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University College Cork, Ireland Coláiste na hOllscoile Corcaigh Ollscoil na hÉireann National University of Ireland

Coláiste na hOllscoile, Corcaigh School of Food and Nutritional Sciences



# Fundamental studies of sourdoughs fermented with *Weissella cibaria* and *Lactobacillus plantarum*:

### Influence on baking characteristics, sensory profiles and *in vitro* starch digestibility of gluten-free breads

Thesis presented by

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Under the supervision of

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to obtain the degree of

Doctor of Philosophy - PhD in Food Science and Technology

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#### Declaration

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

hika woltes

Signature of candidate

#### Abbreviations

A<sub>F</sub>, dough strength; AUC, area under curve; a<sub>w</sub>, water activity; BF, based on flour; Ctrl, control bread; DNS, 3,5-dinitro salicylic acid; DS, Damaged Starch; DSC, differential scanning calorimetry; DTT, dithiothreitol; dwb, dry weight basis; dy, dough yield; EPS, exopolysaccharide; G\*, complex modulus; G', elastic modulus; G", viscous modulus; GlcOS, glucoseoligosaccharides; HI, hydrolysis index; HLA, human leucocyte antigen; HMW, High Molecular Weight; HHP, High Hydrostatic Pressure; ICP-AES, Inductively Coupled Plasma-Atomic Emission Spectroscopy; LAB, lactic acid bacteria; LMW, Low Molecular Weight, Lp, Lactobacillus plantarum FST1.7; MALS, Multi-Angle Light Scattering, ME, maltose equivalent; PGA, propylene glycol alginate; pGI, predicted glycaemic index; pGL, predicted glycaemic load; PSO, panose-series oligosaccharides RH, relative humidity; **RI**, Refractive Index; RS, resistant starch; RSR, reducing sugars released; SD, sourdough; SEC, Size Exclusion Chromatography SEM, scanning electron microscopy; TAC, total available carbohydrates; T<sub>E</sub>, end temperature; T<sub>0</sub>, onset temperature; T<sub>P</sub>, peak temperature; TTA, total titratable acid; Wc, Weissella cibaria MG1; WL, water addition level; z, network connectivity;

#### Abstract

The application of sourdough can improve texture, structure, nutritional value, staling rate and shelf life of wheat and gluten-free breads. These quality improvements are associated with the formation of organic acids, exopolysaccharides (EPS), aroma or anti-fungal compounds. Initially, the suitability of two lactic acid bacteria strains to serve as sourdough starters for buckwheat, oat, quinoa, sorghum and flours was investigated. Wheat flour was chosen as a reference. The obligate heterofermentative lactic acid bacterium (LAB) Weissella cibaria MG1 (Wc) formed the EPS dextran (a  $\alpha$ -1,6-glucan) from sucrose in situ with a molecular size of 10<sup>6</sup> to 10<sup>7</sup> kDa. EPS formation in all breads was analysed using size exclusion chromatography and highest amounts were formed in buckwheat (4 g/kg) and quinoa sourdough (3 g/kg). The facultative heterofermentative Lactobacillus plantarum FST1.7 (Lp) was identified as strong acidifier and was chosen due to its ubiquitous presence in gluten-free as well as wheat sourdoughs (Vogelmann et al. 2009). Both Wc and Lp, showed highest total titratable acids in buckwheat (16.8 ml; 26.0 ml), teff (16.2 ml; 24.5 ml) and quinoa sourdoughs (26.4 ml; 35.3 ml) correlating with higher amounts of fermentable sugars and higher buffering capacities. Sourdough incorporation reduced the crumb hardness after five days of storage in buckwheat (Wc -111%), teff (Wc -39%) and wheat (Wc -206%; Lp -118%) sourdough breads. The rate of staling (N/ day) was reduced in buckwheat (Ctrl 8 N; Wc 3 N; Lp 6 N), teff (Ctrl 13 N; Wc 9 N; Lp 10 N) and wheat (Ctrl 5 N; Wc 1 N; Lp 2 N) sourdough breads. Bread dough softening upon Wc and Lp sourdough incorporation accounted for increased crumb porosity in buckwheat (+10.4%; +4.7), teff (+8.1%; +8.3%) and wheat sourdough breads (+8.7%; +6.4%). Weissella cibaria MG1 sourdough improved the aroma quality of wheat bread but had no impact on aroma of gluten-free breads. Microbial shelf life however, was not prolonged in any of the breads regardless of the starter culture used. Due to the high prevalence of insulin-dependent diabetes mellitus particular amongst coeliac patients, glycaemic control is of great (Berti et al. 2004). The *in vitro* starch digestibility of gluten-free breads with and without sourdough addition was analysed to predict the GI (pGI). Sourdough can decrease starch hydrolysis in vitro, due to formation of resistant starch and organic acids. Predicted GI of gluten-free control breads were significantly lower than for the reference white wheat bread (GI=100). Starch granule size was investigated with scanning electron microscopy and was significantly smaller in quinoa flour (<2  $\mu$ m). This resulted in higher enzymatic susceptibility and hence higher pGI for guinoa bread (95). Lowest hydrolysis indexes for sorghum and teff control breads (72 and 74, respectively) correlate with higher gelatinisation peak temperatures (69°C and 71°C, respectively). Levels of resistant starch were not increased by addition of *Weissella cibaria* MG1 (weak acidifier) or Lactobacillus plantarum FST1.7 (strong acidifier). The pGI was significantly decreased for both wheat sourdough breads (Wc 85; Lp 76). Lactic acid can promote starch interactions with gluten hence decreasing starch susceptibility (Östman et al. 2002). For most gluten-free breads, the pGI was increased upon sourdough addition. Only sorghum and teff Lp sourdough breads (69 and 68, respectively) had significantly decreased pGI. Results suggest that the increase of starch hydrolysis in gluten-free breads was related to mechanism other than presence of organic acids and formation of resistant starch.

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## Chapter 1 Introduction

Cereal products are important staple foods for the human diet (EFIC 2013). However, the digestion of the storage protein gluten (present in wheat and related grains like barley, rye and triticale) releases a peptide from the  $\alpha$ -gliadin fraction which induces a systemic immune-mediated disorder, called coeliac disease, in genetically susceptible persons (Green and Cellier 2007; Fasano and Catassi 2012). Worldwide, 0.6-1.0% of the population is affected by this auto-immune disease which damages the intestinal mucosa through inflammation of the micro-villi and thereby deteriorates the ability to absorb nutrients (Green and Cellier 2007; Fasano and Cellier 2007; Fasano and Catassi 2012).

Currently, the only available treatment is adherence to a gluten-free diet (Arendt et al. 2011) which can reverse the intestinal damage (Green and Cellier 2007). Although a wide range of gluten-free flours are available, gluten-free breads often possess poor sensory characteristics such as dry crumb, poor mouth feel and off-flavours. They also cause high glycaemic responses (Berti et al. 2004) and often lack nutritional quality (Gallagher 2009; Hager et al. 2011). The use of nutrient-dense flours, for example, quinoa, buckwheat or teff, may improve the nutritional value (Hager et al. 2012b) but does not provide a network forming protein. Hydrocolloids, such as xanthan, carrageen and agar, are generally used in gluten-free bakery products as a replacement for gluten and thereby to improve crumb structure (Gallagher et al. 2004; Anton and Artfield 2008; Hager et al. 2013).

Traditional sourdough is a mixture of flour and water which is fermented by the combined metabolic activity of lactic acid bacteria (LAB) and yeast (Gänzle et al. 1998). The addition of sourdough previously improved flavour, texture and shelf life (Gänzle et al. 2007) of conventional bread due to the synthesis of aroma compounds (Czerny and Schieberle 2002; Hansen and Schieberle 2005), enzymes and antifungal compounds during fermentation (Ryan et al. 2008; Poutanen et al. 2009). Especially, the synthesis of exopolysaccharides (EPS) by lactic acid bacteria during sourdough fermentation has gained increasing interest to improve textural bread properties. However, the performance in baking applications is determined by the structure of the polysaccharides and is also affected by the flour composition, recipe and parameters used for dough processing and baking. Depending on their composition, EPS can be divided into homopolysaccharides (HoPS), consisting of one type of monosaccharide being

either glucose (glucan) or fructose (fructan), and into heteropolysaccharides (made of 3-8 multiple, repeated moieties) (De Vuyst et al. 2001; van Hijum et al. 2006). HoPS-producing lactic acid bacteria are already used in conventional bread making (Decock and Cappelle 2005; Tieking and Gänzle 2005; Lacaze et al. 2007) to improve textural properties as well as shelf life. Their use is also particularly promising in gluten-free baking (Schwab et al. 2008; Galle et al. 2012; Ruehmkorf et al. 2012), since EPS can potentially act as hydrocolloids (Di Cagno et al. 2006; Schwab et al. 2008).

