nuts
Green (pungent)	Aroma typical of cut grass
Cereal (bread)	Aroma typical of cereals (oats, rye, barley, wheat) mixed with boiling water 1:3
Intensity	Perceived first impression of odour intensity of breadcrumb
Taste (Flavour)	
Salty	Degree of perceived salty taste, as a basic taste
Acidic / Sour	Degree of sourness taste
Yeasty	Flavour associated with natural yeast as a leavening agent
Green (pungent)	Itchy trigeminal sensation on the tip of the tongue
Aftertaste	Flavour of crumb staying after tasting
Intensity	Intensity of overall flavour in crumb

Statistical analysis

All measurements were performed at least in triplicate. The significance of the results was analysed using One Way ANOVA (R version 3.0.1). The level of significance was determined at $p. < 0.05$. In addition, Pearson correlation analysis (R version 3.0.1) was applied to find correlation between yeast properties and the results of the baked products.

Results and Discussion

To the authors knowledge, this study is the first study to apply different yeast strains, which are commonly used in the brewing industry in a gluten-free bread system. During the fermentation process yeast produces mainly carbon dioxide and ethanol, but also secondary metabolites, such as glycerol, organic acids and flavour compounds, which have an impact on the final product quality (Randez-Gil, Sanz, & Prieto, 1999). The effects of yeast on bread quality characteristics include the volume, structure, flavour and shelf life of each fermented product (Fleet, 2007). Based on the specific characteristics of the *S. Cerevisiae* yeast strains showed, the authors hypothesise that their application will have significant influence on final gluten-free bread quality (Table 5-2). The main differences between the yeast strains are their optimum temperatures and their different tolerances to temperature changes. The optimal temperature for Baker's yeast is higher in comparison to that of beer yeasts.

A further important characterising of yeasts is the metabolism of different sugars of the various yeasts. Especially in a very refined system such as that of a gluten-free formulation, sugar sources are limited and usually constructed of mainly complex sugars. These sugars are usually only accessible to yeast fermentation when degraded by enzymes to smaller fermentable sugars. The gluten-free system in this study also consists of limited amounts of sugar. It further does not contain added enzymes for the breakdown of the complex sugars to provide smaller sugars for yeast fermentation. The main component in the system is potato starch, which consists of about 92% total starch, 1% damaged starch, 0.02% protein and no lipids. Additionally, no enzyme activity (α- and β-amylase) was determined in this potato starch. This gluten-free bread system is refined and does not offer as many nutrients for yeast metabolism as the conventional wheat bread system. However, effects on the gluten-free bread quality parameters after the application of the various yeasts was expected. Therefore, five yeast strains of the *S. cerevisiae* family namely US-05, T-58, S-23, WB-06 and a control Baker's yeast were selected and their effect on dough and final bread quality was analysed.

Table 5-2 Properties of the different yeast strains

S. cerevisiae	Application[1]	Temperature optimum [C][1]	Fermentation time[1]	Activity [cfu/g][2]	Dosage [%][2]	Sugar metabolism[1]			
						MalT	Mal	Glu	Dextr
Baker's yeast	Baked goods	25-30	Hours	$1.06*10^9$	2	++	+	+	+
S-23	Lager	12-15 (27 faster) lower temperature tolerance	Up to 14 days	$5.18*10^8$	4.1	++	+++	+++	+
T-58	Ale	15-20 (32 faster) High temperature tolerance	2-3 days	$5.5*10^8$	3.86	++	++	++	+++
US-05	Ale	15-22 high temperature tolerance	2-3 days	$4.47*10^8$	4.48	+++	+	+++	++
WB-06	Wheat Beer	18-24	2-3 days	$7.16*10^8$	2.97	+	++	++	++

[1]Adapted from Heitmann et al., (Heitmann, Axel, Zannini, & Arendt, 2017) with modifications

[2]From yeast activity measurement

MalT: Maltosetriose; Mal: Maltose; Glu: Glucose; Dextr: Dextrins

+++ high; ++ moderate; + low

Cell Count

The viability of freeze-dried yeast cells was analysed to standardise the inoculum level of yeast for the baking of the various breads. The control yeast *S. cerevisiae* Baker's yeast had a cell count of 1.06E + 09 cfu/g. The beer yeasts showed lower cell count in decreasing order: *S. cerevisiae* WB-06 7.16E +08 cfu/g; *S. cerevisiae* T-58 5.5E +08 cfu/g; *S. cerevisiae* S-23 5.18E +08 cfu/g and *S. cerevisiae* US-05 4.74E +08 cfu/g. Comparable results were found by Heitmann et al. (2015). The addition levels of the yeast in the dough formulation were based on the concentration usually reached by the control yeast (*S. cerevisiae* Baker's yeast) (Table 5-2). When dried yeasts are used in bread the non-viable cells need to be considered, since non-viable cells can release glutathione as a stress response (Penninckx, 2002; Reed, 2012; Verheyen et al., 2015). In wheat doughs, the release of glutathione has a strong reducing effect which ultimately leads to a modification of the viscoelastic gluten network (Delcour & Hoseney, 2010; Verheyen et al., 2015). Glutathione was further applied in a gluten-free formulation and found to improve rice-flour based bread quality parameters (Yano, 2010). In the used formulation interactions between glutathione and the rice protein 'glutelin' resulted in an improvement of the volume and crumb structure of the bread. However, based on the lack of gluten, rice flour and glutelin in the used formulation in this study, the effect of glutathione on bread parameters was neglected.

Total starch

The total starch content of the doughs and breads was analysed to identify difference in the yeast performance. No significant differences between the total starch contents in the dough were found (Table 5-3). However, differences in the starch content of the final breads were detected. This indicates different activities of the various yeast strains during processing. Breads baked with the *S. cerevisiae* strains T-58 (75.97%) and S-23 (78.57%) showed the significant lowest amount of total starch. The control baked with *S. cerevisiae* Baker's yeast had the significant highest amount of total starch left (87.27%), suggesting a lower activity. Heitmann et al. (2015) analysed the application of beer yeast strains in wheat bread and also found Baker's yeast to have the highest amount of starch left in the final bread. The authors mentioned that the lower content of total starch in

the breads prepared with beer yeast resulted from their higher enzyme activities in comparison to Baker's yeast, which degrade starch into more fermentable sugars (White & Zainasheff, 2010). The values in the study by Heitmann et al. (2015) showed lower total starch values, which is explained by the higher concentration of starch in this study as explained earlier.

Sugars and Acids

The analysis of fermentable sugars like glucose and fructose showed fluctuation and significant differences amongst the different yeasts (Table 5-3). All the yeasts showed a decrease in glucose and fructose after baking, confirming that all the yeast strains have metabolic activity. The sugar contents in the final bread of fructose and glucose showed the lowest values in the formulations with the addition of T-58, suggesting a higher activity in comparison to the other yeasts. This functionality is hypothesised by the authors to be the result of the higher temperature tolerance and a fast fermentation at higher temperatures in comparison to the other yeast strains (Table 5-2). It is well known that yeast activity can be influenced by many factors such as the pre-growth conditions of yeast, dough fermentation conditions, dough ingredients and the genetic background of the various yeast strains (Struyf et al., 2017).

The acid analysis of the dough and bread samples formulated with the different yeasts did not find detectable quantities. Only quantities of acetic acid were found in bread samples as part of volatile compound analysis (Table 5-5). The detection of acetic acid during the volatile compound analysis is explained by the different detection limits of the two used detection methods. GC-MS used for the volatile compound analysis can detect compounds in ppm quantities while the detection limit of the HPLC is significantly higher. Acetic acid values measured by the GC were observed to be four times higher in bread crumbs baked with *S. cerevisiae* S-23 in comparison to the other yeasts. The lowest value was found in breadcrumbs of breads baked with US-05, which overall showed low amounts of volatile compounds. Acetic acid contributes to the overall aroma of baked goods (Frasse, Lambert, Richard-Molard, & Chiron, 1993). Its organoleptic descriptors are 'vinegar', 'pungent' and 'sour', hence the differences in the amounts of acetic acid are assumed to influence the sensory evaluation. The small quantities measured, however, are not considered to affect the dough and bread

properties or to contribute to the flavour or aroma profile. Based on the refined gluten-free system in this study, in addition to the limited amount of oxygen in a dough system, the acid analysis suggests that the metabolic pathways of the various yeasts followed the alcoholic fermentation, rather than the TCA cycle (Heitmann, Zannini, & Arendt, 2017). As discussed earlier, the refined system was considered to not provide enough nutrients for the yeast to synthesise metabolites like acids.

pH values

Changes in pH of the dough before and after proofing and in the final bread are shown in Table 5-3. The various *S. cerevisiae* yeast strains showed significant differences in the pH development over the breadmaking process. Overall it was observed that the doughs decreased in pH during fermentation and increased after baking. US-05 and S-23 had the significant highest pH before proofing. Doughs formulated with *S. cerevisiae* T-58 showed the significant lowest pH. Also, after proofing T-58 showed the lowest and US-05 the highest pH. The effect of acids on pH in this study was excluded since they were not detected. Thus, the effect of CO_2 production is assumed to be the main cause for the changes in pH (Verheyen, Jekle, & Becker, 2014). After the baking process, an increase in the pH values in all the baked breads was observed. Even though the pH increased, the significant lowest pH was found for breads formulated with T-58. The significant highest pH value was reached by breads containing the yeast strain WB-06 followed by US-05. The effect of the pH increase after baking is explained by the loss of carbon dioxide and linked carbonic acid. Reduction in pH indicates CO_2 and ethanol production by the yeasts. The more active the yeasts the more sugars are fermented, and the more CO_2 is produced, dropping the pH in the dough (Sluimer, 2005).

Time- and temperature- dependent rising behaviour of dough

The evaluation of dough rising behaviour is a commonly determined parameter in wheat-doughs, to achieve constant dough quality. The measurement is usually conducted with the aid of the rheofermentometer. This machine, however, showed limitations in analysing gluten-free batters due to their liquid nature. Therefore, a new method was established using the Anton paar® rheometer attached with the

TruStrain™ system, allowing the determination of the dough rise and providing a prediction tool for yeast activity (Figure 5-1). Analysed parameters were the max height, the slope during the fermentation process (Slope FP), the baking process (Slope BP) and max height temperature (TMH) (Table 5-3). It was found that doughs formulated with *S. cerevisiae* T-58 had the highest dough rise in comparison to the other strains. The lowest dough rise was observed for US-05. The temperature at which the maximum height was reached indicates that the control yeast reached its maximum height significantly earlier than the remaining yeasts. The yeast strains S-23 and WB-06 reached their maximum height at significantly higher temperatures. The different temperatures to reach the max height are not correlated but can be explained by the different activities of the yeast strains and their preferred temperatures (Table 5-2) (Cauvain & Young, 2016). The slope during the fermentation phase (FP) at 30°C presented T-58 as the most active yeast with a slope twice as high as the control, which is the second most active strain. The authors hypothesise that this high activity is the result of the temperature optimum for fast fermentation (32 °C) and the metabolism of different sugars but mainly the preference of dextrin's (Table 5-2). The explanation why S-23 and WB-06 reached a higher height than the control is due to their increased in activity at higher temperatures (Slope BP 30-90 °C). This high increase would suggest a more pronounced oven spring as usually observed during the baking process.

Overall the method showed that it is comparable to the rheofermentometer since similar results were found by Heitmann et al. (2015), who applied beer yeast strains to wheat breads. In their study, it was also observed that T-58 had the highest activity and US-05 the lowest which was explained by a slower fermentation of sugars. The obtained results of the various yeast strains show the suitability of the method for gluten-free doughs. It is further hypothesised that it can be used as an indicator for the final bread properties.

Table 5-3 Chemical and functional properties of the bread doughs containing the different yeast strains

		US-05	WB-06	T-58	S-23	Baker's Yeast
Total starch	Dough [g/100g]	84.778 +/- 5.377[a]	81.535 +/- 4.687[a]	82.710 +/- 5.628[a]	84.128 +/- 8.658[a]	77.998 +/- 1.675[a]
	Bread [g/100g]	82.087 +/- 4.237[ab]	81.496+/- 4.138 [ab]	75.9722 +/- 1.674[b]	78.571 +/- 2.244[b]	87.268 +/- 0.872[a]
Sugars	**Glucose**					
	Dough [g/100g]	2.298 +/- 0.602[a]	2.696 +/- 0.175[a]	1.944 +/- 0.540[a]	2.299 +/- 0.040[a]	1.847 +/- 0.137[a]
	Bread [g/100g]	2.229 +/- 0.450[a]	1.240 +/- 0.054[b]	0.365 +/- 0.065[c]	1.208 +/- 0.087[b]	1.167 +/- 0.021[b]
	Fructose					
	Dough [g/100g]	2.025 +/- 0.238[a]	2.248 +/- 0.119[a]	2.021 +/- 0.025[a]	2.021 +/- 0.025[a]	2.243 +/- 0.095[a]
	Bread [g/100g]	2.303 +/- 0.410[a]	1.539 +/- 0.535[ab]	1.119 +/- 0.046[b]	1.550 +/- 0.087[ab]	1.608 +/- 0.030[ab]
pH	Dough [-]	5.12 +/- 0.04[a]	4.96 +/- 0.01 [b]	4.77 +/- 0.04 [c]	5.14 +/- 0.01 [a]	4.98 +/- 0.03 [b]
	Proofed Dough [-]	4.88 +/- 0.04 [a]	4.84 +/- 0.01[ab]	4.54 +/- 0.01 [c]	4.85 +/- 0.10 [ab]	4.72 +/- 0.00 [b]
	Bread [-]	5.26 +/- 0.02[ab]	5.29 +/- 0.02[c]	5.05 +/- 0.03[c]	5.20 +/- 0.03[b]	5.20 +/- 0.04[b]
Dough Rise	SlopeFP [mm/min]	0.04	0.09	0.27	0.10	0.13
	SlopeBP [mm/min]	0.30	0.53	0.43	0.53	0.39
	MaxH [mm]	10.09 ± 0.04[d]	16.01 ± 0.59[b]	21.78 ± 0.29[a]	17.13 ± 0.21[b]	14.65 ± 0.93[c]
	T_{MH} [°C]	82.01 ±0.02[c]	89.92 ±0.01[a]	83.10 ± 0.04[b]	89.91 ± 0.01[a]	74.96 ± 0.03[d]

Means in the same row with different letters are significantly different (≥3 = One-way ANOVA; ≥2 0 =t-Test, $p < 0.05$).

Bread results

One of the most important quality parameters and the first impression for the consumer is the appearance of a product. Figure 5-2 illustrates cross sections and surface images of the baked breads with the different yeasts. It can be observed, that breads baked with the ale yeast US-05 showed reduced loaf volume and smaller average cell pore size. Bread baked with WB-06 and S-23 showed a closer resemblance to the control bread in terms of size and cell pore size. The effect of T-58, however, showed a bigger loaf volume and big gas cells in comparison to the control bread (Baker's yeast). A more detailed description of the quality parameters is presented in Table 5-4. The images of the breads containing the different yeasts depicted in Figure 5-2 indicate significant differences between the bread. The specific volume and its related appearance are the most important bread quality parameter which has a high influence on the consumer's quality perception (Hager, Wolter, Czerny, et al., 2012). The differences of the specific volume are significant and show the breads baked with T-58 showed the highest loaf volume (Table 5-4). The other applied yeasts either had no significant differences (WB-06) or resulted in inferior bread characteristics (S-23, US-05) particularly relating to the volume of the breads. Next, to the influence of the yeast, a key role for the rise of a bread is the dough consistency. After mixing and heating, the dough can facilitate the entrapment of produced gas and the expansion of the gas cells (Morreale, Garzón, & Rosell, 2017).

The cell structure of bread is a key quality criterion which can be related to crumb hardness and the specific volume. The development of crumb structure and gas cells expansion initially starts during fermentation, when CO_2 and ethanol are produced as products of the yeast metabolism. In the baking process then the produced ethanol evaporates with some of the water and helps the expansion of gas cells and ultimately the loaf rise (Verheyen et al., 2014). Cell structure of bread is a key quality criterion which can be related to crumb hardness and the specific volume. cells, the cell diameter and the number of cells over the bread slice area. The application of the ale yeast US-05 was the only yeast which increased the number of cells significantly in comparison to the baker's yeast (control). The addition of the remaining yeast led to breads with a lower number of cells when compared to the control. The combination of the number

of cells and their development of crumb structure and gas cells expansion initially starts during fermentation, when CO_2 and ethanol are produced as products of the yeast metabolism. In the baking process then the produced ethanol evaporates with some of the water and helps the expansion of gas cells and ultimately the loaf rise (Verheyen et al., 2014). Parameters chosen for the crumb structure were the number of diameter determines the volume of a bread loaf. This explains the results of breads baked with US-05, which despite their high number of cells, but because of their small crumb cell diameter led to small loaf volume. The opposite effect was found in breads containing T-58. The breads showed the lowest number of cells; however, these cells showed the significant highest cell diameter resulting in breads with the significant highest specific volume (Table 5-4). The number of cells / slice area (mm^2) gives the ratio of cells per mm^2 on the bread. Breads baked with US-05, S-23 showed the highest ratio in comparison to the control. No significant differences were found between WB-05 and the control. The significant lowest value was found in breads baked with T-58. Texture is a further important quality characteristic for consumer acceptance (Cauvain & Young, 2016).

The process of increasing hardness over time is known as staling and to affect the texture and flavour of a bread (Gray & Bemiller, 2003). Hardness of the breadcrumb was chosen to determine textural parameters. The hardness was measured 2h and 24h after baking. Both measurements of hardness showed significant differences between the bread samples baked with the various yeast strains. Further observations showed that all bread samples increased in hardness. Measurements conducted after 2h of baking showed that breads baked with S-23, WB-06 and T-58 had a significant softer breadcrumb texture in comparison to Baker's yeast. T-58 however showed the significant lowest hardness in comparison to all applied yeast strains. Bread baked with the yeast strain US-05 showed the significant highest hardness. Similar observations were made by Heitmann et al. (2015), who also showed that wheat breads formulated with the yeast strain US-05 had the highest hardness after baking. A similar order of hardness of the different breads baked with the various yeast strains was observed after 24h. Breads baked with US-05 resulted in the significant highest hardness. The applied yeast S-23 and T-58 showed the significant lowest hardness in comparison to the other yeasts, with T-58 having still the significant softest breadcrumb. The application of WB-

06 resulted in breads which showed similar bread properties to the control Baker's yeast, indicating a faster staling process. The differences of the varies breads in crumb hardness are hypothesised to be caused by the crumb structure. The hardness of breadcrumb is measured by compression over a certain area (probe diameter 20mm). Due to the significant difference in cell diameter, different areas of cell walls are compressed. Hence, it is suggested that breads with high cell diameter provide less cell walls for the measuring probe to compress resulting in less resistance and a lower measurement of hardness.

Figure 5-2 Images of cross section and surface of breads baked with the various yeast strains

Table 5-4 Results of bread parameters baked with the different yeast strains

Yeast strain	US-05	S-23	WB-06	T-58	Baker's Yeast
Specific Volume [ml/g]	1.96 ± 0.05^{d}	2.18 ± 0.12^{c}	2.50 ± 0.08^{b}	3.43 ± 0.28^{a}	2.42 ± 0.11^{b}
Bake Loss [g/100g]	15.36 ± 0.25^{c}	16.61 ± 0.28^{b}	17.34 ± 0.79^{b}	19.36 ± 1.18^{a}	16.88 ± 0.38^{b}
Hardness (0h) [N]	8.26 ± 1.26^{a}	4.10 ± 1.18^{c}	3.86 ± 0.50^{c}	2.19 ± 0.46^{d}	5.82 ± 0.92^{b}
Hardness (24h) [N]	29.91 ± 3.64^{a}	14.62 ± 1.82^{c}	16.67 ± 1.82^{b}	6.33 ± 1.17^{d}	16.75 ± 2.00^{b}
Number of Cells [-]	3192.1 ± 205.2^{a}	2517.056 ± 71.7^{c}	2430.889 ± 195.0^{c}	2297.529 ± 226.6^{d}	2534.278 ± 124.7^{b}
Cell Diameter [mm]	1.43 ± 0.10^{d}	2.00 ± 0.21^{c}	2.43 ± 0.23^{b}	3.69 ± 0.22^{a}	2.54 ± 0.22^{b}
Number of Cells/ Slice Area [mm^2]	0.805 ± 0.063^{d}	0.560± 0.049^{c}	0.490 ± 0.039^{b}	0.377 ± 0.026^{a}	0.508 ± 0.031^{b}

Means in the same row with different letters are significantly different (≥3 = One-way ANOVA; ≥2 0 =t-Test, $p < 0.05$).

n.d. = not detected

Volatile Aroma Compounds Analysis

The identification of the aroma compounds revealed ethanol to be the only component present in all the breadcrumb samples (Table 5-5). Ethanol, which is the most produced volatile compound during bread fermentation, was also found in this study to be the main compound. The *S. cerevisiae* yeast strain T-58 was found to have produced almost twice as much ethanol in comparison to the other yeast strains. The high activity of T-58 was also earlier discussed during the dough-rise measurement and the lower pH in the final bread. Overall it is suggested that it is due to its tolerance to high temperature (Heitmann et al., 2015). Further detected aroma compounds in some of the bread samples were 2,3-butanediol and 1-hydroxy-2-propanone. 2,3- butanediol is a metabolite of alcoholic fermentation, which was found in breads fermented with the yeast strains S-23 and T-58. The metabolic pathway for the production of 2,3-butanediol by yeast was reported to be the oxidative decarboxylation and enzymatically reduction of 2-acetolactat (Wainwright, 1973). The production of 2,3-butandiol has been related to increased ethanol production (Heitmann, Zannini, Axel, & Arendt, 2017). However, in this study this effect could not be confirmed. The aroma compounds 1-hydroxy-2-propanone was found in breads baked with S-23. This compound is a product of Maillard reaction and created by the reaction between reducing sugars and amino acids, mainly proline (Tressl, Helak, Kersten, & Rewicki, 1993). Its presence of low amounts was explained by the pea protein present in the used gluten-free system.

The compound analysis, based on its low results suggests that the metabolic pathways of the various yeasts followed the alcoholic fermentation, rather than the TCA cycle. To produce significant amounts of aroma compounds, conditions like amino acid composition, glucose supply and oxygen must be provided (Otterstedt et al., 2004). The refined system in this study based on pure potato starch, lacks on nutrients for the yeast growth and the connected metabolite production. Due to the lack of alpha-amylase activity of potato starch (Horstmann, Belz, Heitmann, Zannini, & Arendt, 2016), no glucose can be generated by degrading the starch. A low content of damaged starch, due to the extraction process of potato starch further prevents the generation of glucose (Horstmann, Lynch, & Arendt, 2017). Only the addition of sucrose in the recipe

provides a limited amount of glucose after degradation, as seen in Table 5-2. Hence the main reason for the switch to alcoholic fermentation is assumed to be caused by the liquid batter, which causes depletion of oxygen. Based on these conditions it is hypothesised, that the yeast during fermentation switched to the alcoholic fermentation, rather than following respiration. This ultimately assumed to leads to low amounts of acid and aroma compounds.

Table 5-5 Volatile compound analysis

Compound	Organoleptic description	Concentration [µg/kg]				
		S-23	T-58	US-05	WB-06	Baker's Yeast
Ethanol	Alcoholic, sweet	2500	5800	2300	2300	3000
Acetic Acid	Vinegar, pungent, sour	1300	360	120	200	260
2,3-butandiediol	Fruity, creamy, buttery	300	160	n.d.	n.d.	n.d.
1-hydroxy-2-propanone	pungent sweet caramellic ethereal	190	n.d.	n.d.	n.d.	n.d.

n.d = not detected

Descriptive sensory evaluation

For the descriptive analysis of the breadcrumb samples, a total of 12 attributes split into aroma and flavour were chosen. The descriptors are listed in Table 5-1. The sensory evaluation of the aroma did not show significant differences between the baked breads with the various yeast strains (data not shown). The outcome of this analysis is explained by the low production of volatile compounds and acids. The used gluten-free system lacks sufficient and or specific nutrients for the yeast to metabolise and produce other products than ethanol and acetic acid. The lack of nutrients for the yeast in a gluten-free system can be confirmed by the volatiles found in wheat-based system, applying the same yeast strains (Heitmann, Zannini, Axel, et al., 2017). In a wheat system higher amounts of volatile aroma compounds were found and hence differences in sensory profiles were reported. The outcome of the sensory evaluation suggests that the yeasts can be interchangeably be used without affecting the flavour and aroma profile. This allows focussing on the techno-functional effects of the yeast strains on the dough and final bead.

Correlation between yeast and bread properties

The application of different yeasts strains in gluten-free bread formulations to modify the final product quality is a novel approach. Key differences between yeasts in wheat doughs are the level of activity and the metabolic pathway (Heitmann et al., 2015). Table 5-6 shows the various level of correlation between yeast, dough and final bread properties. The correlation coefficient can be classified in different categories: perfect (r. = 1.0), strong (0.80 < r. > 1.0), moderate (0.50 < r. > 0.80), weak (0.10 < r. > 0.50) and very weak correlations (r. < 0.10).

For the discussion only, major correlations were discussed. The number of viable cells was adjusted to the level of Backer's yeast. This allowed a direct comparison of the effect of the different yeast strains on a gluten-free formulation. The differences in the optimal fermentation temperatures and metabolism of sugar affected the chemical and technological properties of the gluten-free dough. When optimal conditions are provided, yeast can work at its full potential. This was confirmed by reduced levels of sugars in the final bread and the pH development of the bread making process. Correlation analysis revealed strong negative correlations between the pH and dough rise (r. 0.921, p.<0.001). The correlation is explained by the produced CO_2, which is decreasing the pH due to its carbonic acid and the expansion of gas cells accelerating the dough rise (Heitmann et al., 2015; Verheyen et al., 2014). The production of CO_2 is considered as an indicator for yeast activity (Heitmann et al., 2015). The more CO_2 and ethanol are produced by yeast, the more active it is considered. The differences in the activity between the various yeast strains can be explained by the negative correlations between the remaining sugars in the final bread and the dough rise (r. -0.879, p.<0.001). This is due to the metabolism of the different yeasts, which ferment the available sugars and produces CO_2 (Randez-Gil et al., 1999). The more sugars are fermented the more CO_2 is produced and the higher is the dough rise. Further correlations from the dough properties to the final bread properties were found (r > 0.8). The dough rise had strong correlations between the crumb cell structure, in particular with the cell diameter (r. 0.937, p. < 0.001). This was explained by the production of CO_2, which expands the crumb cells and in turn increases the dough rise. Based on this, it can be expected to find correlations between the dough rise properties of the doughs and the specific volume of the various breads (r. 0.844, p. < 0.001). The found correlation suggests that

the dough rise measurement offers the potential to be used as prediction tool for the final volume of baked breads and yeast activity. Correlation analysis also confirmed the discussed connection between cell structure and texture. After baking a higher number of cells was positively correlated with the hardness of the breadcrumb 2 hr (r. 0.870, p. < 0.001) and 24 hr (r. 0.929, p. < 0.001). This suggests that the increase in cells increased the number of cell walls which in turn strengthen the breadcrumb and results in higher hardness values. A further correlation was found for the specific volume and the bake loss (r. 0.802, p. < 0.001). This correlation has also been found in a previous study (Horstmann et al., 2017) and is known to be caused by a greater specific volume which offers a greater surface area for water to evaporate.