The obligate heterofermentative strain *Weissella cibaria* MG1 has improved bread quality of wheat and sorghum bread due to the production of dextran (a  $\alpha$ -1,6-linked glucan) from sucrose (Galle et al. 2010; Galle et al. 2012). Yet, comparable to other *Weissella* species, acetate formation was low, since *W. cibaria* MG1 lacks mannitol dehydrogenase activity to convert fructose to mannitol with concomitant acetate formation (Galle et al. 2010). An excess of acetate influences the quality of bread negatively (Tieking and Gänzle 2005; Kaditzky et al. 2008; Galle et al. 2010). In addition, selected lactic acid bacteria strains have been able to generate very specific aroma profiles and odorant compositions (Czerny et al. 2005). This could improve undesirable aroma exhibited by breads made from gluten-free flours (Hager et al. 2012a).

The suitability of the two lactic acid bacteria, *Weissella cibaria* MG1 and *Lactobacillus plantarum* FST1.7, to serve as sourdough starters and to influence bread quality and flavour profile of buckwheat, oat, quinoa, sorghum and teff bread was investigated (Chapters 3, 4 and 5).

The facultative heterofermentative strain *Lactobacillus plantarum* has been one of the key species during wheat fermentation (Gänzle et al. 2007). In addition, *L. plantarum* was also among the dominant lactic acid biota in gluten-free sourdoughs from rice, amaranth, quinoa (Vogelmann et al. 2009) or buckwheat and teff flour (Moroni et al. 2011). Previously, the strain has improved staling rate and crumb hardness of a brown rice, buckwheat based, gluten-free formulation (Moore et al. 2007). The fermentation and baking performance of gluten-free flours was compared to the gluten-containing counterpart wheat flour.

An increased risk of autoimmune disorders, especially insulin-dependent diabetes mellitus, occurs in patients with coeliac disease when compared to the

general population (Collin et al. 1994; Holmes 2002; Goh and Banerjee 2007). Therefore, the maintenance of glycaemic control is an important task for coeliac disease patients (Berti et al. 2004). The postprandial glycaemic effect of foods is related to the rate of carbohydrate digestion and reliably characterized by the glycaemic index (GI) (Jenkins et al. 2002).

The GI is defined as the incremental area under the curve (AUC) of the blood glucose concentration occurring upon ingestion of a carbohydrate-containing food relative to a reference food (glucose or white wheat bread=100) (Jenkins et al. 1981). Foods can be distinguished into those with low (<55; legumes, nuts, dairy products and pasta), intermediate (55-70; muesli, certain breads) and high GI (>70; whole meal barley flour bread, white wheat bread) (Atkinson et al. 2008). Breads fall into intermediate (55-70) to high GI (>70) categories (Atkinson et al. 2008) due to increased enzymatic susceptibility upon starch gelatinization during the baking process (Haralampu 2000).

The glycaemic response depends on indigenous factors of the food matrix (starch susceptibility, protein and lipid content) as well as on the macroscopic structure of the food (botanical integrity of ingredients, physical texture). Starch susceptibility again is determined by its native structure, physical encapsulation, degree of gelatinisation and retrogradation of the starch granules, as well as by the proportion of damaged granules (Fardet et al. 2006). The rate of *in vitro* starch hydrolysis during a multi-enzyme dialysis system corresponded well with the postprandial blood glucose response (Singh et al. 2010). The "International Tables of Glycemic Index Values" also contain GIs of three gluten-free breads: (Atkinson et al. 2008). Nevertheless, information about starch digestibility and glycaemic response is scarce. If so, studies on the GI of gluten-free breads have been conducted on composite recipes (Matos Segura and Rosell 2011; Capriles and Areas 2013) making it difficult to estimate the influence of the individual starch on enzymatic susceptibility.

Therefore, using a multi-enzyme dialysis system the influence of starch properties of buckwheat, oat, quinoa, sorghum or teff flour on *in vitro* starch hydrolysis was evaluated using a basic gluten-free bread formulation (Chapter 6).

The nutritional value of bread can be improved by sourdough application (Liljeberg et al. 1995; Arendt et al. 2011). The presence of organic acids formed

during sourdough fermentation reduced postprandial glucose response for wheat bread (De Angelis et al. 2007; De Angelis et al. 2009; Scazzina et al. 2009; Lappi et al. 2010; Borczak et al. 2011). The effect was linked to the decreased pH and subsequent inhibition of hydrolytic enzymes *in vivo* (Liljeberg et al. 1995) (De Angelis et al. 2007). Biological acidification of breads lowered starch hydrolysis more effectively than chemical acidification (De Angelis et al. 2007). The inclusion of lactic acid into a barley flour/ water mixture prior to heat treatment reduced starch hydrolysis significantly by promoting starch-gluten interactions during starch gelatinization in comparison to addition after heat treatment (Östman et al. 2002).

Alternatively, sourdough or lactic acid addition promoted starch retrogradation (Liljeberg et al. 1996) which increased formation of resistant starch (RS) (Scazzina et al. 2009). RS is defined as the sum of starch and its degradation products resistant to enzymatic attack and not absorbed in the small intestine of healthy individuals (Champ et al. 1994). It possesses a highly ordered molecular structure (Östman et al. 2002). Increased contents of RS were previously linked with reduced starch digestibility *in vivo* for white wheat bread (Brighenti et al. 1998; De Angelis et al. 2007). The presence of organic acids possibly facilitates the formation of RS, through debranching of amylopectin moieties during baking (Brighenti et al. 1998) (Berry 1986). RS starch can surround starch granules and thereby limits the degree of gelatinization, or forms a physical barrier to enzymatic attack by  $\alpha$ -amylases (EC 3.2.1.1) (Liljeberg et al. 1996).

Also, for gluten-free breads, a decreased glycaemic response was found *in vivo* upon sourdough addition (15-22%) in comparison to non-acidified control bread (Novotni et al. 2012). This was linked to the presence of organic acids. The influence of sourdough addition on starch digestibility and glycaemic indexes was investigated in Chapter 7 using the obligately heterofermentative strain *Weissella cibaria* MG1 (Wc) (low acids producer) and the facultatively heterofermentative strain *Lactobacillus plantarum* FST1.7 (Lp) (strong acidifier). Formation of resistant starch and starch hydrolysis was investigated for gluten-free sourdough breads in comparison to the reference white wheat bread. Predicted glycaemic indices were derived from hydrolysis curves of enzymatic starch digestion of sourdough breads.

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## Chapter 2 Literature review -Functional replacements for gluten

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#### 2.1 Background of gluten-free diet

Gluten is the structure forming, main storage protein in wheat consisting of gliadin and glutenin. The ingestion of the proline-rich gliadin and related proteins of rye (secaline) and barley (hordein) in genetically susceptible individuals triggers an immune-mediated enteropathy called "coeliac disease" (Green and Cellier 2007). This auto-immune disease affects ~1% of the world population. Inflammation and damage of intestinal villi cause an absorptive dysfunction of the mucosa (Trier 1991). Therefore, symptoms are various and comprehensive (manifestation amongst others as dysfunction of intestine, constipation, malabsorption of nutrients), which impedes diagnosis (Catassi and Fasano 2008). Mucosal damage proven by duodenal biopsy (Oberhuber et al. 1999) and immunological evidence of specific antibodies in patient's serum (Ciacci et al. 2002) are the main diagnostic evidences.

The only treatment to relief symptoms and regenerate mucosa is a life-long, gluten-free diet (Marsh 1992).

#### 2.2 The function of gluten in bread

Gluten is the "structural" protein for wheat bakery products, particularly in yeast leavened ones, presenting a major determinant of important dough properties such as gas retaining ability, mixing tolerance, resistance to stretch and extensibility (Gallagher et al. 2004). Thus, gluten is of fundamental importance for the overall appearance and textural properties of cereal-based baked products. In wheat breads, the solid matrix of the crumbs consists of a continuous phase of gelatinised starch (Durrenberger et al. 2001) and a continuous gluten network which encloses the starch granules and fibre fragments. In gluten-free breads, this continuous protein network able to embed the starch granules is missing. In fact, the absence of gluten results in a liquid batter rather than dough, and is responsible for the deficient quality characteristics compared to wheat breads. A marketing review conducted at University College Cork found that most of the gluten-free products were of low quality, exhibiting poor mouth-feel and very often showing off-flavours (Arendt et al. 2002). For these reasons, the replacement of the gluten network in the development of gluten-free cereal products, by using alternative ingredients and treatments, is a challenging task for the cereal technologist and the baker. The Commission of Codex alimentarius (2008) of the World Health Organisation

(WHO) and the Food and Agricultural Organisation (FAO) agreed on a standard for application of the term "gluten-free". Accordingly, "gluten-free" foods (a) consist of or are made only from one or more ingredients which do not contain wheat (i.e. all *Triticum* species, such as durum wheat, spelt and kamut), rye, barley, oats or their crossbred varieties, and the gluten level does not exceed 20 mg/ kg in total and/ or (b) consist of one or more ingredients from wheat (i.e. all *Triticum* species, such as durum wheat, spelt and kamut), rye, barley, oats or their crossbred varieties, which have been specially processed to remove gluten, and the gluten level does not exceed 20 mg/ kg in total.