Table 5-6 Correlation of dough properties with final bread characteristics

		Dough Rise properties	
		Max Height	**Slope 30C**
Yeast activity	**pH (proofed Bread)**	-0.728**	-0.921***
	pH (Bread)	-0.744**	-0.911***
	Glucose (Bread)	-0.922***	-0.879***
	Fructose (Bread)	-0.793***	-0.723**
Bread properties	**Cell Diameter**	0.849***	0.937***
	Number of Cells / Slice Area	-0.885***	-0.789***
	Specific Volume	0.844***	0.937***
	Hardness 0h	-0.910***	-0.730**
	Hardness 24h	-0.948***	-0.851***

Pearson correlation: *p. < 0.5, ** p. < 0.1, *** p.< 0.01

Conclusion

This study was conducted to investigate the effect of different *S. cerevisiae* yeast strains on a gluten-free bread formulation. Although only strains of *S. cerevisiae* were applied, differences in dough and bread quality parameters were observed. Differences in sugar metabolism and preferred fermentation temperatures lead to diverse activity levels and performance of the various yeasts. These differences in activity had major changes in the dough performance and ultimately in the bread baking characteristics. The application of the yeast strain US-05 showed a decrease in loaf volume and a high increase in crumb hardness in comparison to the control yeast. On the contrary T-58 resulted in the bread with the highest loaf volume and the softest bread crumb. The yeast strain WB-06 showed the closest resemblance to the breads baked with the control yeast strain Baker's yeast. Pearson analysis showed significant correlations between yeast activity indicators such as pH and remaining levels of sugar and the dough rise parameters ($r. > 0.70$). These in turn correlated with loaf volume crumb structure and texture of the baked breads ($r. > 0.75$). Volatile aroma compound analysis detected only low amounts of volatiles which explained the not significant different results of the descriptive sensory analysis. The low production of volatiles was explained to be caused by the refined gluten-free system in this study, which lacks nutrients for the yeast metabolism. In summary it was found that the different yeasts only affected the technological properties rather than the flavour and aroma profile of the baked breads. This was found to be due to the yeast specific activities and properties. The performed study demonstrated the suitability of different yeast strains of S. cerevisiae in the application of gluten-free bread.

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Chapter 6 A comparative study of gluten-free sprouts in the gluten-free breadmaking process

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Abstract

The addition of sprouted grains and seeds to cereal products has been identified as one of the upcoming trends in recent market reports. Traditionally, malted barley is added at very low levels to wheat flour to increase the enzyme content. In comparison to barley malt, gluten-free malts and sprouts commonly have very low enzymatic activity. Thus, a higher quantity of these gluten-free grains needs to be added to improve nutritional and functional properties of gluten-free breads, without causing a liquefaction effect. In this study, seven types of sprouts (amaranth, brown millet, corn, lentil, lupin, pea, quinoa) were milled and characterised with respect to their compositional (starch, protein, fat, ash, fibre, moisture) and functional properties (water hydration properties). These sprouted flours were included in a gluten-free bread formulation at a level of 5% and the impact on dough (temperature-dependent rising behaviour, pasting and rheological properties) and bread quality parameters (volume, crumb structure and texture) was evaluated. Factors such as the method of germination and the botanical origin influenced the chemical composition of the applied raw material. The functional properties of the different malts and sprouts are affected by the chemical composition of the individual grains. The differences in functional properties were, in turn, found to affect the dough properties and the quality parameters of the baked gluten-free breads. However, statistical analysis showed no correlation between the various factors. Based on this, effects on dough and bread properties were hypothesised to be caused by a combination of multiple factors. All bread formulations containing sprouted flour had significantly improved bread quality parameters in comparison to the control (without sprouted flour). The addition of amaranth sprouted flour, however, resulted in the highest loaf volume and the softest breadcrumb, suggesting its potential for further investigations in further studies.

Introduction

The inclusion of sprouted grain into cereal products, for their claimed health benefits, has been named as one of the major trends by recent market reports (The Washington Post 2017). The germination process of seeds or grains is conducted under strictly controlled conditions of temperature, moisture and aeration (Kunze, 1999). During the germination stage, metabolic processes synthesise and activate enzymes, leading to an increase in antioxidant activity, improved protein digestibility and mineral bioavailability (Kaukovirta-Norja, Wilhelmson, & Poutanen, 2004; Mäkinen & Arendt, 2015). Until recently the process of germination has been mainly used to produce fermentable extracts for brewing and distilling purposes. Today, however, it is also considered as a tool for the production of ingredients with an enhanced nutritional profile and health-promoting compounds (Hübner & Arendt, 2013). Thus, sprouted grains and seeds have been promoted in recent literature for the improvement of the nutritional aspects of gluten-free bakery products, in particular breads (Deora, Deswal, & Mishra, 2014; Omary, Fong, Rothschild, & Finney, 2012).

Gluten-free bread is one of the most consumed gluten-free goods by people who suffer from coeliac disease (CD), one of the most common food intolerances. The prevalence of CD is increasing and affects approximately 1% of the world population. The disease is triggered, in susceptible individuals, by the ingestion of gluten (Lionetti, Gatti, Pulvirenti, & Catassi, 2015). However, CD is not the only disease which is caused by gluten. Under the umbrella term "gluten-related disorders" many more diseases are found, which increases the number of people who must follow a gluten-free diet as part of a treatment (Foschia et al., 2016). Despite increasing research interest and the consequent improvement of gluten-free bread quality over the past number of decades, consumers remain unsatisfied with the quality. Gluten-free breads are still lacking in techno-functional properties and nutritional value (Foschia et al., 2016). Literature in the application and effects of sprouts or malts on gluten-free bread quality is scarce. Nevertheless, published research has shown positive effects of malted oat and quinoa (Mäkinen, Zannini, & Arendt, 2013), malted sorghum (Phattanakulkaewmorie, Paseephol, & Moongngarm, 2011) and germinated brown rice (Cornejo, Caceres, Martínez-Villaluenga, Rosell, & Frias, 2015; Cornejo & Rosell, 2015) on gluten-free

bread properties. The application of malted oats was reported to improve the volume, crumb structure and texture of gluten-free bread; however, quinoa malt was found to only add to the flavour and nutritional properties (Mäkinen et al., 2013). Sorghum malt was shown to reduce crumb hardness when used as a replacement for ungerminated sorghum flour (50:50; 100:50) in a gluten-free bread and to potentially improve the chemical composition (Cornejo & Rosell, 2015). Improved breadcrumb texture of gluten-free breads was reported to be influenced by the addition of germinated brown rice flour, however, the germination time of the rice also had an effect. Flours produced with a prolonged germination time were shown to have a negative effect on the baked breads (Cornejo & Rosell, 2015). Germinated brown rice flour was further found to improve the nutritional quality of gluten-free bread (Cornejo et al., 2015). The addition of germinated amaranth in a gluten-free cookie was also reported, which improved the nutritional value, based on an increased content of protein and total dietary fibre and level of antioxidant activity in comparison to raw amaranth flour (Chauhan, Saxena, & Singh, 2015).

Based on the aforementioned evidence of positive effects of germinated grains, the aim of this study was to investigate the gluten-free bread-making potential of sprouts including, amaranth, brown millet, quinoa, lupin, lentil, pea and corn. The suitability of these sprouts for application in a gluten-free system was evaluated and their effects on the composition and properties of dough and the final bread products were investigated. The results gained from this study are expected to contribute knowledge for improving gluten-free bread quality.

Experimental

Material and Methods

Potato starch was supplied by Emsland, Germany; pea protein by Roquette, France; pectin by Cp Kelco, Germany; sugar by Siucra Nordzucker, Ireland and salt by Glacia British Salt Limited, UK. Instant active dry Baker's yeast was obtained from Puratos, Belgium. Sprouts were purchased form Ziegler, Germany (Amaranth sprouts, Brown millet sprouts, Quinoa sprouts) and Keimkraft, Austria (Lupin sprouts, Lentil sprouts, Pea sprouts, Corn sprouts). All chemicals were supplied by Sigma-Aldrich, Arklow, Ireland.

Milling of germinated seeds and grains

Commercially purchased sprouted grains and seeds were milled using a Bühler Universla disc mill (Uzwil, Switzerland) with settings for a particle size of 250 μm. After milling samples were passed through a sieve with a pore size of 250 μm. Separated husks and larger particles were discarded.

Compositional analysis

The total nitrogen content of the potato protein was analysed using the Kjeldahl method (MEBAK 1.5.2.1). A nitrogen to protein conversion factor of 6.25 was used. Moisture content was determined according to AACC Method 44-15 A. The total available carbohydrate level of the milled samples was determined spectrophotometrically using an enzyme kit (K-TSTA) supplied by Megazyme, Ireland. The ash content was determined according to AACC Method 08-01.01. The lipid content was determined according to the Soxlet-method (AACC Methods 30- 25.01) after acid hydrolysis. Total dietary fibre contents were determined according to the AOAC method 991.43 by Concept Life Sciences, UK.

Enzyme activity

The amylase activity of alpha (AACC Method 22-02.01. (K-CERA)) and beta amylase (K-BETA3) was determined using commercially available enzyme kits, supplied by Megazyme, Ireland. Protease activity was determined according to Brijs, Trogh, Jones, and Delcour (2002), with slight modifications. Protease activity was extracted from 0.3g of milled sample in 0.05 M acetate buffer containing 2 mM-cysteine (pH 5.0) under shaking for 30 minutes at 5°C. The sample extract was assayed after centrifugation (10,000 g x 15 min at 4°C) against 1.0% haemoglobulin in 0.2 M sodium acetate buffer. Therefore 0.25 ml of haemoglobulin solution and 0.4 ml of sample extract were mixed and incubated for 2.5 h at 40°C. The reaction was stopped by adding 0.4 ml of cold TCA (10% w/v). Subsequently, the tubes were centrifuged at 10,000 g for 10 minutes to remove precipitated proteins. A reaction blank was assayed for each flour by adding the stopping reagent prior to the incubation. The supernatants were analysed for free α-amino nitrogen, using trinitrobenzene-sulfonic acid (TNBS) reagent (0.3%, w/v, in 0.2 M sodium phosphate buffer, pH 8.0). Absorption of samples and reaction blanks was measured at 340 nm against distilled water.

Sugars

Sugar levels (glucose and fructose) of dough and bread crumb were analysed with an Agilent 1260 high performance liquid chromatography system (HPLC) with a Sugar-Pak column (Waters, Cork, Ireland) coupled to a refractive index detector (RID) at 40°C. The sugars were extracted with distilled water for 20 min shaking and then centrifugated at 3000g for 10 minutes. HPLC analysis was performed at 80°C column temperature with 0.0001 M CaEDTA (HPLC-grade) at a flow rate of 0.5 mL/min.

Flour hydration properties

Flour hydration properties were analysed according to Cornejo and Rosell (2015). The water holding capacity (WHC) was determined by mixing 1.000g +/- 0.001g of milled sample with distilled water (10 ml) and holding at room temperature for 24 h. WHC was expressed as grams of water retained per grams of sample. For the determination of the swelling power (SP) 1.000g +/- 0.001g of sample were placed in a graduated cylinder and mixed with distilled water (10ml). The sample was kept at room

temperature for 24 h and swelling power was calculated by dividing the total volume of swollen sample by the original weight of flour. The water-binding capacity (WBC) was measured similar to the WHC with the addition of a centrifugal step (2000 g for 10 min).

Pasting properties

The pasting behaviour of dough formulations with different sprouts (dry mix, excluding yeast) was measured using a Rapid Visco Analyzer (RVA Super 3 Rapid Visco Analyser Newport Scientific, Warriewood, Australia). Each blend (3.0 g) was mixed with 25 ml of distilled water in a container, heated at a rate of 0.2 °C/sec from 50 °C to 95 °C, maintained at 95 °C for 162 s, cooled at the rate of 0.2 °C/sec to 50 °C, and held for 120 s at 50 °C before the test ended.

Dough frequency test

Rheological measurements of dough samples containing the different sprouts were carried out by using a Rheometer Physica MCR 301 (Anton Paar GmbH, Germany) equipped with serrated parallel plate geometry (diameter 50 mm, gap 1 mm). Dough samples were placed between the plates of the rheometer. Samples were left to rest for 5 min after loading prior to the performance of a frequency sweep test at 25°C from 100 Hz to 0.1 Hz within a linear viscoelastic range. Data obtained were complex viscosity (G*) and damping factor (tan δ).

Time- and temperature-dependent rising behaviour of dough

The measurements were conducted according to Horstmann et al. (2018b) using an Anton Paar MCR rheometer with the TruStrain™ option. 3g of sample were loaded into a stainless-steel cylinder with the height of 33 mm and the inner diameter of 25 mm. To mimic the proofing properties the temperature was set at 30°C for 45 min with a constant normal force of FN = 0.0 to ensure permanent contact between sample and upper plate. For determination of the oven spring and the determination of yeast activity during the baking process, the temperature was increased to 90°C with a heat rate of 4°C / min. Recorded and calculated parameters were the max height [mm], which is the maximum height the dough reached during the measurement. Further the

slope [mm/min] during the fermentation process (Slope FP) and then during the baking process (Slope BP) for determination of yeast activity and dough performance was determined. Also, the max height temperature (TMH) [°C] was recorded.

Bread making procedure

Bread samples were produced based on a simple recipe (80% water, 5% sprouted flour, 2% pea protein, 2% pectin, 2% salt, 4% sugar, 2% yeast, based on potato starch weight). For the pre-fermentation, yeast was suspended in warm water (25°C) and regenerated for a period of 10 min. Mixing was carried out with a k-beater (Kenwood, Havant, UK) at low disk speed (level 1 of 6) for 1 minute in a Kenwood Major Titanium kmm 020 Mixer (Kenwood, Havant, UK). After that, the dough was scraped down from the bowl walls and a further mixing of 2 minutes at higher disk speed (level 2 of 6) was carried out. The batter was scaled to 300 g in 9 baking tins of 16,5 cm x 11 cm x 7 cm and placed in a proofer for 45 minutes at 30°C and 85% relatively humidity (RH). The dough samples were then baked for 45 min at 220°C top and 220°C bottom heat in a deck oven, previously steamed with 0.7 L of water. The breads were cooled for 2 hours prior to analysis.

Bread analysis

The specific volume of the bread was determined by use of a Vol-scan apparatus (Stable Micro System, UK). The specific volume is calculated on the basis of loaf volume and weight. An image analysis system (Calibre Control International Ltd., UK) was used to analyse the breadcrumb structure chosen parameters were the cell diameter and the number of cells per slice area. Crumb firmness was analysed using a Texture Profile Analyser (TA-XT2i, Stable Micro Systems, Godalming, England) with a 25 kg load cell, which compresses the breadcrumb with a 20 mm aluminium cylindrical probe. Bread samples were cut in 20 mm slices and analysed with a test speed of 5 mm/s and a trigger force of 20 g, compressing the middle of the breadcrumb to 10 mm. The measurement with the various parameters was conducted on the baking day and 24h

after baking to monitor the staling process. The colour values of breadcrumb samples were measured using the CIE *L* a* b** colour system, where L* is an indicator for lightness, positive *a** values refer to redness, and positive *b** values refer to yellowness. The analysis was performed using a Colorimeter CR-400 (Konica Minolta, Osaka, Japan). The colorimetric parameters *L**, *a** and *b** were referred to CIE standard illuminant D65.

Statistical analysis

All measurements were performed at least in triplicate. The significance of the results was analysed using One Way ANOVA (R version 3.0.1). The level of significance was determined at $p<0.05$.

Results and Discussion

Chemical composition

The germination process of seeds and grains has considerable influence on the final chemical composition of the raw material. Parameters such as time and temperature of the germination are crucial factors during this process (Kunze, 1999). In addition, the milling and sieving of the sprouted material can further alter this composition. Husks of seeds which are mainly fibres are more difficult to process than the kernel itself and are often sieved out. This concentrates the amounts of other components such as starch, protein and fat in the milled flour in comparison to the whole seed or grain. Commercially purchased sprouts of amaranth, brown millet, quinoa, lupin, lentil, pea and corn were milled and sifted through a sieve with a 250 μm pore size for the use as flour in gluten-free baking. The different flours milled from the various sprouts will be referred to as SF (sprout flour). Their chemical composition is listed in Table 6-1. Based on the differences in botanical origin, modified germination regimes and the milling processes, significant differences between the sprouts were found.

Total starch contents showed significant differences between the various sprouts. Corn SF contained the highest amount of total starch (76.47g/100g), which was about 40% higher than found in the other sprouts. The significantly lowest value was found in lupin SF with a content of 22.02g/100g. Analysed sugars showed the significantly highest amount of di-saccharides in lupin SF. The significantly lowest amount was found in brown millet SF. This flour also contained the lowest concentration of fructose, while lupin SF contained the highest amount. Differences were observed in the glucose contents, with quinoa SF having the highest content. Pea SF contained the lowest amount of glucose. Overall only small quantities of the free sugars were found. However, significantly different amounts can influence the fermentation process of the dough. The more sugars are available the more the yeast can metabolise, and the more CO_2 is produced (Randez-Gil et al., 1999). A higher production of CO_2 in conjunction with the supporting dough viscosity can increase the specific volume of a gluten-free model bread (Horstmann et al., 2018b). Protein analysis showed that lupin SF had the highest protein content (43.08g/100g), which was 25% higher than the second highest protein content determined in lentil SF. A high protein content in lupin SF was

expected, since lupin seeds contain already high amounts (> 30g/100g) of protein (Sujak, Kotlarz, & Strobel, 2006). The lowest amount of protein was found in corn SF. Similar low values for ungerminated corn flour have been recently reported in another gluten-free study (Hager, Wolter, Jacob, Zannini, & Arendt, 2012). The highest fibre content was found in lupin SF while the significant lowest fibre content was found in lentil SF. The addition of fibre rich ingredients can help to improve the nutritional profile of gluten-free breads. However, fibres can absorb up to 10 times their own weight of water (Sluimer, 2005). Thus, the application of high fibre containing ingredients can affect the baking performance of the fragile gluten-free system. Significant differences in the composition of the various sprouts was also found in the fat content. The fat content ranged from 1.25 g/100g to 8.01 g/100g, with pea SF having the lowest and lupin SF the highest content. Lipids can affect the gelatinisation properties of starch through complex formation with amylose during heating (Copeland, Blazek, Salman, & Tang, 2009). A limiting effect of starch swelling by lipids was reported to result in a softer breadcrumb or weakened crumb, depending on the amount added (Gallagher, 2009). Such an effect was discussed in a previous study performed on the application of different starches in a gluten-free model system (Horstmann et al., 2016).

The addition of minerals (ash), in the natural amounts in which they occur in raw materials, to the authors' knowledge, does not influence the bread making process or the structure of the final bread. However, ingredients rich in mineral contents offer the potential to improve the nutritional profile of products which are lacking minerals, such as gluten-free breads (Hübner & Arendt, 2013). The highest content of ash was found in amaranth SF (3.77 g/100g) followed by brown miller SF (3.19g /100g). No significant differences between quinoa SF, lupin SF., lentil SF and pea SF were found (approx. 2.60g/100g). The significantly lowest content was found in corn SF which was lower than 1%. The moisture content of the various SF showed significant differences. The highest content was determined in lentil SF, while the lowest amount was found in quinoa SF. Differences in the moisture content are often influenced by the drying procedure after germination (Kunze, 1999).

The germination of seeds or grains activates enzymes by metabolic processes (Mäkinen & Arendt, 2015). Enzyme activities of raw material have significant effects on dough and final bread properties (Rosell, 2009). In wheat breads barley malt flour is added in small amounts (0.1 - 0.8 %) to improve baking properties and improve loaf volume and structure (Sluimer, 2005). However, high amounts of barley malt flour can cause liquefaction of the dough, leading to a detrimental result. In gluten-free systems, a controlled level of enzymatic activity can either positively or negatively affect the baking properties (Phattanakulkaewmorie et al., 2011). Based on the previously observed positive and negative effects of enzymes in the aforementioned studies, their activities in the different SFs was determined. Protease activity showed significant differences, amaranth SF the highest (8.65 U/g) and pea SF the lowest activity (0.82U/g). No activity was recorded in lentil SF and corn SF. Proteases hydrolyse proteins. This can be used to promote gluten relaxation in wheat-based systems. However, excessive protease activity has been reported to destroy the gluten network producing a viscous system or even a liquid batter (Haros, Rosell, & Benedito, 2002; Renzetti & Arendt, 2009; Sluimer, 2005).

The cleaving of complex sugars to simple sugars is a crucial process which can affect the baking process drastically. Generated glucose and fructose can be metabolised by yeast into CO_2 and ethanol and expand gas cells (Horstmann et al., 2018b). Amylases can further retard the retrogradation process of starch in bread and hence delay staling (Giannone et al., 2016). Alpha-amylase activity was only found in corn SF, with a high activity (12.55 U/g). The analysis of beta-amylase activity showed only low but significantly different levels between the SF. The significantly highest activity was found in lupin SF (0.61 U/g) and the lowest activity in SF produced from brown millet (0.04 U/g). No activity was recorded for quinoa SF. No lipase activity was detected in any of the SFs (data not shown). This lower enzymatic activity of the selected sprouts enables their use in higher concentrations than, for example, barley malt, while not causing a deleterious liquefication effect. Use of higher amounts of SF used in gluten-free formulation could, therefore, improve the nutritional profile.

Table 6-1 Chemical composition and hydration properties of the different sprouted flours.

	Amaranth sprouts	Brown millet sprouts	Quinoa sprouts	Lupin sprouts	Lentil sprouts	Pea sprouts	Corn sprouts
Composition [g/100g]							
Total Starch	56.76 ± 4.16^{b}	57.56 ± 0.33b	58.52 ± 1.54^{b}	22.02 ± 0.04^{c}	50.45 ± 4.26^{b}	56.23 ± 3.64^{b}	76.47 ± 4.64^{a}
Di-Saccharides	1.16 ± 0.02^{c}	0.87 ± 0.02^{d}	1.15 ± 0.00^{c}	3.29 ± 0.09^{a}	1.99 ± 0.06^{b}	2.06 ± 0.11^{b}	1.10 ± 0.03^{c}
Glucose	0.95 ± 0.02^{b}	0.28 ± 0.02^{c}	1.15 ± 0.02^{a}	0.113 ±0.008^{e}	0.206 ± 0.011^{d}	0.033 ± 0.013^{f}	0.197 ± 0.02^{d}
Fructose	0.121 ± 0.003^{d}	0.043 ± 0.004^{f}	0.162 ± 0.018^{c}	0.263 ± 0.003^{a}	0.090 ± 0.006^{e}	0.162 ± 0.009^{c}	0.192 ± 0.013^{b}
Protein	9.89±0.21^{f}	10.86±0.22^{e}	16.00±0.05^{d}	43.08±0.02^{a}	28.08±0.06^{b}	26.17±0.04^{c}	5.64±0.03^{g}
Fibre[1]	5.5^{e}	14.1^{b}	6.5^{c}	17.4^{a}	3.1^{g}	5.7^{d}	3.6^{f}
Soluble[1]	< 0.1	10.8	< 0.1	0.6	< 0.1	< 0.1	< 0.1
Insoluble[1]	5.5	3.3	6.5	16.8	3.18	5.7	3.6
Fat	7.13 ± 0.20^{a}	4.29 ± 0.12^{b}	6.74 ± 0.81^{a}	8.01 ± 0.91^{a}	1.47 ± 0.11^{c}	1.26 ± 0.13^{c}	2.52 ± 0.03^{c}
Ash	3.77 ± 0.14^{a}	3.19 ± 0.05^{b}	2.59 ± 0.06^{c}	2.61 ±0.14^{c}	2.66 ± 0.16^{c}	2.63 ± 0.07^{c}	0.63 ± 0.07^{d}
Moisture	11.29±0.20^{d}	11.17±0.06^{d}	10.97±0.14^{d}	12.04±0.06^{c}	13.25±0.06^{a}	12.70±0.27^{b}	13.03±0.10^{ab}

Table 6-1 continued

	Amaranth sprouts	Brown millet sprouts	Quinoa sprouts	Lupin sprouts	Lentil sprouts	Pea sprouts	Corn sprouts
Enzyme activity							
α-amylase [U/g]	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]	12.55 ± 2.93[a]
β-amylase [U/g]	0.10±0.00[cd]	0.04±0.00d[e]	n.d.[e]	0.61±0.08[a]	0.19±0.02[b]	0.07±0.01[de]	0.18±0.00[bc]
Protease Activity [U/g]	8.65±0.37[a]	4.82±0.50[b]	7.67±0.52[a]	7.70±1.92[a]	n.d[c]	0.82±0.00[c]	n.d[c]
Hydration properties							
Swelling Power [ml/g]	3.24±0.24[bc]	2.45± 0.06[d]	2.87±0.13[cd]	6.00±0.13[a]	3.29±0.23[bc]	3.49±0.08[b]	2.87±0.13[cd]
Water Holding Capacity [g/g]	2.94±0.08[b]	1.83± 0.16[d]	2.56±0.31[bc]	5.42±0.21[a]	2.81±0.26[b]	2.91±0.15[b]	2.24±0.07[cd]
Water Binding Capacity [g/g]	1.51±0.01[b]	1.45±0.17[b]	1.45±0.17[b]	2.54±0.07[a]	1.42±0.03[b]	1.39±0.07[b]	1.48±0.03[b]

Means in the same row with different letters are significantly different (≥3 = One-way ANOVA; ≥2 0 =t-Test, $p < 0.05$). n.d. = not detected

[1] analysed by external laboratory (Concept life sciences, Cambridgeshire, UK)

Flour hydration properties

Based on differences in chemical composition in SF, such as in fibre and its potential to absorb and affect baking properties, the hydration properties of the SFs were determined. Parameters analysed for the hydration properties were the water holding capacity (WHC), swelling power (SP) and the water binding capacity (WBC) as described by Cornejo and Rosell (2015). The WHC determines the amount of water retained by the sample without being subjected to any stress. The highest amount of water was retained by lupin SF, which was nearly twice as high as the other SFs (Table 6-1). Brown millet SF retained the least amount of water. Similar trends were found for the SP, which is defined as the volume gained after hydration of the sample. Also, here lupin SF was found to have the highest SP, while brown millet SF showed the lowest SP.

The WBC of a sample is defined similar to the WHC, with the exception that it is determined after low-speed centrifugation (Cornejo et al., 2015). Lupin SF was found to retain the highest amount of water after centrifugal stress in comparison to the remaining SFs. No significant differences between other SFs were found. The assumption that the total fibre content is the main contributor to the WHC was ruled out, since lupin SF and brown millet SF have the highest fibre contents but low WHC. This was explained by the different types of fibres which were found. Lupin SF contains 16.8% insoluble fibre, while brown millet SF contains 3.3%. The remaining 10.8% are soluble and hypothesised to be discarded with the supernatant and hence less water could be retained. This hypothesis is strengthened by the finding that corn SF, being the second lowest water retaining SF, also contained only a low amount of insoluble fibre content. Similar results were also found by Wang, Rosell, and de Barber (2002), who analysed the effect of fibres on wheat dough, the authors found that carob fibre which was rich in insoluble fibre increased the water absorption more than inulin, which was rich in soluble fibre. Also, factors like hydroxyl groups, ionic charge, chain length and molecular weight can influence the water hydration properties and are mainly linked to

the source of origin (Horstmann, Axel, & Arendt, 2018a; Rosell, Rojas, & De Barber, 2001; Wang et al., 2002). However, not only the soluble and insoluble parts of fibre affect the water hydration properties of a SF. The protein content also plays a significant role in the hydration properties of a raw material (Horstmann et al., 2017).

Pasting properties of dough formulations

The analysis of pasting properties using a rapid visco analyser was conducted on the dough formulation, excluding yeast. Results of the viscosity profiles during applied shear and a range of temperature are shown in Table 6-2. Dough formulations containing SF showed a reduced viscosity profile in comparison to the control. Viscosity reducing effects were also reported in literature (Cornejo & Rosell, 2015; Mäkinen et al., 2013; Phattanakulkaewmorie et al., 2011). Apart from the viscosity reducing effect of SF addition, significant differences between the applied SF on the viscosity profiles were found.

Analysis of the reached peak viscosities showed significant differences. The highest peak viscosities after the control formulation were found in the doughs containing lupin SF, lentil SF and pea SF. The significantly lowest value was found in samples containing brown millet SF. The peak viscosity is usually described as the maximum swelling of the starch granules before bursting (Schirmer, Jekle, & Becker, 2015). In a dough formulation, it can refer to the entire system and factors such as protein denaturation, hydrocolloid and fibre swelling, and the enzymatic activity must be considered. These factors can also further affect pasting parameters such as the breakdown viscosity. The breakdown viscosity has been described as an indicator for the breaking of granules upon heating after the maximum swelling at the peak viscosity (Rojas, Rosell, & De Barber, 1999). Hence in a dough formulation, it can be used as an indicator for the stability of the system, and ability to withstand heat and mechanical shear conditions. The highest breakdown viscosity was found for the control and the formulations containing brown millet SF and pea SF. The most stable dough system with the significantly lowest breakdown viscosity was that containing corn SF addition.