#### 2.3 The formulation of gluten-free bakery products

In the past decades there has been extensive research on the development of gluten-free bakery products. The gluten-free bread production is similar to the wheat counterparts. However, the process for gluten-free bread differs in terms of complexity of recipes and the water that is added to the recipe (85-125%) (Renzetti et al. 2008b; Hager et al. 2012a) and therefore, dough behaviour, appearance and properties resemble more a cake-batter than bread-dough (Figure 2-1).



Figure 2-1 Schematic of procedure of wheat- and gluten-free bread production Plants that are not closely related to wheat are according to Kasadra et al. (2001) suitable for consumption by coeliac patients. This includes sorghum, millet varieties and Job's tear (*Coix lacryma-jobi*), as well as buckwheat, amaranth and quinoa as pseudo-cereals. Various approaches included the use of

(i) gluten-free flours (rice, sorghum, oats, buckwheat, amaranth, quinoa, teff, corn) (Hager et al. 2012a) (Figure 2-2), (ii) starches, (iii) dairy products (Gallagher et al. 2004), (iv) protein supplementation i.e. egg, soya and maize proteins, (v) gums and hydrocolloids (Gallagher et al. 2004; Schober et al. 2005) (vi), the use of functional ingredients and (vii) alternative technologies such as sourdough fermentation, enzymatic processing (Renzetti et al. 2010) and high hydrostatic pressure processing (Hüttner et al. 2010a). Recent scientific approaches are reviewed below.



### Figure 2-2 Photographs of crust surface and crumb of bread loaves prepared from 100% gluten-free flours and wheat flours

Reprint from "Investigation of product quality, sensory profile and ultrastructure of breads made from a range of commercial gluten-free flours compared to their wheat counterparts", Hager, Anna-Sophie; Wolter, Anika; Czerny, Mariko; Bez, Jürgen; Zannini, Emanuele; Arendt, Elke K.; Czerny, Michael; *European Food Research and Technology*, Vol. 235; 2012, Fig 1 and Fig2;pp. 337-338, with kind permission of Springer Science+Business Media

#### 2.4 Non gluten containing grains and pseudocereals

Historically, corn and rice were substitutes for gluten-containing grains. Nowadays several grains, legumes, seeds and nut flours offer increased variety, high nutritional quality, and palatability of the gluten-free formulation. These grains and seeds include quinoa, amaranth, buckwheat, teff, and sorghum (Table 2-1) (Renzetti et al. 2008b; Hager et al. 2012a).

Not allowed	Allowed
Wheat	Amaranth <sup>a</sup>
Rye	Buckwheat <sup>a</sup>
Triticale	Corn
Barley	Millet
Kamut	Quinoa <sup>a</sup>
Spelt	Rice
Oat ?	Sorghum <sup>a</sup>
	Soy <sup>a</sup>
	Legumes <sup>a</sup>
	Teff

Table 2-1 Grain and seeds sources in the gluten-free diet

<sup>a</sup> These sources contain higher fibre, protein, calcium and iron and are more nutritious than other grains in the gluten-free diet.
? Controversy

#### 2.4.1 Corn

Flour from corn (*Zea mays ssp. mays* L.) (Figure 2-3) is composed of the endosperm, which contains between 75-87% starch and 6-8% protein (Shukla and Cheryan 2001). Cornstarch bread was first developed using xanthan gum as a networking component. The resulting bread had good specific volume but a coarse crumb structure and lack of flavour (Christiansson 1974). The use of binding agents (xanthan, guar gum, locust bean gum and tragacanth) as substitutes for gluten in a gluten-free bread formulation based on cornstarch resulted in increased loaf volume and softening of the crumb structure (Ács et al. 1996a; Ács et al. 1996b; Hager et al. 2012a). Furthermore, gluten-free breads with improved specific volume and crumb–structure quality were obtained when cornstarch (74%) was added to a bread recipe, containing 17% rice flour, 9% cassava starch and 0.5% soy flour (Sànchez et al. 2002).



Figure 2-3 Images of gluten free flours widely used for the production of gluten free bakery products

#### 2.4.2 Rice

Rice (Oryza sativa) is one of the leading food crops in South East Asia including India. The production of rice in this part of the world is much higher than that of wheat (Sivaramakrishnan et al. 2004). Besides several beneficial qualities such as bland taste, colourlessness, ease of digestion and hypoallergenic properties (Kadan et al. 2001) rice flour (Figure 2-3) possesses also low levels of sodium, fat and high amounts of easily digested carbohydrates (Gujral and Rosell 2004a; Hager et al. 2012b). Rice is therefore considered as one of the most suitable ingredients for preparing gluten-free products. Starch granule properties of rice flour assume a major role in dictating the suitability for baking applications. It influences the texture of a product especially, if the flour is present in sufficient quantities (usually over 10% substitution) (Bean and Nishita 1985). However, rice has also low amounts of proteins, which are devoid of the viscoelastic properties typical of wheat gluten (Juliano 1985). Therefore, rice proteins are unable to retain the gas produced during the fermentation process, and this limits the use of rice flour in bread making (Gujral and Rosell 2004a; Marco and Rosell 2008). Rice variety has also been found to influence the bread making characteristics of rice flour. Rice varieties having low amylose contents and low gelatinisation temperatures give superior crumb properties (Nishita et al. 1976). Addition of 10% short grain rice to a white rice bread formula improved texture and slowed retrogradation compared to the same formulation made of 100% long grain rice (Kadan et al. 2001). The addition of hydrocolloids such as HPMC (hydroxypropyl-methyl-cellulose) (Sivaramakrishnan et al. 2004), guar

gum, carboxymethylcellulose (CMC) (Cato et al. 2004), xanthan gum (Lee and Lee 2006) locust bean gum or emulsifiers increased the batter viscosity and the resulting bread quality (Demirkesen et al. 2010). Among the emulsifier, diacetyl tartaric ester of monoglycerides (DATEM) improved the quality of rice bread in terms of specific volume and sensory values when added in the dough formulation at 0.5% (Demirkesen et al. 2010). Moreover, the use of protein cross linking enzymes to improve the bread making properties of rice flour has been investigated. Transglutaminase promotes cross-linking among rice proteins (Renzetti et al. 2008b). Cyclodextrin glycosyl transferase produces cyclodextrins which form complexes with lipids/ proteins (Gujral et al. 2003) and increased specific volume and crumb softness.

#### 2.4.3 Sorghum

Sorghum (Sorghum vulgare) belongs to the grass family Graminae. Like other cereal grains, the primary component of sorghum is starch (Rooney and Waniska 2000; Hager et al. 2012b). Sorghum has a similar chemical composition to maize, but after cooking protein and starch have a slightly lower digestibility due to the formation of protein cross-links (Duodu et al. 2003). Sorghum is often recommended as a safe food for celiac patients because it is more closely related to maize, than to wheat, rye and barley (Kasadra 2001). Sorghum might therefore provide a good basis for gluten-free bread. Some physico-chemical properties of sorghum flour (Figure 2-3) negatively affect its bread making performance. More in detail, the vitreous part of the endosperm tends to form coarse grits which contribute to a sandy mouth-feel. Upon heating, protein aggregates form a web- or sheet-like structure, which interfere with the starch gel resulting in problems like a flat top of the bread and larges holes in the crumb (Schober et al. 2007; Hager et al. 2012a) (Figure 2-2). Moreover, the high gelatinisation (64-68°C) temperature of sorghum starch (Taylor and Dewar 1994) may cause inadequate gelatinisation during baking (Schober 2009).

The quality of gluten-free sorghum bread can be improved by adding proteins (Cauvain 1998), hydrocolloids (Satin 1988), emulsifier, starch (Schober et al. 2007; Onyango et al. 2010), rye pentosans (Casier et al. 1977) or sourdough starter cultures (Schober 2009; Galle et al. 2010). This facilitates development of a cohesive crumb network that traps gas bubbles and prevents crust collapse (Taylor et al. 2006; Schober et al. 2007). Furthermore, starch dilutes the

endosperm and bran particles in sorghum flour which disturb the uniformity of the starch gel and interfere with liquid films around the gas cells (Taylor et al. 2006). Additionally, the botanical origin and amount of starch influence the rheological properties of the batter and the resulting bread (Onyango et al. 2010). Cassava starch, blended with sorghum flour in the ratio of 50:50, ensured the best overall crumb properties than sorghum bread made from maize, potato and rice starch (Onyango et al. 2010). However, the properties of cassava starch, which are responsible for the different qualities of sorghum breads, are not yet fully elucidated.

Investigating the bread making quality of different sorghum varieties in the development of gluten-free bread resulted in significant differences in crumb structure in terms of pore size and number as well as hardness (Schober et al. 2005). Breads differed little in volume, height, bake loss, and water activity. It was concluded that differences in kernel hardness and damaged starch content were the key elements responsible for such differences and that certain sorghum hybrids have better bread making potentials than others.

Recently, positive effects of glucose oxidase (EC 1.1.3.4) treatment on sorghum bread quality have been related to protein polymerization. Enhanced continuity of the protein phase and elastic-like behaviour of sorghum batter increased the specific volume and reduced collapsing at the top (Renzetti and Arendt 2009).

#### 2.4.4 Teff

Teff (*Eragrostis tef*) is a member of the tribe Eragrostidae, grass-family Gramineae. Teff is only distantly related to cereals like wheat, barley, rye and oat, and lacks gluten-like prolamins that cause problems for coeliac disease patients. Teff is a major cereal crop in Ethiopia and due to its adaptability has been introduced as a forage crop in India, South Africa and Australia (Tatham et al. 1996). Teff grain contains ~80% starch, 9-12% protein and 3% fat (Bultosa 2007; Hager et al. 2012b). The amylose content of starch with 18 and 23 % is in the typical range of those from native cereal starches like corn, sorghum and wheat (Hager et al. 2012b). Teff flour (Figure 2-3) is traditionally used in Ethiopia and Eritrea to produce popular, spongy, pancake-like bread, called *injera*. Teff flour is mixed with water in an equal ratio to give a batter which is then fermented. However, about 20% of the batter is removed and cooked in order to give a viscous paste called *absit*. The functionality of *absit* in the *injera* flat-bread can be described

as that of hydrocolloids in gluten-free breads, providing the batter with a better gas holding capacity due to increased viscosity. The use of teff flour rather than sorghum in the production of *injera* can be related to lower staling compared to those made of sorghum, resulting in a better product quality (Yetneberk et al. 2004). On a nutritive level, teff flour can be used for the same purposes as wheat flour since its nutritional value is similar. Its traditional use in bread making for production of *injera* may stimulate research in the development of gluten-free bread based on teff flour.

#### 2.4.5 Pseudo cereals

The pseudo cereals are botanically assigned to the Dicotyledonae (unlike cereals, which are Monocotyledonae), and they produce starch-rich seeds that can be used as flour for bread and other staple foods. The three best-known pseudo cereal crops are amaranth (*Amaranthus caudatus* L., Amaranthaceae), quinoa (*Chenopodium quinoa* Willd.; Chenopodiaceae) and buckwheat (*Fagopyrum esculentum* Moench; Polygonaceae).

These pseudo cereals are currently emerging as alternatives to other gluten-free grains in the gluten-free diet. The addition of buckwheat, quinoa and amaranth would add nutritional value to the diet of persons with celiac disease (Kupper 2005).

#### 2.4.6 Amaranth

Amaranth (*Amaranthus* spp.) produces cereal-like grains that contain a high level of nutritionally favourable proteins with amino acid composition close to ideal protein (Yanez et al. 1994), unusual quality of starch (excellent freezethaw and retrogradation stability, high gelatinization temperature, high viscosity, high water-binding capacity, high swelling power and enzyme susceptibility) (Baker and Rayas-Duarte 1998; Hunjai et al. 2004) and highquality oil (including 2.3-6% squalene (Berganza et al. 2003)). Amaranth flour (Figure 2-3) represents a suitable nutritional basis for patients with gluten intolerance. However, the baking quality of amaranth is poor. During baking of amaranth bread, no crumb forms and the size of the bread typically remains small (Aufhammer 2000). However, the application of sourdough technology seems to produce amaranth dough with viscosity and elasticity similar to that found in pure wheat dough (Houben et al. 2010). This is mainly due to the metabolism of protein, fat and carbohydrate carried out by lactic acid bacteria as well by the flour enzyme particularly active in an acidic environment during sourdough fermentation (Houben et al. 2010). However, the influences of sourdough fermentation on the rheological characteristics of the final products remain to be investigated. By replacing 10% of cornstarch with amaranth flour, increased protein and fibre levels by 32 and 152% respectively, while sensory quality was unaffected (Gambus 2002). The results indicate that amaranth flour can be used to enhance the protein and fibre contents of gluten-free breads.

#### 2.4.7 Buckwheat

Common buckwheat (*Fagopyrum esculentum*, Moench) is an annual melliferous crop that originates from North and East Asia, where it has been especially grown in China at least since 1000 B.C. and is now also widely adapted in North America. Buckwheat achenes contain 55% starch (Bonafaccia et al. 2003), with a ratio of 24% amylose and 76% amylopectin, similar to what is found in cereal starches and 11-15% protein content (Aufhammer 2000).

The nutritional value of gluten-free bread can be improved by the addition of buckwheat flour (Figure 2-3), especially regarding important nutrients such as protein, fibre, calcium, iron and vitamin E. The resultant breads also had a significantly higher content of polyphenol compounds and increased *in vitro* antioxidant activity (Alvarez-Jubete et al. 2009; Alvarez-Jubete et al. 2010; Hager et al. 2012b). However, some technological limitations arise when buckwheat is included in gluten-free formulations.

Gluten free bread containing 8.5% buckwheat flour was brittle after two days of storage (Moore 2004). The addition of sourdough from buckwheat flour to a buckwheat based recipe lead to a decrease of the specific volume and to an increase of the crumb hardness (Moroni et al. 2011). However, independent to the type of buckwheat flour (peeled or unpeeled) used, gluten-free bread, containing a flour ratio of rice/ buckwheat of 70:30, was produced without affecting the textural properties of the product (Torbica et al. 2010). Whereas, an increase of the amount of buckwheat flour in the bread formulation resulted in a decrease of quality of the protein structure which was manifested as the cracked surfaces of the upper crust of the breads. Moreover, by increasing the amount of peeled buckwheat flour from 10 to 20% taste properties significantly increased, due to the intensity of aromatic taste characteristic for unpeeled

buckwheat flour, which possesses bitter taste predominantly present in the husk (Luthar 1992). Therefore peeled buckwheat flour gives products a more pleasant flavour and taste (Torbica et al. 2010).

#### 2.4.8 Quinoa

Quinoa (*Chenopodium quinoa*, Willd.) is a typical seed crop originated in the Andes region near Lake Titicaca in Peru and Bolivia. It has been cultivated in this area since 3000 B.C. (Tapia 1979) and occupied a place of prominence in the Inca empire next only to maize (Cusack 1984). While the starch content of quinoa (58-64%) is similar to that of wheat (68%) (Hager et al. 2012b), the protein content (13-14%) is significantly higher than that of wheat (10-12%) (Repo-Carrasco et al. 2003). Quinoa flour (Figure 2-3) is used in association with wheat flour to make leavened bread. The addition of quinoa flour to leavened breads caused a reduction in the volume which is related to the high level of starch damage found in quinoa flour and meal, to the small size of the granules and to the low proportion of amylose in the starch (Chauhan et al. 1992). Quinoa is gaining importance not only because it is gluten-free, but also because it contains a high level of a wide range of nutrients (Kupper 2005). The replacement of 50% of potato starch by milled quinoa, in a rice flour based, gluten-free recipe, produced bread with a higher protein content (10% instead of 4%), higher dietary fibre content (20% vs. 8%), ash content (3% vs. 2%) and higher fat content (9% vs. 7%) (Alvarez-Jubete et al. 2009). Nevertheless, the application of quinoa for the production of gluten-free bread has still to be extensively investigated (Mäkinen et al. 2013) (Figure 2-2).