Table 6-2 Pasting properties of the different formulations including the sprouted flours

	Peak 1 [cP]	Breakdown [cP]	Final Visc [cP]
Amaranth sprouts	558.0 ± 91.0^{abc}	19.4 ± 6.8^{abc}	847. ± 102.0^{bc}
Brown millet sprouts	308.5 ± 55.8^{d}	31.5 ± 5.0^{a}	416.0 ± 79.2^{d}
Corn sprouts	518 ± 5.66^{c}	7.5 ± 2.12^{c}	781.0 ± 14.4^{c}
Lentil sprouts	641.3 ± 30.7^{abc}	24.4 ± 5.1^{ab}	970.3 ± 47.1^{abc}
Lupin sprouts	621.7 ± 28.8^{abc}	12.3 ± 6.9^{bc}	965.0 ± 23.9^{abc}
Pea sprouts	637 ± 354^{abc}	609 ± 10.61^{a}	937.0 ± 7.07^{abc}
Quinoa sprouts	665.0 ± 43.6^{ab}	26.6 ± 3.8^{ab}	1020.4 ± 48.4^{ab}
Control	731.4 ± 16.2^{a}	33.7 ± 3.2^{a}	1083.7 ± 16.8^{a}

Means in the same row with different letters are significantly different (≥3 = One-way ANOVA; ≥2 0 =t-Test, $p < 0.05$).

The final viscosity is the viscosity reached after cooling. It is described as the reassociation of starch granules during cooling and is considered as an indicator for bread staling (Chanapamokkhot & Thongngam, 2007). The highest final viscosity was reached by quinoa SF formulations, showing no significant differences from the control formulation. The lowest viscosity was found in doughs formulated with brown millet SF.

The low viscosity results determined for brown millet SF in comparison to the remaining SFs is hypothesised to be attributed to its chemical composition, which was earlier discussed and linked to its low hydration properties. The overall decreasing viscosity results for most of the SFs cannot be limited to only one, but many factors. All the applied sprouts increased the lipid content in the dough formulation, which was earlier described to build complexes with amylose, limiting starch swelling (Gallagher, 2009; Horstmann et al., 2016). Furthermore, the denaturation and source of protein were recently discussed as influencing the pasting properties of dough formulations (Horstmann et al., 2017). In addition, the effect of enzymes must be taken into consideration, since a broad range of temperature during the measurement is applied, activating different enzymes (Poutanen, 1997). These were found to decrease viscosity

profiles by changing the molecular structure of starch through the breakdown of polymer chains (Cornejo & Rosell, 2015; Mäkinen et al., 2013). This breakdown reduced the ability to bind water and increased the viscosity. This has been demonstrated by previous studies using germinated flour (Phattanakulkaewmorie et al., 2011), increasing the concentration of germinated flour (Mäkinen et al., 2013) or by increasing the time of germination (Cornejo & Rosell, 2015). All of these approaches led to a higher enzyme activity in the analysed sample, decreasing its viscosity profile.

Oscillatory viscosity

Visco-elastic properties are an important characteristic of dough in order to facilitate gas / air cell expansion (Capriles & Arêas, 2014). The effect of the different SFs on the visco-elastic properties was measured and is shown in Figure 6-1. The complex viscosity and the damping factor of the dough (excluding yeast) were analysed. A decrease in complex viscosity over angular frequency was observed for all the dough samples. Similar findings were reported in a previous study applying different hydrocolloids to the gluten-free formulation (Horstmann et al., 2018a). However, doughs formulated with lentil SF, pea SF, lupin SF and corn SF showed higher viscosity values than the control. The analysis of the damping factor is an indicator of the viscoelastic behaviour. The dough samples prepared with the different SFs showed a higher viscous behaviour at lower rather than higher angular frequency. Different results for the control were reported in a previous study (Horstmann et al., 2018a). In this study the damping factor of the control (excluding sprouts) decreased (0.75 – 0.35) during increasing frequency (0.1 – to 10) but recovered to a small extent during the angular frequency from 10-100. In the previously reported study, the damping factor increased with increasing angular frequency from 0.5 to 0.88. The differences were explained by the change the amount of water added to the formulation and the addition of a protein source (pea protein). The added protein was reported in a further study to decrease the damping factor of a gluten-free model system (Horstmann et al., 2017). Furthermore, aside from the protein addition, in this study different sprouts were added to the formulation.

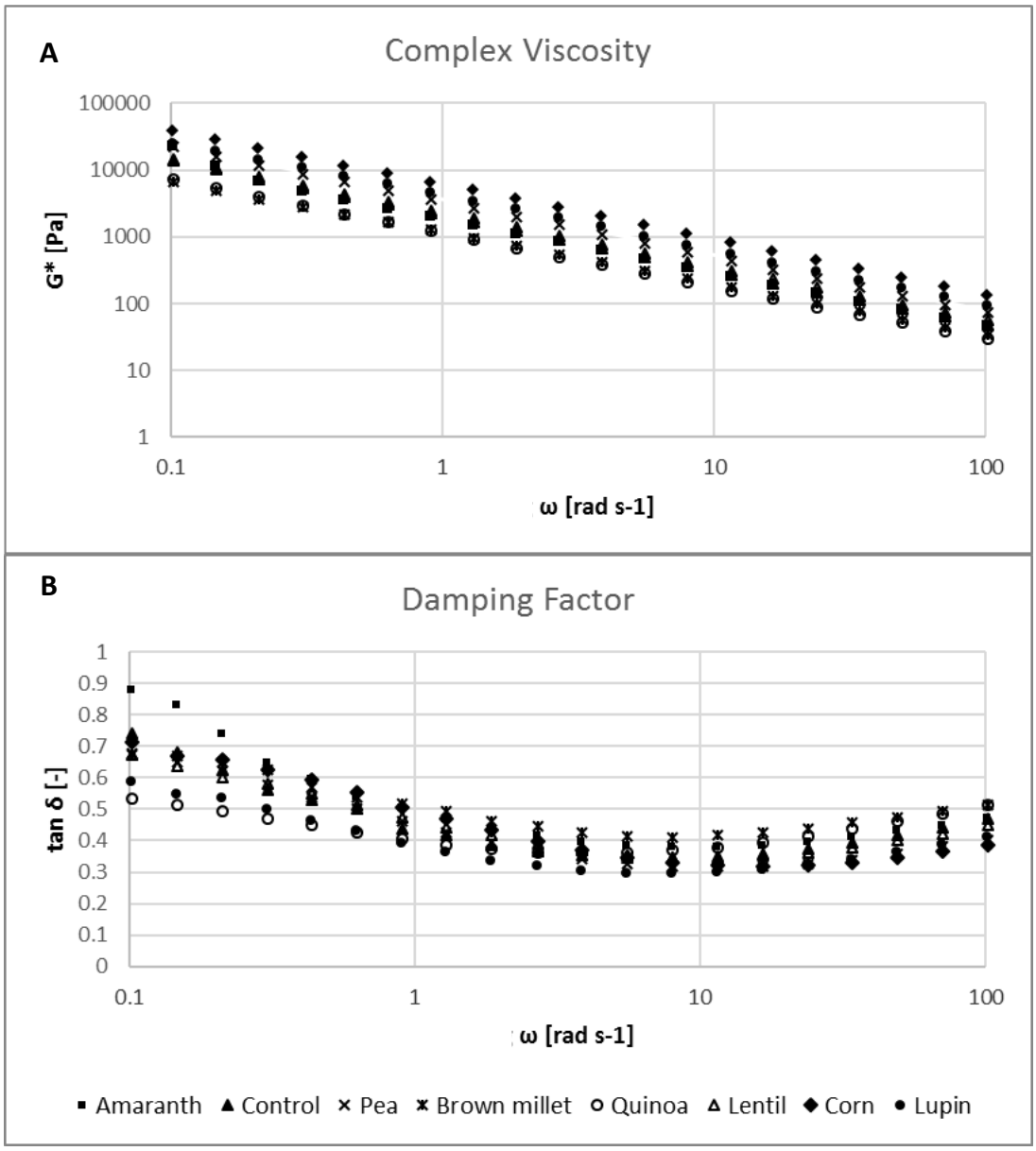

Figure 6-1 Rheological properties of different dough formulation, containing the different sprouted flours

These were found to have significantly different chemical compositions and water interacting properties. Despite their different properties, however, the addition of SF showed only significant differences at low angular frequency (angular frequency < 1). This is hypothesised by low molecular interactions between the different chemical components and water interacting properties of the various SFs. At this stage of the measurement only the addition of amaranth SF showed a higher damping factor than the control, referring to a more viscous behaviour. The addition of the remaining SFs showed either no significant difference compared to the control (corn SF, brown millet SF, lentil SF) or a significantly lower damping factor (lupin SF, quinoa SF). Overall, these results are similar to the ones found in literature, showing the damping factor 0.1 < tan delta < 1 (Horstmann et al., 2018a; Pruska-Kędzior et al., 2008; Witczak, Juszczak, Ziobro, & Korus, 2012; Ziobro, Witczak, Juszczak, & Korus, 2013).

Time- and Temperature – dependent rising behaviour of dough

The method of the rising behaviour of dough being dependent on time and temperature was described in a recent study (Horstmann, 2018b). This measurement was found to be a suitable alternative method for the analysis of gluten-free doughs. However, even though the CO_2 content is not recorded, the dough rise itself successfully correlated with the final bread properties of a gluten-free model system (Horstmann, 2018b). The method was described as a good indicator of yeast activity. Based on the different chemical compositions and enzyme activities of the various SFs their potential effect on yeast activity and related dough rise was analysed. Rising behaviour of the doughs formulated with the different sprouts showed significant differences (Figure 6-2 / Table 6-3). The slope of dough-rise during fermentation (Slope FP) is an indicator of how fast the dough rises. Doughs formulated with corn SF showed the fastest dough rise (0.348 mm/min), which is almost twice as high as the second fastest dough rise, observed in bread doughs containing quinoa SF (0.192 mm/min). The slowest rise was determined in the control dough, which did not contain SF (0.126 mm/min). The lower performance of the control is likely due to a limitation of available sugars for yeast metabolism. In comparison to the control dough, doughs containing SF, however, have more available sugars based on their chemical composition (Table 6-1). The high dough

rise during fermentation of corn SF is hypothesised to be caused by its amylase activity, producing fermentable sugars for the yeast available (Horstmann et al., 2017; Van Der Maarel, Van Der Veen, Uitdehaag, Leemhuis, & Dijkhuizen, 2002). An increase in the speed of dough rise was observed when the temperature increased and the slope of the "baking process" (Slope BP) was measured. An increase in temperature on a dough system has various effects: i) starch gelatinisation, ii) protein denaturation, iii) hydrocolloid gelling, iv) increased enzymatic and yeast activity and v) interactions and crosslinks between the aforementioned effects (BeMiller & Whistler, 2009; Morris et al., 2008). Thus, changes in dough rise during the baking process are mainly influenced by the chemical composition. The highest increase and the fastest dough rise was observed in doughs containing brown millet SF. The increase is hypothesised to be due to temperature-induced changes of the chemical components of the dough and their interactions, since no correlation to any one component was found. As observed in the rheological investigations, doughs containing brown millet SF showed a higher damping factor (viscous behaviour) in comparison to other doughs. A more viscous behaviour facilitates cell growth better than low damping factors (elastic behaviour) (Horstmann et al., 2017). The lowest and even decreased dough rise rate was found in doughs formulated with lupin SF. The slope during baking was reduced by more than 50% in comparison to the slope during the fermentation process. This detrimental effect is assumed to be caused by the significantly higher protein and insoluble fibre content in lupin SF, in comparison to the other SFs. The higher amount of protein is understood to denature, build a strong dough network and increase dough viscosity. The increase of viscosity caused by an increase in protein content, resulting in an elastic rather than viscous behaviour, has been recently reported in a previous study by Horstmann et al. (2017). The remaining chemical components are further factors which are described to affect the dough rising behaviour and contributing to a rather high viscosity. The authors in this study assume that the chemical components compete with the starch for free water. Starch gelatinisation is described as a result of granule swelling during heating, increasing viscosity (BeMiller, 2011). When the starch granules reach their maximum swelling capacity, they burst which results in a drop in viscosity (Schirmer et al., 2015).

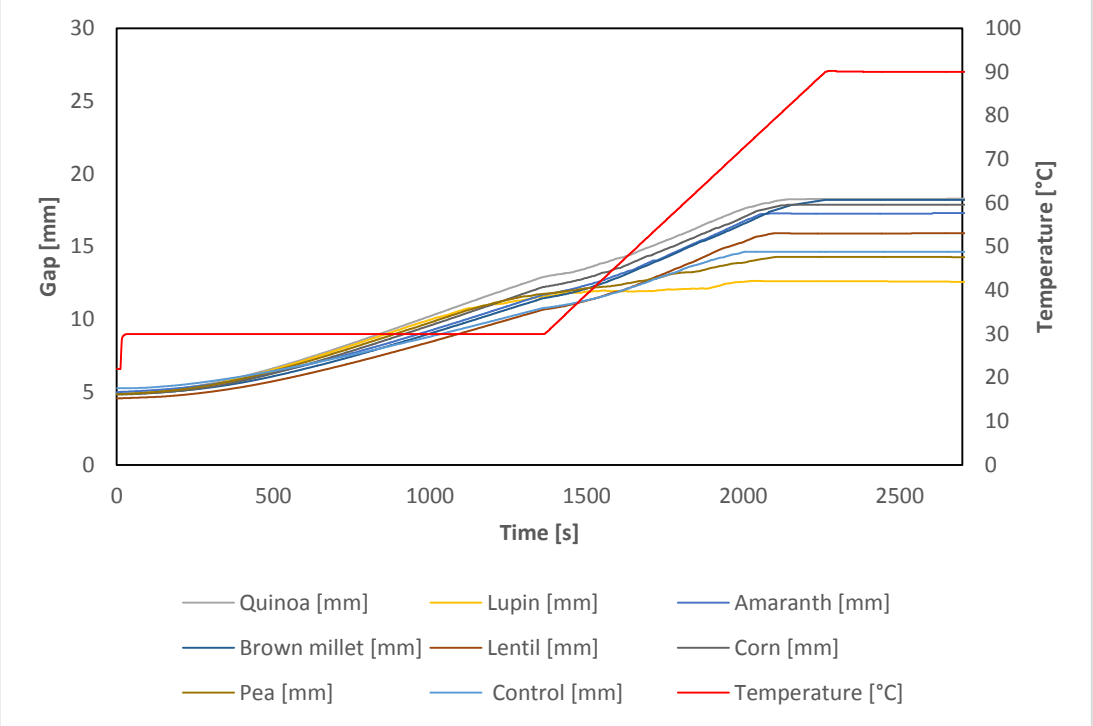

Figure 6-2 Time- and Temperature dependent dough rising

Table 6-3 Time- and Temperature-dependent rising parameters of the different dough formulations

	SlopeFP [mm/min]	SlopeBP [mm/min]	MaxH [mm]	TMH [°C
Amaranth sprouts	0.156±0.006^{abc}	0.456±0.015^{a}	17.24±0.76^{ab}	76.50
Brown millet sprouts	0.156±0.004^{abc}	0.510±0.032^{a}	18.19±1.04^{a}	89.90
Quinoa sprouts	0.192±0.006^{a}	0.426±0.101^{a}	18.26± 1.28^{a}	86.20
Lupin sprouts	0.168±0.017^{ab}	0.072±0.003^{b}	12.63±0.58^{d}	74.10
Lentil sprouts	0.144±0.01^{bc}	0.426±0.027^{a}	15.91±1.04^{abc}	79.10
Pea sprouts	0.174±0.017^{ab}	0.198±0.073^{b}	14.28±1.16^{cd}	80.40
Corn sprouts	0. 170±0.0197^{ab}	0.411±0.055^{a}	17.82±1.03^{ab}	80.40
Control	0.126±0.015^{c}	0.390±0.079^{a}	15.10±0.93^{bcd}	74.95

Means in the same column with different letters are significantly different (≥3 = One-way ANOVA; ≥2 0 =t-Test, $p < 0.05$).

The increase of viscosity caused by an increase in protein content, resulting in an elastic rather than viscous behaviour, has been recently reported in a previous study by Horstmann et al. (2017). The remaining chemical components are further factors which are described to affect the dough rising behaviour and contributing to a rather high viscosity. The authors in this study assume that the chemical components compete with the starch for free water. Starch gelatinisation is described as a result of granule swelling during heating, increasing viscosity (BeMiller, 2011). When the starch granules reach their maximum swelling capacity, they burst which results in a drop in viscosity (Schirmer et al., 2015). This granular bursting and related viscosity drop are hypothesised to be restrained by the competition with other chemical components such as fibre, protein. Also, the amount of lipids has to be considered, as lipids can coat the starch granules and interact with amylose restraining starch swelling (Horstmann et al., 2016). Prevention of granular bursting would maintain the high viscosity in the dough system and could further restrain gas cell expansion. The differences in dough rise rates over the various stages of fermentation and baking leads to further significant differences in the maximum height (maxH). Doughs containing brown millet SF, quinoa SF, amaranth SF, corn SF and lentil SF reached a higher maxH than the control. However, the highest maxH was reached by doughs containing quinoa SF and brown millet SF. The addition of pea SF and lupin SF had a decreasing effect on the maxH, where lupin SF showed the significantly lowest maxH. The low maxH for lupin SF is linked to the slow dough rise during the baking stage. The dough rise is affected by available nutrients for the yeast to metabolise, but also by the viscosity of the dough system (Horstmann et al., 2018b). The compositional analysis of the SFs showed significant differences in their compositions.

Baked bread properties

Baked breads formulated with the various SFs showed different results. Figure 6-3 gives an overview of the cross section and whole loaf of the baked breads. Except for brown millet SF all breads showed an even crumb texture without any large holes. The hole in brown millet SF is assumed to be caused by the low hydration properties which allow more water to evaporate during the early stages of baking and weakens the dough. The combination of the two is assumed to cause a coalition of crumb cells under the crust,

which is formed very early in the baking process and thus not allowing the evaporated water to escape. Furthermore, differences in colour, volume and crumb structure were observed. The quantitative differences of the various parameters are shown in Table 6-4. The addition of amaranth SF to the gluten-free formulation increased the specific volume giving the highest value, of 3.01 ml/g. Lupin SF was found to decrease the specific volume and showed the lowest value of 2.29 ml/g. Overall it was observed that the addition of SFs increased the specific volume in comparison to the control. Only lupin SF decreased the specific volume. Lentil SF-containing breads showed no significant difference to the control bread. Mixed results for the addition of germinated flours are also reported in literature. A positive effect on specific volume was reported for the addition of germinated brown rice flour in a gluten-free bread (Cornejo & Rosell, 2015). No influence was reported for the addition of germinated quinoa flour (Mäkinen et al., 2013). However, germinated oat flour applied in the same study was found to increase the specific volume. The authors correlated this result with the higher alpha-amylase activity in oat malt, causing a drop in viscosity of the dough, which allowed greater gas cell expansion. Similar findings were observed for the addition of germinated rice flour in comparison to ungerminated rice flour (Cornejo & Rosell, 2015). In this study, however, except in corn SF, no alpha-amylase activity was detected (Table 6-1). Furthermore, corn SF-formulated bread did not show the highest specific volume. This suggests that other factors play a key role in the baking process. It was not possible to establish correlations between dough properties and final bread results. The authors hypothesise that this is caused by complex and multiple interactions related to the chemical composition. The interactions are assumed to be the result of temperature changes during baking, which cannot be completely mimicked in the dough analyses performed. Nevertheless, the authors consider fibre and protein content to be major key factors. These were found to be significantly high in lupin SF, leading to high water hydration properties. These were further understood to cause a lower damping factor and a higher viscosity, indicating a more elastic dough in comparison to the remaining sprouts. The elastic dough is assumed to restrain gas cell expansion during fermentation, leading to smaller bread volume. This was demonstrated in the dough rise measurement of the various dough formulations (Figure 6-2, Table 6-3).

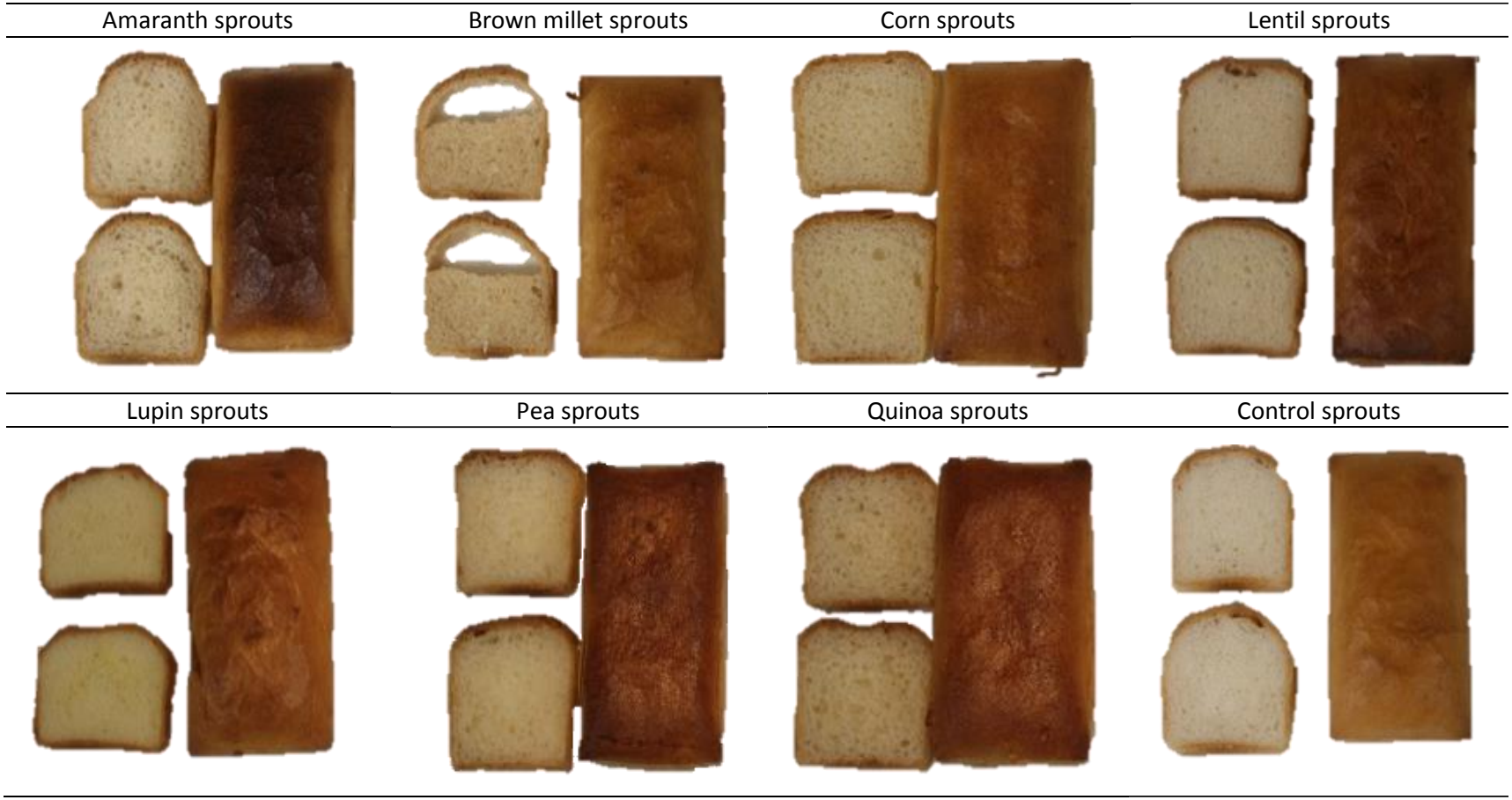

Figure 6-3 Cross sections of baked breads formulated with the different sprouted flours

Similar findings were observed in previous studies (Horstmann et al., 2017; Horstmann et al., 2018a,b).

Restrained gas cell expansion was confirmed by the results generated during breadcrumb analysis. The greatest cell diameter was measured in breads formulated with amaranth SF, while the smallest diameter was found in breads containing lupin SF and lentil SF. The diameter of cells, however, is not only influenced by the restrained gas cell expansion, but also the amount of CO_2 produced during fermentation. The different chemical composition of the SF provides the yeast with different amounts of nutrients for fermentation. In general, higher amounts of simple sugars lead to a greater production of CO_2, which ultimately leads to a greater cell diameter (Horstmann et al., 2018b). However, in this study, no link between available sugars and cell diameter could be established. The authors assume that the diverse enzyme activities provide further amounts of sugars for the yeast to metabolise. The additional sugars are fermented and increase the amount of CO_2 produced, which in turn increases gas cell expansion. In addition to the cell diameter, the number of cells must be considered when links to the specific volume are established. However, the number of cells did not show significant variation amongst the baked breads. Thus, it is not surprising that amaranth SF-containing breads showed the least cells per area and lupin SF and lentil SF. The application of amaranth SF, brown millet SF, quinoa SF and pea SF showed an increase in cell size compared to the control, while the remaining SFs produced either decreased the cell diameter or showed no significant difference. An increasing and decreasing effect on cell diameter was also recently reported by the addition of germinated oat and quinoa flour, respectively (Mäkinen et al., 2013).

A greater specific volume provides more surface area and hence facilitates water evaporation, leading to an increase in bake loss (Horstmann et al., 2017; Horstmann et al., 2018b). In this study, however, no significant differences between the bake loss of the baked breads were found. This is assumed to be caused by the variation in water hydration properties, being able to bind dissimilar amounts of water to the dough system. A higher amount of water in the dough system can lead to a softening of the breadcrumb (Fadda, Sanguinetti, Del Caro, Collar, & Piga, 2014).

Bread texture is an important quality parameter for consumer acceptance (Cauvain & Young, 2016). The hardness of bread after baking is influenced by the retrogradation process of amylose and amylopectin (Fadda et al., 2014). Furthermore, it was recently found that the number of cells per area and cell diameter also influence the breadcrumb hardness (Horstmann et al., 2018b). The authors hypothesised, that a higher cell diameter decreases the number of cell walls compressed by a measuring probe, leading to a softer breadcrumb. The hardness values of the baked breads showed significantly different results. Breads baked with amaranth SF, quinoa SF and pea SF showed a lower hardness in comparison to the control. The remaining SFs increased the hardness. An increase in hardness over time is defined as the staling process. During this process, water migrates from crumb to crust and recrystallization of starch proceeds, which alters the bread texture (Fadda et al., 2014). The crumb hardness of all the baked breads increased after 24h. However, after 24 h the crumb hardness of the various breads differed and did not correlated with that which was measured on the baking day, indicating differences in staling rates. Breads formulated with brown millet SF, pea SF and the control bread showed the significantly highest hardness values. The softest breadcrumb however, was found for breads formulated with amaranth SF. These results are within the range of hardness values previously reported for this model bread system (Horstmann et al., 2017; Horstmann et al., 2018a). A decreasing effect on hardness, by the addition of germinated sorghum flour, was recently reported by Phattanakulkaewmorie et al. (2011). The authors analysed the effect of different amounts of germinated sorghum flour on gluten-free bread properties. Another study also found a decreasing effect on bread hardness by the addition of germinated brown rice flour (Cornejo & Rosell, 2015). The authors found that a longer germination time leads to degradation of starch by alpha-amylase resulting thinner cell walls of the gluten-free breads. The effect of other enzyme activities and their effect on bread staling have been recently discussed. Lipase activity was described to alter the polarity of lipids which results in cell wall strengthen allowing greater gas cell explanation (Nunes, Moore, Ryan, & Arendt, 2009; Primo-Martín, Hamer, & de Jongh, 2006). However, in this study, no lipase activity was found in the analysed sprouts (data not shown). Proteolytic activities of germinated flours were reported to reduce crumb hardness in gluten-free bread (Renzetti & Arendt, 2009).