#### 2.4.9 Oats

The interest in oats (*Avena sativa*) for human consumption has increased in recent years. The nutrient composition of oats and its potential health benefits have been recently reviewed (Ryan et al. 2007; Food And Drug Administration 2008). Oat and its by-products contain high amounts of (water-) soluble fibres, essential amino acids, unsaturated fatty acids, vitamins, minerals and phytochemicals (Flander et al. 2007; Pulido et al. 2009; Hager et al. 2012b). Oat represents a good source of water-soluble viscous fibre (mainly 1, 3/1, 4- $\beta$ -glucan) that has the ability to lower blood cholesterol and postprandial glucose level (Pulido et al. 2009). The gluten-free diet is often characterised by an on

one side excessive consumption of energy from protein and fat, but by a low intake of fibre (Thompson 2009). Consequently, the enrichment of gluten-free baked products with oat can help to provide the much needed fibre (Malandrino et al. 2008) and to improve the palatability of the final products providing a greater variety of food choices in the restrictive diet (Janatuinen et al. 2002). Whole oat flour characterised by large particle size, limited starch damage and low protein content resulted in good quality bread, indicated by high specific loaf volume and soft crumb structure (Hüttner et al. 2010c; Hager et al. 2012a) (Figure 2-2). Moreover, the addition of <1% oat malt may help to improve bread volume and crumb grain. However an over dosage deteriorates crumb properties (Mäkinen et al. 2013).



Figure 2-4 Scanning Electron Micrographs of different flours a) amaranth, b) buckwheat, c) oat, d) corn, e) quinoa, f) rice, g) sorghum, h) teff and i) wheat; scale bar represents 10  $\mu$ m

#### 2.5 Functional ingredients used for the production of gluten-free breads

The replacement of gluten with other protein sources is another approach used in the production of gluten-free products. So far, dairy proteins, egg proteins, soybean and maize proteins have been studied as supplements in gluten-free formulations.

#### 2.5.1 Dairy proteins

Dairy-based ingredients are used as components of many food products. In baking industry their incorporation has long been established (Zadow 1981) for both nutritional (increase of calcium and protein content, as well as supplying essential amino acids, i.e. lysine, methionine and tryptophan) and functional benefits (Crowley et al. 2002). Dairy ingredients are able to form networks, to enhance flavour, crust colour, to improve texture, to reduce the rate of staling and to increase water absorption and therefore to improve the handling of batters (Arendt et al. 2008). However, celiac patients are often lactose intolerant due to the damage that coeliac disease that inhibits the production of lactase enzyme by the villi (Bodé and Gudmand-Høyer 1988). Therefore, the incorporation of dairy ingredients in gluten-free products, must take their lactose content into consideration.

The inclusion of dairy powders with high protein/low lactose content (i.e. sodium caseinate and milk protein isolate) in gluten-free wheat starch based bread formulations resulted in breads with an improved overall shape and volume, a firmer crumb texture and better organoleptic perception (Gallagher et al. 2003). The use of whey proteins however, showed contradictive results, namely a higher increase of the specific volume in comparison to sodium caseinate (Nurest 2009). Overall, this work has proved that without a detrimental effect to the loaf volume, application of dairy powders can give products that are more appealing to the panellists than the control formulations (Gallagher et al. 2003).

The opposite trend was caused by the inclusion of whey protein inducing a greater increase in the specific volume of bread than the addition of sodium caseinate. The formulation presented by Gallagher, Gormley et al., (2003) did not include gums, which influence the water availability in the batter and hence change the crumb structure of the bread.

Textural properties of gluten-free bread with and without addition of skim milk powder (37.5% dry weight) were compared to those of commercial gluten-free (starch-based) and a regular wheat bread (Moore 2004). All the gluten-free breads were brittle after two days of storage while wheat bread and the bread made from the commercial gluten-free flour mix yielded significantly higher loaf volumes. Using confocal laser-scanning microscopy, a network-like structure resembling the gluten network in wheat bread crumb was visible in dairybased, gluten-free bread crumb (Moore 2004). However, results collected so far indicate that dairy powders do not have significant positive effects in improving the textural characteristics of gluten-free breads (Moore 2004).

#### 2.5.2 Egg proteins

Eggs other than improving the gas retention properties add nutrition and functional appeal to gluten-free foods (Jonagh 1968). Egg proteins form strong, cohesive, viscoelastic films, which are essential for stable foaming (Ibanoglu and Ercelebi 2007). Moreover, egg albumen in combination with methyl-cellulose and gum arabic are the major determinants of the breads' sensory quality which in terms of grain distribution, first bite hardness, masticatory hardness and cohesiveness of mass (Toufeili et al. 1994).

The application of egg powder and transglutaminase (TGase) in a gluten-free formulation increased the firmness of the bread, lowered bake loss and hence lead to higher crumb moisture, since the formation of a protein network (similar to that found in wheat bread) increased the water-holding capacity (Moore et al. 2006). Moreover, the crumb of the egg powder bread appeared lighter, with a finer and more homogenous crumb after TGase treatment. This suggests a potential of egg as a structure builder by retaining gas and forming continuous networks in gluten-free formulation, particularly when enhanced by enzymatic cross-linking.

#### 2.5.3 Soybean

Flour of soybeans (family Fabaceae) is extensively employed for the supplementation in gluten-free bakery products (Schober et al. 2003; Miñarro et al. 2012). Soybean proteins are used for fortification of bakery products to improve protein quality, mechanical behaviour and textural quality of bread during storage (Ribotta et al. 2004; Sànchez et al. 2004). Since soybean protein amino acid profile (rich in lysine, limited in sulphur amino acids) complements grain protein profile (limited in lysine, rich in sulphur amino acids) (Belitz et al. 2008) enhances product's nutritional quality.

Formulated wheat starch-based gluten-free breads with 20%, 30% and 40% soya protein isolate (88% protein content) showed satisfactory baking characteristics (Ranhorta 1975). Breads prepared with soya flours and soya

protein concentrates contained more protein and fat than wheat bread. The crumb structure and texture showed a marked improvement from a rough, crumbly, open-faced interior to a more tender and close-grain structure (Ranhorta 1975). However, soya could not be added to gluten-free formulation at high levels without severely decreasing bread quality. The optimised gluten-free bread formula based on 74.2% corn starch, 8.6% cassava starch and 17.2% rice flour did result in a maximum specific volume, but at the expenses of crumb structure, which showed large holes (Sànchez et al. 2002). The inclusion of 0.5% soya flour corrected this problem, improving crumb structure.

Studies on the effects of enzyme-active, semi-active and inactive full-fat soybean flours on gluten-free bread quality showed that enzymatically active soybean flour improved the volume and structure of gluten-free bread (Ribotta et al. 2004). This effect seemed to be due to both the structural proteins and the enzymatic activities of the soybean flour. In conclusion, soya proteins are suitable protein source for gluten-free formulations to overcome problems related to crumb texture.

#### 2.5.4 Corn protein (Zein)

Maize prolamins (*zein*), a readily available by-product from corn wet milling and fuel-ethanol production, have been successfully used for gluten-free bread production (Schober et al. 2008). Indeed, a mixture of maize prolamin (zein), maize starch and water can form viscoelastic dough closely resembling wheat dough (Lawton 1992). However, zein could not mimic the properties of gluten on its own. Hydrocolloids such as hydroxypropyl methylcellulose (HPMC) positively affect the structural and rheological properties of zein, which yield dough similar to wheat dough and bread with increased volume (Schober et al. 2008; Andersson et al. 2011). Degreasing of the surface of the zein particles helps their aggregations (Schober et al. 2010), since according to the surface lipid hypothesis water absorption and protein-interactions between the zein particles could be thwarted by the thin layer of surface lipid. Inclusion of the defatted zein in a dough formulation based on maize starch, HPMC, sugar, table salt, dry yeast and water, the resulted in bread showing remarkable technological improvements in terms of volume and shape (Schober et al. 2010). In conclusion, zein as potential cereal protein source may serve as a structural enhancer in combination with hydrocolloids. Modifications of zein through development of new recipes or different processing conditions may improve dough rheology and baking performance even further. The economic disadvantage of zein addition to gluten-free bread lays in its high costs.

#### 2.5.5 Starches

Starch is the primary source of stored energy in many plants including cereals, legumes and tubers, and provides 70-80% of the calories consumed by humans worldwide (Thomas and Atwell 1998). In addition to their nutritional value, use comprises gelation-, thickening agents, moisture-retention, their emulsifiers, film- forming agents and fat substitutes (El-Sayed 2009). In baking they significantly contribute to texture, appearance and overall acceptability of cereal based foods (Ward 2002; Miyazaki 2006). Corn, cassava, sweet potato, rice, wheat and potato are the major sources of food starch while sorghum and barley serve as minor source of starch in different localised regions of the world. Native starch occurs in form of granules. The size, shape and molecular arrangement inside the granules depend on the species, cultivar and variety of the source plant as well as the genetic-environment interactions (Delcour and Hoseney 2010). The starch biosynthetic pathway generally results in the formation of two types of glucose polymers: the linear amylose and the highly branched amylopectin molecule. During dough preparation, starch absorbs up to about 45% water and is considered to act as inert filler in the matrix of the dough (Bloksma 1990). On the other hand, dough is described as a bicontinuous network of protein and starch (Eliasson and Larsson 1993). During the bread baking process starch granules gelatinize, meaning they swell and are partially solubilised, but still maintain their granular identity (Hug-Iten 2001). Starch gelatinisation could play an important role in gluten-free formulation, due to the ability of starch to form a paste entrapping air bubbles and therefore increasing the gas holding capacity of batter. For this reason the addition of pregelatinized, gel-forming starches and air cell stabiliser such as gums have been suggested as a means to provide gas occlusion and stabilisation (Gallagher 2009). Moreover, the addition of starch in the gluten-free formula could improve (i) batter consistency during mixing, (ii) enhance the softness of the crumb, and (iii) control starch gelatinization during the baking process (Gallagher 2009).

Isolated wheat starch has been often utilised in gluten-free products. However, many coeliac patients are sensitive to the presence of low amounts of gliadins, which might escape the isolation procedure, and therefore starch-based ingredients should originate from raw materials that are naturally gluten-free (Lohiniemi et al. 2000). Different starches from naturally gluten-free sources such as corn, cassava, tapioca, potato and rice have been utilised in gluten-free formulations (Gallagher et al. 2002; Sànchez et al. 2002; Kobylanski 2004; Moore 2004). While rice starch due to its low level of sodium and high digestibility has been used as basic ingredient in gluten free breads (Gallagher et al. 2002), corn and tapioca starches can cause some difficulties by imparting unusual taste to bread (Sànchez et al. 1996). Further studies are required in order to better understand the impact of different starch types and their functional properties on the quality characteristics of gluten-free products.

#### 2.5.6 Hydrocolloids

The viscoelastic properties provided by the gluten network are largely responsible for the important rheological characteristics of dough, such as elasticity, extensibility, resistance to stretch, mixing tolerance and gas holding ability (Gan et al. 1989). The imitation of gluten in gluten-free products represents a major technological challenge, since gluten-free batters are characterised by a rather liquid consistency due to missing a gluten network (Cauvain 1998). As a result, polymeric substances (Table 2-2) able to mimic the viscoelastic properties of gluten are required for the development of gluten-free breads. Hydrocolloids or gums are substances consisting of hydrophilic longchain, high molecular weight molecules, usually with colloidal properties, that produce gels, highly viscous suspensions or solutions in water-based systems, with low dry-substance content (Hoefler 2004). The term hydrocolloids embraces all polysaccharides extracted from plant, seaweed and microbial source, as well as gums derived from plant extrudates and modified biopolymers prepared by chemical treatment of cellulose (Dickinson 2003). All the hydrocolloids mentioned above have been widely investigated as gluten replacers to improve structure, mouth feel, acceptability and shelf-life of glutenfree baked goods (Toufeili et al. 1994; Gallagher et al. 2004; Gujral and Rosell 2004a; Lee and Lee 2006; Schober et al. 2008). However, their functionality were strictly dependent on the source of the hydrocolloid, its chemical

structure, extraction process, chemical modification, the dosage of hydrocolloid in to dough formulations, and the interaction with other food polymers and component of recipe (Anton and Artfield 2008; El-Sayed 2009; Hüttner and Arendt 2010). Although currently a large number of hydrocolloids are available on the market, HPMC and xanthan gum are the most commonly used, since they seem to be able to mimic the gluten properties best in gluten-free breads regardless of the formula used (Anton and Artfield 2008). The addition of hydrocolloids such as propylene glycol alginate (PGA) to a recipe enabled the production of a buckwheat- and rice-flour (40:60) based bread without negative effect on bread properties (Peressini et al. 2011). Precisely, PGA addition improved specific volume, crumb hardness and structure. A lower addition level of 0.5% improves the combined effect of the polymer-derived dough viscosity und the ability to form elastic films at the gas-liquid interface. This is related to association of PGA-molecules which prevents gas bubbles from instability (Peressini et al. 2011).
Hydrocolloid	Gelling properties <sup>a</sup>	Effect on gluten-free bread	References
Agarose	Form gels upon heating	Increased loaf volume Decreased uniformity of crumb (large gas cells)	Lazaridou, Duta et al., (2007)
Guar gum	Highly viscous solutions, no gelling properties	Even cell size distribution in crumb, Retarded bread staling	Schwarzlaff, Johnson et al., (1996)
Locust bean gum	Slightly soluble in cold water, dissolves in water at 85°C Form gels with kappa- type carrageenans and xanthan	increased height of the bread loaves, retarded bread staling	Schwarzlaff, Johnson et al., (1996)
Hydroxy propyl methyl cellulose HPMC	Form gels upon heating Some interfacial activity and ability to form films	Increased specific volume Improved gas retention and water absorption	Kang et al., (1997); Gan et al., (1989); Kadan, Robinson et al. (2001)
Pectin	Low-methoxyl pectins form gels in presence of calcium ions	Increased crumb porosity	Lazaridou et al., (2007)
Xanthan gum	High viscosity, pseudoplastic solutions (unaffected by temperature, pH, salt conditions). Forms gels with agarose, kappa-type carrageenans, locust bean gum, konjac gum	Good crumb structure Decreased loaf volume and increased crumb firmness	Christiansson (1974) Lazaridou, Duda et al. (2007); Schober, Messerschmidt et al., (2005)

Table 2-2 Hydrocolloids used in gluten free bread making

<sup>a</sup> Source: Hüttner and Arendt (2010), adapted from BeMiller (2008)

#### 2.5.7 Water

Water is one of the key ingredients to produce good quality gluten-free bread and an essential factor affecting the rheological behaviour of gluten-free batters (Renzetti et al. 2008b), particularly at stages such as proofing, when the batter is at rest and its expansion and gas holding capacity are dependent on dough/batter elasticity. In general, water addition is higher for gluten-free formulations than for wheat recipes (Moore 2004; Renzetti et al. 2008b; Hager et al. 2012a). If the proportion of water is too low, the dough becomes brittle, not consistent and exhibits a marked 'crust' effect due to rapid dehydration at the surface. On the other hand, if the water content is too high, the batter has a low viscosity and there is little or no resistance to deformation, thus no extensibility and dough development (Renzetti et al. 2008b). Water also plays an important role in the major changes during bread-baking (e.g. starch gelatinisation) which contribute to the structure and palatability of the baked product. The water content and distribution determine textural properties such as crumb hardness, crispness of the crust and shelf-life (Wagner et al. 2007). Higher addition of water (70-90%) to gluten-free flours resulted in higher loaf volume and a much softer crumb texture (Gallagher et al. 2003). This confirms that increasing water content of gluten-free bread significantly decreased crumb firmness (McCarthy et al. 2005). Formulations of sorghum batter and bread have been studied under variation of the water content (95-120% flour weight basis) while maintaining a constant starch content (30% maize starch) regarding rheological and crumb properties (Schober et al. 2005). Formulations with high water contents resembled pancake batters, whereas those with low water contents gave dough that lacked elasticity. Breads made from batters containing high water contents had higher volumes than those made from batters containing low water contents (Schober et al. 2005). HPMC and water showed significant interactions on crumb grain structure. Optimised levels of 2.2% HPMC and 79% water yielded good-quality gluten-free bread. The presence of HPMC in wheat bread decreased the rate of staling and also retarded retrogradation (Bárcenas and Rosell 2005). This might be due to the reduced water activity of the bread containing HPMC (Bárcenas and Rosell 2005). Hydrocolloids affect the retrogradation level in breads by limiting both the diffusion and the loss of water from bread crumb (Davidou et al. 1996). Thus, the control of water content and its mobility may be key factors controlling loaf volume and crumb firmness in bread.

# 2.6 Novel approaches in gluten-free bread-making

# 2.6.1 High Hydrostatic Pressure (HHP) technology

The potential application of high hydrostatic pressure (HHP) for food processing has been investigated with growing interest. This technology consists in submitting foods to high hydrostatic pressure, mainly between 100 and 1000 MPa. HHP creates new structures and textures by modifying functional properties of proteins and starches (Gomes et al. 1998; Ahmed et al. 2007; Kieffer et al. 2007). Consequently, this technology might be a useful tool for enhancing gluten-free bread quality. HPP induces the gelatinization of starch, which however, keeps granular integrity following different mechanism compared to thermally induced gelatinization (Gomes et al. 1998; Vallons and Arendt 2009). The HHP-treated starches improve moisture retention and texture, increase volume and enhance shelf life of baked goods (Thomas and Atwell 1998). In general, the extent of swelling highly depends on the applied pressure, treatment time and temperature, concentration and type of starch (Stolt et al. 2000).