Table 6-4 Results of bread parameters baked with the different sprouted flours

	Amaranth sprouts	Brown millet sprouts	Quinoa sprouts	Lupin sprouts	Lentil sprouts	Pea sprouts	Corn sprouts	Control
Specific Volume [ml/g]	3.01±0.06^{a}	2.77±0.06^{ab}	2.71±0.10^{abc}	2.29±0.13^{d}	2.39±0.13^{cd}	2.98±0.17^{ab}	2.66±0.14^{bc}	2.42±0.11^{cd}
Bake loss [%]	18.25±0.65	18.02±0.52	17.25±0.57	16.88±0.44	16.90±0.41	18.21±0.69	17.66±0.39	16.88±0.38
Crumb structure								
Number of Cells [-]	2384.3±133.2	2181.9±183.8	2387.1±171.7	2351.5±122.6	2412.5±110.8	2341.1±225.2	2327.8 ±140.1	2534.3±124.7
Number of Cells / Area [-]	0.43±0.03^{c}	0.49±0.03^{abc}	0.45±0.04^{bc}	0.56±0.08^{ab}	0.59±0.03^{a}	0.45±0.02^{bc}	0.49±0.02^{abc}	0.51±0.03^{abc}
Cell Diameter [mm]	3.53±0.29^{a}	3.24±0.45^{ab}	2.95±0.31^{abc}	2.15±0.36^{cd}	1.86±0.14^{d}	2.75±0.28^{abc}	2.43±0.20^{bcd}	2.54±0.22^{bcd}
Crumb texture								
Hardness (0h) [N]	3.50±0.58^{d}	8.46±0.85^{a}	4.53±0.42^{cd}	7.02±0.75^{ab}	7.27±0.71^{ab}	4.69±0.62^{cd}	6.86±0.65^{ab}	5.77±0.69^{bc}
Hardness (24h) [N]	9.01±0.93^{c}	19.48±2.12^{a}	12.18±1.49^{bc}	16.68±2.34^{ab}	16.45±1.57^{ab}	18.39±2.99^{a}	14.28±1.37^{abc}	17.95±2.57^{a}
Colour								
L*-value	56.5±2.2^{cd}	55.8±2.0^{d}	58.0±2.8^{bcd}	63.9±2.1^{ab}	63.9±1.6^{abc}	67.6±3.9^{a}	62.5±2.6^{abcd}	62.9±3.2^{abcd}
a*-value	-0.4±0.12^{b}	0.6±0.10^{a}	-0.5±0.16^{b}	-1.8±0.07^{f}	-0.6±0.05^{bc}	-1.0±0.11^{d}	-1.5±0.12^{e}	-0.8±0.09^{cd}
b*-value	9.52±0.86^{b}	12.64±0.78^{b}	9.18±0.87^{b}	11.98±0.83^{b}	8.85±0.78^{b}	10.17±1.04^{b}	8.03±0.72^{b}	5.70±0.56^{a}

Means in the same row with different letters are significantly different (≥3 = One-way ANOVA; ≥2 0 =t-Test, $p < 0.05$).

However, the study also stated that the impact strongly depends on the applied matrix. Hence it is assumed, that the differences in chemical composition of the applied sprouts in this study created such aforementioned matrices.

This assumption is based on the generated results showing no correlation between protease activity and crumb hardness. The hardness and staling process can be further affected by other factors. Such factors could be the aforementioned formation of lipid-amylose complexes, protein-starch and or starch–hydrocolloid interactions (Horstmann et al., 2018a). The addition of the various SF further affected the colour values of the bread crumbs (Figure 6-3). For the evaluation of the changes in colour of the breadcrumb, the CIE-L*a*b* system was applied. The addition of amaranth, brown millet and quinoa sprouts reduced the L* value, which indicates a darker crumb. Lupin, lentil and pea sprouts, however, increased the L* value. The addition of corn sprouts showed no effect on the L* value compared to the control breadcrumb. Similar values have been reported by the addition of germinated brown rice flour (Cornejo & Rosell, 2015). They were further stated to be similar to those values reported for commercial gluten-free bread (María Estela Matos & Rosell, 2012). Detected a* and b* values of the bread crumbs baked with the different sprouts indicated an increase in yellow colour in comparison to the control. While the study by María Estela Matos and Rosell (2012) showed colour intensity changes due to germination time, in this study the main factor affecting colour change is attributed to the raw material applied.

Conclusion

In this study the effect of sprouted flour from different plants (amaranth, brown millet, corn, lentil, lupin, pea and quinoa) on a gluten-free dough and bread formulation was compared. The flours of the commercially purchased sprouts showed significant differences in their chemical composition. The low enzyme activity of the sprouted flours allowed their application in the gluten-free formulation at a concentration of 5 % w/w. The differences in composition were further found to influence the flour hydration properties, which in turn affected dough properties. Sprouted flour of lupin showed the highest flour hydration properties which were assumed to be caused by the specific chemical composition, high in fibre and protein. The high-water binding capacity was further postulated to be related to the higher viscosity and a more elastic

behaviour in comparison to the remaining sprouted flours. Doughs with more elastic behaviour were found to have a reduced dough rise, due to restrained gas cell expansion. The decreased gas cell expansion lead to smaller breads with a denser texture. However, the hardest breadcrumb was found in breads formulated with brown millet sprouted flour, which showed the lowest hydration properties. Hence, statistical analysis revealed no correlation between the chemical composition and the dough and bread properties. Thus, as discussed, this suggests the influence of more than one single factor, such as starch gelatinisation, protein denaturation, hydrocolloid / fibre gelling, enzymatic activity and their chemical interactions. Despite the various influencing factors, all the baked formulations containing the sprouted flours resulted in bread-like products and improved quality parameters in comparison to the control (no sprouted flour). The addition of amaranth sprouted flour increased the specific volume of baked breads significantly. It further reduced the crumb hardness. The chemical composition of amaranth was also suggested, based on its protein and ash/ mineral content to improve the nutritional value of gluten-free bread. This study demonstrated the successful application of gluten-free sprouted flours in a gluten-free bread system with the potential to increase the nutritional value of gluten-free breads.

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Chapter 7 General Discussion

As described in the in **Chapter 1**, coeliac disease is one of the most common food intolerances, caused by the ingestion of gluten (Gujral, Freeman, & Thomson, 2012). However, there are other gluten-related disorders which increase the number of people affected by gluten (Table 2-1). Due to improved diagnostic methods for the identification of people suffering from coeliac disease and other gluten-related disorders this number has further increased (Sapone et al., 2012). Maintenance of a strict gluten-free diet is currently the only, and safe, treatment available for these types of disorders (Koehler et al., 2014). This in turn increases the demand for gluten-free products and thus, increases the growth of the gluten-free market (Mintel, 2015). However, consumer studies showed that the quality of gluten-free products is still low and lacking in nutritional value (Rosell & Matos, 2015; Thompson, Dennis, Higgins, Lee, & Sharrett, 2005).

Chapter 2 describes that the development of high quality gluten-free bread remains the major technological challenge for the food industries. This is attributed to gluten which plays a key role in the bread-making process, especially in terms of dough rheology, sensory and shelf-life of the final product (Gallagher, Gormley, & Arendt, 2004; Moore, Schober, Dockery, & Arendt, 2004). There is no raw material, ingredient, or additive (or a combination thereof) that can yet fully replace the techno-functional properties of gluten. Thus, a combination of ingredients and/or additives is necessary to obtain gluten-free bread of good quality. Several studies highlighted the importance of including hydrocolloids in the formulations (Table 2-3). In addition, numerous alternative flours, starches and proteins from various sources have been included in gluten-free bread formulations due to their high nutritional value (Table 2-3). Nevertheless, the analysis of commercial gluten-free products revealed that not all research findings have been adopted by industry. The application of sourdough in gluten-free bread production has been discussed as one of the most promising technologies. It can improve the textural and sensorial properties and has the potential to increase the nutritional value of the final products (Galle, Schwab, Arendt, & Gänzle, 2010; Moore, Juga, Schober, & Arendt, 2007; Moroni, Dal Bello, & Arendt, 2009; RŘhmkorf, Jungkunz, Wagner, & Vogel, 2012; Sterr, Weiss, & Schmidt, 2009; Vogelmann, Seitter, Singer, Brandt, & Hertel, 2009; Wolter, Hager, Zannini, Czerny, & Arendt, 2014a, 2014b; Wolter, Hager, Zannini, Galle, et al., 2014). However, despite the

increased research in the area of gluten-free products, no definite conclusion can be made on the optimal raw material characteristics for the production of good quality gluten-free bread. Based on this, fundamental studies are needed to get a better understanding of ingredient functionalities in the gluten-free system.

Prior to this thesis, a preliminary study was conducted, which laid the foundation for this research (Horstmann et al., 2016). This study analysed five starches (rice, tapioca, corn, wheat, potato) for their application and suitability in a model gluten-free bread system. Potato starch offered good mechanical properties, suitable for a model bread system. To obtain fundamental knowledge on gluten-free bread formulations and their interactions different ingredients were applied and investigated in this model system.

In order to increase the nutritional value of gluten-free breads and improve the techno-functional properties, the addition of protein sources has been suggested in previous literature (Waglay, Karboune, & Alli, 2014). The application of plant proteins (potato, pea, carob, lupin, soy) to the model formulation was conducted and described in **Chapter 3**. The analysed proteins were found to be significantly different in their composition (Table 3-1), which caused significant differences in their functional properties. These in turn affected the dough and baked bread properties. Statistical analysis conducted, revealed correlations between protein properties and bread characteristics (Table 3-4). Foaming properties and the solubility of the proteins in the dough significantly correlated with dough properties, which further affected the final bread quality. Proteins with high foaming properties lead to a higher dough viscosity (r. 0.97, $p < 0.01$). The correlation between these two facts was hypothesised to be caused by the denaturation of proteins, increasing dough stability and viscosity. However, further studies on this hypothesis were suggested, to clarify this phenomenon. The solubility of proteins was also positively correlated with a viscosity increase ($p < 0.05$). It was suggested that the soluble parts of the analysed proteins distribute more evenly in the liquid phase, when mixed with water. This could create a stronger network, by linear aggregation when denatured. A random aggregation, which is mainly created by insoluble proteins, would create a weaker network (Zayas, 1997). The viscosity parameters affected by foaming properties and the solubility, further correlated with crumb cell and the specific volume of the baked breads ($p < 0.05$). A higher viscosity had a negative effect on the specific volume of the bread (r. -0.89, $p < 0.05$). The

relationship between viscosity and specific volume was explained by reduced gas cell expansion. An increased viscosity would decrease gas cell expansion and hence result in a smaller bread. Overall, the addition of potato protein and soy protein to the bread formulation resulted in smaller breads with denser crumb structure in comparison to the other proteins (Figure 3-3, Table 3-3). The addition of carob, lupin and pea, however, resulted in a high volume with greater cell pores and a softer bread crumb. Based on the even crumb structure, low hardness, high volume and non-allergenic claim, pea protein was selected for further research.

Due to their ability to mimic the viscoelastic properties of gluten, the most commonly used hydrocolloids in gluten-free formulations are HPMC and xanthan gum (Cato, Gan, Rafael, & Small, 2004; Mariotti, Pagani, & Lucisano, 2013; Peressini, Pin, & Sensidoni, 2011). These two hydrocolloids are also known to reduce staling, improve water binding and the overall structure of bread. **Chapter 4** compares the most commonly used hydrocolloids in gluten-free bread formulations (HPMC, Xanthan) with less commonly used gums (pectin, sodium alginate, guar gum, locust bean gum). To keep the influencing factors limited, the application of hydrocolloids at different concentrations (0.25%, 0.5%, 1.0%, 1.5%, 2.0%) in the gluten-free bread formulation was analysed, without the suggested pea protein from Chapter 3. Based on the different water absorption properties (Table 4-1) of the various hydrocolloids and their concentration (Table 4-2), an equation was developed to add the optimal water amount to the bread formulations. This allowed the formulation of bread-like products with all hydrocolloids at the different concentrations. However, despite the adjusted water contents, the breads showed significant differences and revealed different optimal hydrocolloid concentrations. A two-way ANOVA was conducted to identify the main contributing factors for the differences in baking performance. The main contributing factor influencing the specific volume of baked breads was the type of hydrocolloid used (65.5%, $p < 0.05$). Correlation analysis revealed a link between the specific volume and the viscosity profiles, measured with the rapid visco analyser (r. -0.89, $p < 0.05$). The authors hypothesised that the different charge and molecular weight of the various hydrocolloids influenced the dough viscosity and hence the baking performance of the breads (Table 4-3). It was also hypothesised that repelling forces created by the negative charges of some hydrocolloids (sodium alginate, pectin), and the negatively charged

phosphate groups of potato starch, reduced the granule swelling of the potato starch (Shi & BeMiller, 2002). The reduced granule swelling lowers the dough viscosity. A decreased viscosity, as discussed above, can better facilitate gas cell expansion during fermentation, resulting in a higher bread volume. Hydrocolloids without a negative charge and a high molecular weight (guar gum, locust bean gum) however, are understood to create many hydrogen bonds with leached amylose resulting in high viscosities and restraining gas cell expansion (Morris et al., 2008). This shows that the molecular weight had a stronger effect than the water. However, HPMC with its neutral charge, resulted in low dough viscosities, similar to hydrocolloids with a negative charge. This is due to its surface-active components which stabilize the gluten-free doughs due to gas cell stabilisation at the gas – liquid interface. The order of the contributing factors regarding crumb texture was as follows: type of hydrocolloid (28.94%, $p < 0.05$), concentration (45.46%, $p < 0.05$) and interaction (19.89%, $p < 0.05$). Based on these results the concentration was used as the most influential factor and subjected to the Holm-Sidak test resulting in groupings. The statistical analysis revealed that concentration levels of 2% resulted in the softest breads while the 0.25% level produced the hardest. This result was assumed to be caused by the higher amount of water added for higher concentrations of hydrocolloid and the replacement of starch by more hydrocolloids. This caused lower interactions between starch and hydrocolloids, reducing the retrogradation and recrystallization (Funami et al., 2005). In addition, the two-way ANOVA evaluation indicated that pectin was the significantly best performing hydrocolloid in improving the bread quality parameters. It reached its maximum potential at a concentration of 2% (Figure 4-3, Table 4-4).