In wheat, HHP treatment resulted in protein network formation (Kieffer et al. 2007) because the formation of disulphide bonds is enhanced. These bonds play a key role in the formation of a strong gluten network (Kieffer et al. 2007). Nonetheless, little is known about the effects of HHP on complex systems such as dough or batter.

When HHP was applied on wheat dough, the reduction of specific volume, uneven cell gas distribution and increase of crumb hardness were observed (Bárcenas et al. 2010). Applying HPP to gluten free batters, namely basmati rice slurries, increased starch and protein components were completely gelatinised and denatured and mechanical strength of the HP-treated rice slurries increased (Ahmed et al. 2007). The HHP treatment on white rice and teff batters caused (i) changes in the microstructure of the batters; (ii) starch gelatinisation and (iii) protein polymerisation by thiol/disulphide-interchange reactions in white rice and teff batters (Vallons et al. 2010). For buckwheat proteins however, no such cross-linking mechanism was observed, which was explained by the absence of free sulfhydryl groups. An increase in viscoelastic properties at higher pressures was also observed, and was explained by the modifications occurring in starch and protein structure. All these finding shows the potential of HPP to improve functional properties of gluten-free batters.

Moreover, the treatment of oat batters at high HP caused a pre-gelatinisation of starch which resulted in higher batter viscosity (Hüttner et al. 2010a). Higher elasticity can increase gas retention of the batters and therefore improve texture, and volume of oat bread. Accordingly, HP processing seems to be a promising tool for the improvement of gluten-free bread. However, further studies are needed to determine the potential of HP treatment for application during the production of gluten-free bread.

# 2.6.2 Enzyme technology

Enzymes are commonly applied in baking industry in order to improve the characteristics and quality of wheat flour-based products. Such enzymes include amylases, proteases, hemicellulases, lipases and oxidises which influence the whole baking process (Poutanen 1997; Tenkanen et al. 1998). The application of some of these enzymes has shown great potentials in modifying the bread making functionality of wheat flour and could be successfully applied to gluten-free systems (Renzetti and Arendt 2009).

In the last decade, there have been an increasing number of studies focusing on enzymatic processing of gluten-free batters. In particular, the use of crosslinking enzymes which are able to promote protein networks and/or increase the continuity of the protein phase have been the most studied applications. Among the cross-linking enzymes transglutaminase (TGase) (EC 2.3.2.13) has received particular attention due to its ability to introduce covalent cross-links between proteins (Nonaka et al. 1989). From a rheological standpoint, glutenfree batters treated with TGase show a considerable increase in elastic-like behaviour and in the resistance to deformation (Gujral and Rosell 2004a; Renzetti et al. 2008b), which results from the promotion of large protein aggregates in comparison to a dispersed protein phase of the non-treated batters (Renzetti et al. 2008a; Renzetti et al. 2008b). The changes in the rheological and microstrucutral properties of the batters are reflected in the bread making performance of the gluten-free system, resulting in significant improvements especially in terms of crumb structure (Marco and Rosell 2008; Renzetti et al. 2008a). On the other hand, the increased resistance to deformation negatively affected specific volume (Marco and Rosell 2008; Renzetti et al. 2008a). Furthermore, the impact of the enzyme is very much dependent on the protein source, as beneficial effects were reported for breads made from buckwheat, rice and corn flour, while the bread making functionality of oat, teff and sorghum flours did not improve (Renzetti et al. 2008a).

Glucose oxidase (GO) (EC 1.1.3.4) has also been investigated in the attempt to promote protein networks in gluten-free batters. Gluten-free breads with increased specific volume and decreased crumb hardness were obtained when rice flour was treated with GO (Gujral and Rosell 2004b). Improvements were even more significant when 2% HPMC was added in the formulation. The quality of sorghum and corn bread was also improved by GO treatment of batters (Renzetti and Arendt 2009) while no beneficial effects were observed for buckwheat, teff and oat breads (Renzetti and Arendt 2009; Renzetti et al. 2010). Similarly to TGase, GO treatment promoted an increase in the elastic-like behaviour of gluten-free batters (Gujral and Rosell 2004b; Renzetti and Arendt 2009). Protein cross-linking enzymes have shown the potential to improve the breadmaking functionality of gluten-free flours. However, the enzyme type should be selected according to the specific gluten-free formulation. On the other hand, the promotion of an elastic-like behaviour does not necessarily result in improved baking quality (Renzetti et al. 2008b; Renzetti and Arendt 2009).

Recently, a depolymerisation mechanism, during protease treatment, has been proposed to improve gluten-free bread quality as alternative to the polymerization promoted by cross-linking enzymes. Renzetti and Arendt (2009) treated brown rice batters with protease (bacillolysin, EC 3.4.24.28) and successfully improved the bread baking properties of the flour by increasing bread specific volume and decreasing crumb hardness and chewiness. Peptidase was found to break down the disulfide-linked macromolecular proteins natively present in the rice endosperm or formed during baking, that work as barrier affecting the starch swelling and thereby the rheological and cooking properties of rice (Derycke et al. 2005). The reduced size of the protein structures enhanced the continuity of the starch phase resulting in improved bread quality. Similar improvements were gained by prevention of formation of disulfidelinked macromolecular protein agglomerated by addition of glutathione in a rice batter (Yano 2010). Oat bread quality was also significantly improved by addition of protease (Renzetti et al. 2010), while the treatment for sorghum and buckwheat breads was detrimental. From a rheological standpoint, the improvements observed in oats and rice breads are related to a lower batter consistency and paste viscosity during proofing and in the early stages of baking, which favoured batter expansion (Renzetti and Arendt 2009; Renzetti et al. 2010). Additionally, the preserved batter elasticity and the increased paste stability during baking insured the structural integrity of the bread crumbs. These results suggest that in the future the modifications induced by protease

treatment of gluten-free batters should be targeted compared to an increased plastic-like behaviour.

#### 2.6.3 Sourdough technology

Sourdough, a mixture of flour and water fermented with lactic acid bacteria (LAB) and yeasts (Hammes and Gänzle 1998), has a well-established role in improving flavour and structure of bread (Arendt et al. 2007). The influence of lactic acid bacteria and sourdough on the quality of gluten-free bread is recently under study. Gluten-free breads tend to stale quickly and possess poor flavour. For wheat bread those disadvantages could be improved and prevented with incorporation of sourdough (Clarke et al. 2002; Crowley et al. 2002).

When used in optimised 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 (Gobbetti 1998; De Vuyst and Vancanneyt 2007; Poutanen et al. 2009). These positive effects are associated with the metabolic activities of sourdough lactic acid bacteria (LAB) and yeasts, such as lactic acid fermentation, proteolysis, exopolysaccharides (EPS) production and synthesis of volatile and antimicrobial compounds (Arendt et al. 2007; Corsetti and Settanni 2007; Ruehmkorf et al. 2012).

Consequently, the exploitation of sourdough for the development of new glutenfree products seems appealing. Over the last years, increasing attention has been drawn on the application of sourdough technology as a natural and efficient way to improve the quality of gluten-free bread (Moroni et al. 2009; Ruehmkorf et al. 2012).

However, for a successful selection of gluten-free sourdough LAB and yeast, their ability to dominate the fermentation and inhibit the growth of contaminants is a key condition (De Vuyst et al. 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 (Vogelmann et al. 2009; Moroni et al. 2010). 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 starter development (Meroth et al. 2004; Edema and Sanni 2008; Sterr et al. 2009; Vogelmann et al. 2009; Moroni et al. 2010; Ruehmkorf et al. 2012; Wolter et al. 2014). *Lactobacillus fermentum, L. plantarum* and also *Lactobacillus paralimentarius* are frequently isolated from gluten-free sourdoughs from rice, maize, 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 et al. 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 et al. 2011).

Sourdough fermentation has a positive effect on crumb structure of gluten-free sorghum bread (Schober et al. 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 et al. (2010b) found that sourdough *Leuconostoc argentinum, Pedicoccus pentosaceus, Weissella cibaria* and *Lactobacillus coryniformis* bacteria isolated from oats have the potential to increase loaf-specific volume as well as to improved crumb structure enhancing oat bread quality.