The fermentation plays a key role in the breadmaking process, as it can improve the texture, structure, taste and flavour in the final product (Fleet, 2007). In **Chapter 5** different yeasts from the species *Saccharomyces cerevisiae* (T-58 Ale yeast; US-05 Ale yeast; S-23 Lager yeast; WB-06 wheat beer yeast, Baker's yeast) were applied to a model bread system. The model-bread system was formulated from the knowledge gained and findings from Chapters 3 and 4. Hence, the model-bread system used in this study contained potato starch, pea protein, pectin, sugar, salt, yeast and water. The findings revealed differences in dough and bread quality parameters. Doughs fermented with the various yeast strains showed differences in sugar metabolism and pH, indicating

different level of metabolic activity (Table 5-3). This hypothesis was confirmed by the results obtained from the time-temperature dependant dough rise, which further highlighted differences in yeast performance (Table 5-3). These differences in the dough rise were further reflected in the baked breads (Figure 5-2, Table 5-4). Overall, the application of the yeast strain, US-05, showed a decrease in loaf volume and a high increase in crumb hardness in comparison to the control yeast. On the contrary, strain T-58 was found to improve the loaf volume and soften the bread crumb. The closest resemblance to Baker's yeast, regarding the baked breads, was found to be the yeast strain WB-06. Statistical analysis (Table 5-6) showed correlations between yeast activity indicators, such as pH and remaining levels of sugar, and the dough rise parameters ($r. > 0.70$). These further correlated with crumb structure, loaf volume and texture of the baked breads ($r. > 0.75$). Volatile aroma compound analysis detected only low amounts of volatiles. This explained the lack of significant differences in the results of the trained panel for the descriptive sensory. The low production of volatiles was suggested to be caused by the refined gluten-free system in this study, which lacks certain nutrients for the yeast metabolism. In summary, it was found that the different yeasts only affected the technological properties, rather than the flavour and aroma profile of the baked breads. This study demonstrated the suitability of different strains of *S. cerevisiae* for application in gluten-free bread.

As the first few chapters focused on improving the texture and structure of the model bread system, **Chapter 6** focused on improvement of the nutritional value, by maintaining the quality parameters optimised for the model bread system. Sprouted flours (SF) have been promoted by the literature for improving the nutritional profile of gluten-free bakery products (Deora et al., 2014; Omary et al., 2012). Therefore, the effect of SF from different botanicals (amaranth, brown millet, corn, lentil, lupin, pea and quinoa) on a gluten-free dough and bread formulation was compared. Differences in the chemical composition of the various sprouted flours were determined (Table 6-1). The detected low enzyme activity of the SFs allowed their application in gluten-free formulation at a concentration of 5%, since a liquification of the dough could be ruled out. Further differences in the flour hydration properties were observed (Table 6-1). Sprouted flour of lupin showed the highest flour hydration properties which was assumed to be caused by its specific chemical composition, which is high in fibre and

protein. A high-water binding capacity was hypothesised to cause higher viscosity and a more elastic behaviour in a dough. Increased elastic behaviour, as discussed throughout this study, was found to have a reduced dough rise, due to the restraint of gas cell expansion. This ultimately lead to smaller bread volumes (Figure 6-3, Table 6-5). Usually a small volume is connected with a harder bread crumb, due to the density of the crumb and the thickness of cell-walls. However, the hardest crumb was found in breads formulated with brown millet sprouted flour, which showed the lowest hydration properties. Statistical analysis (Pearson) showed no correlations between the chemical composition and the dough and bread properties. This was suggested to be caused by the various and numerous interacting factors influencing the bread making process, such as starch gelatinisation, protein denaturation, hydrocolloid / fibre gelling, enzymatic activity and their chemical interactions. Based on the complexity of the model bread system it was not possible to pinpoint any one single factor which influenced the outcome of the baked breads. However, despite the differences in chemical composition (Table 6-1) and dough properties (Figure 6-1,6-2; Table 6-4), all the baked formulations containing the sprouted flours resulted in bread-like products and improved quality parameters in comparison to the control (no sprouted flour). The addition of amaranth sprouted flour increased the specific volume of baked breads significantly. It further reduced the crumb hardness. Amaranth, based on its chemical composition, could contribute to improved nutritional value of gluten-free bread.

Over the course of this thesis a significant improvement of the original gluten-free model bread system could be observed (Figure 7-1). The final gluten-free bread formulation contained pea protein, pectin and milled amaranth sprout. Despite a decreased, but still acceptable, specific volume, it had a softer bread crumb, a delayed staling rate and improved nutritional value. The study further confirms the complexity of gluten-free breads. Nevertheless, it was found that various dough formulations resulted in bread like products. They showed the potential to develop bread formulations with a reduced number of ingredients. Further research, implementation of the knowledge gained and application of other ingredients could allow the production of gluten-free bread without chemical additives. This is suggested based on the authors assumption that in gluten-free bread baked from numerous different ingredients, many ingredients counteract each other.

The knowledge gained in this thesis enables the prediction of the impact of plant proteins, hydrocolloids, yeast strains and sprouted flours in a gluten-free bread formulation. Based on the analysis of a comprehensive range of ingredients, it also suggests and shows the suitability of individual ones. The thesis further provides new methodologies for the analysis of dough, and methods to adjust the water content. All this lays the foundation for future research and can help food industry to enhance the gluten-free bread quality, in addition to improving the nutritional value. Ultimately the author is confident that the study would have an impact on the life quality of people who suffer from gluten-related disorders.

This thesis offers fundamental knowledge about the application of different ingredient categories (protein, hydrocolloid, yeast, sprouted flour). This sets the basis for further research on the application of other ingredient categories such as active or inactive sourdough to the gluten-free model bread system is suggested. The addition of sourdough could beneficially influence the flavour and aroma profile, the mechanical properties and also the nutritional profile of the gluten-free bread. Besides the application and understanding of interactions and effects of different ingredients on a gluten-free system, the process parameter offers further potential for research. Process parameters of the bread making process such as mixing speed and time, proofing and the baking process parameters temperature and time are key factors. Therefore, future research on these process parameters and their effect on the developed gluten-free model bread system is suggested. The combined knowledge of the understanding of the effect of ingredient and process parameter would contribute to the research of gluten-free bread products.

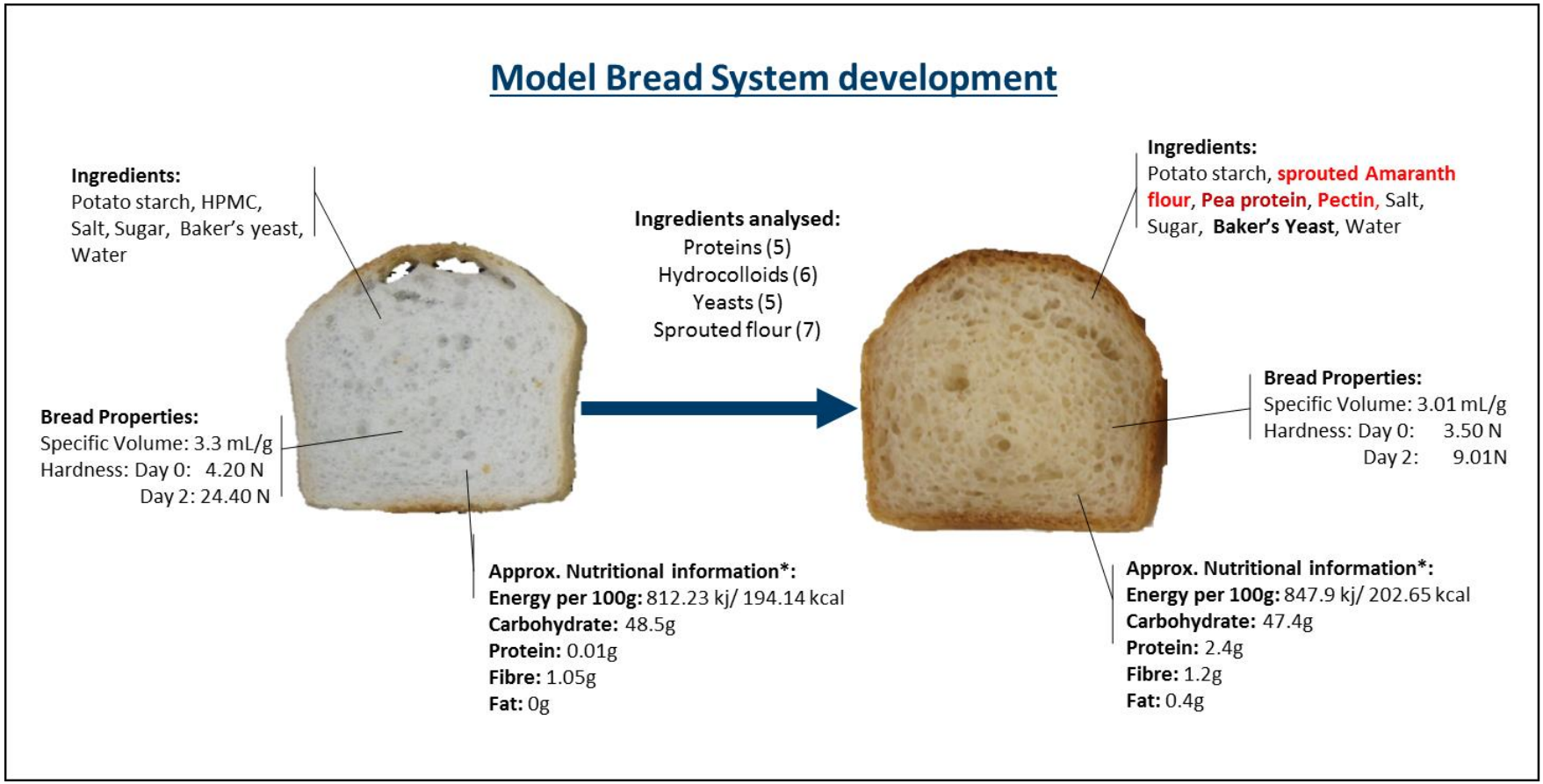

Figure 7-1. Overall improvement of the gluten-free model bread, comparing the initial formulation with the one developed through the research in this thesis.

*calories were calculated by applying the following factors: carbohydrates: 4; protein:4; fibre: 0; fat: 9;

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Appendix I Tables and Figures

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Appendix II Publications and Presentations

Peer reviewed first author publications:

Horstmann, S.W., Belz, M.C., Heitmann, M., Zannini, E. and Arendt, E.K., 2016. Fundamental study on the impact of gluten-free starches on the quality of gluten-free model breads. *Foods, 5(2), p.30.*

Horstmann, S.W., Foschia, M., Arendt, E.K. and Zannini, E., 2016. Nutritional therapy–facing the gap between coeliac disease and gluten-free food. *International journal of food microbiology, 239, pp.113-124.*

Horstmann, S.W., Lynch, K.M. and Arendt, E.K., 2017. Starch characteristics linked to gluten-free products. *Foods, 6(4), p.29.*

Horstmann, S.W., Foschia, M. and Arendt, E.K., 2017. Correlation analysis of protein quality characteristics with gluten-free bread properties. *Food & function, 8(7), pp.2465-2474.*

Horstmann, S.W., Axel, C. and Arendt, E.K., 2018. Water absorption as a prediction tool for the application of hydrocolloids in potato starch-based bread. *Food Hydrocolloids*, *81 (2018): pp. 129-138*

Horstmann, S.W., Atzler, J.J., Heitmann, M., Zannini, E., Arendt, E.K., **2018** Impact of different S. cerevisiae yeast strains on gluten-free dough and bread quality parameters. *Eur Food Res Technol*

Horstmann, S.W., Atzler, J.J., Heitmann, M., Zannini, E., Lynch, K., Arendt, E.K., **2018.** A comparative study of gluten-free sprouts in the gluten-free breadmaking process. *Eur Food Res Technol*

Other

Second author publications:

Foschia, M., Horstmann, S.W., Arendt, E.K. and Zannini, E., 2017. Legumes as Functional Ingredients in Gluten-Free Bakery and Pasta Products. *Annual review of food science and technology, 8, pp.75-96.*

Schmidt, M., Horstmann, S.W., De Colli, L., Danaher, M., Speer, K., Zannini, E. and Arendt, E.K., 2016. Impact of fungal contamination of wheat on grain quality criteria. *Journal of Cereal Science, 69, pp.95-103.*

Article in Scientific Magazine

Horstmann, S.W., & Arendt, E.K. Glutenfreie Backwaren: Aktuelles aus der Forschung. In Cereal Technology Ed. Lösche, Klaus. INGER Verlagsgesellschaft mbH Osnabrück, Germany (1)32-45.

Oral & Poster Presentations:

Horstmann, S.W., Belz, M., Zannini, E., Arendt, E. K. (2014). Characterisation of gluten-free starches and their evaluation in dough and model starch bread systems. *13th European Young Cereal Scientists and Technologists Workshop. Freising, Germany. May, 13-16th.*

Horstmann, S.W., Arendt, E.K. (2015). Gluten-free starch characterization and utilization in gluten-free bread. *Training Workshop on creating value in wheat and gluten-free based bakery production chain. Cork, Ireland. May 15-16th.*

Horstmann, S.W. and Arendt, E.K. (2016). Gluten-free starch characterization and utilization in gluten-free bread. *IUFOST 18th World Congress of Food Science and Technology, Dublin, Ireland, August 2016*

Horstmann, S.W. and Arendt, E.K. (2016). Aktuelles aus der Forschung im Bereich gluten-freier Backwaren. *Tagung für Bäckerei-Technologie, Detmold, Germany, November 2016*

Horstmann, S.W., Foschia, M. and Arendt, E.K. (2016) Fundamental study on the effect of protein addition to model gluten-free bread formulations. *4th International Symposium on Gluten-Free Cereal Products and Beverages, Cork, Ireland. October 2016*

Horstmann, S.W., Axel, C. and Arendt, E.K. (2017) Development of a prediction tool for the application of hydrocolloids in a potato starch based bread. *6th C&E Spring Meeting "Great Grain Products for the Future" and 7th European Symposium on Enzymes in Grain Processing. Amsterdam, Netherlands. June 2017*

Horstmann, S.W., Axel C. and Arendt, E.K. (2017) Development of a prediction tool for the application of hydrocolloids in a potato starch-based bread. *46th Annual Food Science and Technology Conference, Dublin, Ireland. December 2017*

Horstmann, S.W., Atzler, J.J., Heitmann, M., Zannini, E., Arendt, E.K. (2018) Impact of different S. cerevisiae yeast strains on gluten-free dough and bread quality parameters. 7th International Sourdough Symposium, Cork, Ireland. June 2018

Poster Presentation

Horstmann, S.W., Belz, M., Zannini, E., Arendt, E. K. (2014). Gluten-free starch characterization and utilization in gluten-free products. *4th International Symposium on Gluten-Free Cereal Products and Beverages, Cork, Ireland. October 2016*

Awards: 1st place for best presentation:

Horstmann S.W., Foschia M. and Arendt E.K. (2016) Fundamental study on the effect of protein addition to model gluten-free bread formulations. *4th International Symposium on Gluten-Free Cereal Products and Beverages, Cork, Ireland. October 2016*

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