Some lactic acid bacteria (LAB) can produce a wide variety of long-chain sugar polymers called exopolysaccharides (EPS), which are varied in their chemical composition, structure and physical properties (De Vuyst and Degeest 1999). These polysaccharides are synthesised extracellularly from sucrose by glycansucrases, or intracellularly by glycosyltransferases from sugar nucleotide precursors. Those polysaccharides produces from sucrose can improve the technological as well as the nutritional properties of gluten-free breads acting as prebiotics and hydrocolloids, respectively (Lacaze et al. 2007; Waldherr and Vogel 2009).

The application of the EPS-producing strains *L. reuteri* LTH5448 and *Weissella cibaria* 10M in 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 et al. 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. Independent 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 kimchii* 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 et al. 2010; Galle et al. 2012).

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 (Moore et al. 2007; Sterr et al. 2009; Vogelmann et al. 2009; Galle et al. 2010; Moroni et al. 2010; Ruehmkorf et al. 2012; Wolter et al. 2014).

#### 2.7 Conclusion

Many factors contribute to the increased prevalence of coeliac disease, which has emerged as common food intolerance worldwide that can be diagnosed at all age. In the past decade an impressive effort has focused on the development of potential therapeutic solution for CD (Lerner 2010). However, the only currently available and safe treatment for CD consists in dietary exclusion of grains containing gluten. Additionally, supportive nutritional care in case of mineral and vitamins deficiencies is necessary (Hopman et al. 2006).

Gluten is an essential structure-building protein, contributing to the appearance, crumb structure, and consumer acceptability of many baked products. Therefore, the biggest challenge for the food scientist and bakers in the area of gluten-free products is the production of high quality gluten-free bread. Good quality gluten-free bread can only be produced, if a range of flours and polymeric substances, which mimic the viscoeleastic properties of gluten, are included into the gluten-free formulation.

Naturally gluten-free starches such as rice, potatoes or tapioca starch, rather than wheat starch, should be used for this purpose. Hydrocolloids are an essential ingredient for gluten-free bread production, since they are able to mimic the viscoelastic properties of gluten to a certain extent. They are also known to reduce staling, improve water binding and the overall structure of the bread. Research performed so far indicate xanthan gum and HPMC as the most suitable hydrocolloids for gluten-free bread formulations, but further research is needed to optimise the application of these or other hydrocolloids in glutenfree systems. Protein based ingredients are also essential in the improvement of gluten-free bread, and the most promising are probably the egg and maize (zein) protein even if this latter represent an expensive ingredient.

One of the most important ingredients in any gluten-free formulation is water, and therefore it is essential to optimise the water level for every formulation, in order to achieve optimal results. Recently, research has also focused on the application of enzymes to improve the texture of gluten-free bread. Among other enzymes, transglutaminase has been shown to improve the texture of gluten-free bread, but showed a dependency on the raw material taken into consideration. Lactic acid bacteria / gluten-free sourdough are also one possibility to improve gluten-free bread quality, particularly its sensory properties. Even if the research on gluten-free products is still in its infancy, researchers have been able to create products, which are superior to the ones currently on the market, and which celiac patients might soon be able to see available in the stores.

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# Chapter 3 Evaluation of exopolysaccharide producing *Weissella cibaria* MG1 strain for the production of sourdough from various flours

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# 3.1 Abstract

This study determined exopolysaccharide (EPS) production by Weissella cibaria MG1 in sourdoughs prepared from gluten-free flours (buckwheat, oat, quinoa and teff), as well as wheat flour. Sourdoughs (SD) were fermented without sucrose, or by replacing 10% flour with sucrose to support EPS production. The amount of EPS depended on the substrate: high amounts of EPS corresponding to low amounts of oligosaccharides were found in buckwheat (4.2 g EPS/ kg SD) and quinoa sourdoughs (3.2 g EPS/ kg SD); in contrast, no EPS but panoseseries oligosaccharides (PSO) were detected in wheat sourdoughs. Organic acid production, carbohydrates and rheological changes during fermentation were compared to the EPS negative control without added sucrose. Corresponding to the higher mineral content of the flours, sourdoughs from quinoa, teff and buckwheat had higher buffering capacity than wheat. Fermentable carbohydrates in buckwheat, teff and quinoa flours promoted W. cibaria growth; indicating why *W. cibaria* failed to grow in oat sourdoughs. Indigenous proteolytic activity was highest in quinoa flour;  $\alpha$ -amylase activity was highest in wheat and teff flours. Protein degradation during fermentation was most extensive in quinoa and teff SD reducing protein peaks 18-29, 30-41 and 43-55 kDa extensively. Rheological analyses revealed decreased dough strength  $(A_F)$ after fermentation, especially in sucrose-supplemented buckwheat sourdoughs correlating with amounts of EPS. High EPS production correlated with high protein, fermentable sugars (glucose, maltose, fructose), and mineral contents in guinoa flour. In conclusion, W. cibaria MG1 is a suitable starter culture for sourdough fermentation of buckwheat, guinoa and teff flour.

# 3.2 Introduction

A gluten-free diet is currently the only effective treatment for coeliac and gluten intolerant patients. Gluten-free breads often have a low nutritional value and are characterized by a low bread volume and a poor texture owing to the use of refined ingredients (pure starches and proteins), and the lack of the network forming gluten proteins (Gallagher et al. 2003). The use of nutrient-dense flours, for example, quinoa, buckwheat or teff, may improve the nutritional value (Hager et al. 2012) but does not provide a network forming protein. Hydrocolloids, such as xanthan, carrageen and agar, are used in bakery products as a replacement for gluten and to bind water in dough. Hydrocolloids also retard starch retrogradation, which is intimately linked to bread staling and shelf-life (Belitz et al. 2008).

Microbial exopolysaccharides (EPS) are high molecular weight carbohydrate polymers found in some bacteria and microalgae (Monchois et al. 1999; van Hijum et al. 2006). Depending on their composition, they can be divided into homopolysaccharides (HoPS), consisting of one type of monosaccharide being either glucose (glucans) or fructose (fructans), and into heteropolysaccharides (made of 3-8 multiple, repeated moieties) (De Vuyst et al. 2001; van Hijum et al. 2006). In contrast to heteropolysaccharides which are synthesized in smaller amounts from sugar nucleotide precursors (De Vuyst et al. 2001), HoPS are synthesized in larger amounts from sucrose (Monsan et al. 2001). HoPSproducing lactic acid bacteria are already used in conventional bread making (Decock and Cappelle 2005; Lacaze et al. 2007), but their use is particularly promising in gluten-free baking (Schwab et al. 2008; Galle et al. 2012; Ruehmkorf et al. 2012b) since EPS can potentially act as hydrocolloids (Schwab et al. 2008). Microbial production of exopolysaccharides during sourdough fermentation was reported to be more effective than the addition of comparable amount of EPS to the bread formulation (Brandt et al. 2003).

Concurrently to EPS synthesis, sucrase-type enzymes catalyse the reactions sucrose hydrolysis and oligosaccharide formation (Tieking et al. 2003; van Hijum et al. 2006).

The production of oligosaccharides and polysaccharides by glucansucrases is dependent on the concentration and type of suitable acceptor carbohydrates (Tieking and Gänzle 2005; Kaditzky et al. 2008; Galle et al. 2010). Maltose is an efficient acceptor carbohydrate for dextransucrase present in cereals and diverts sucrose conversion from exopolysaccharide synthesis to oligosaccharide production (Kaditzky and Vogel 2008; Galle et al. 2010). The concentration of maltose and other acceptor carbohydrates in sourdough depends on the carbohydrate composition as well as the enzyme activity of the cereal substrate thus influencing the yield of EPS and oligosaccharides in sourdough fermentations with EPS-producing starter cultures (Galle et al. 2010; Galle et al. 2012). The carbohydrate composition of wheat and rye flours as well as the evolution of carbohydrate levels in wheat and rye sourdoughs is well described (Roecken 1995). However, little data are available for other cereals or pseudocereals that are used in gluten-free baking.

During sucrose hydrolysis, fructose is released which can be used as an electron acceptor by most heterofermentative LAB and results in acetate formation (Gänzle et al. 2007). An excess of acetate compromises the quality of bread (Tieking and Gänzle 2005; Kaditzky and Vogel 2008; Galle et al. 2010). The obligate heterofermentative strain *W. cibaria* MG1 produced high amounts of the HoPS dextran (a  $\alpha$ -1,6-linked glucan) from sucrose (Galle et al. 2010; Galle et al. 2012). Yet, comparable to other *Weissella* species, acetate formation was low, since *W. cibaria* MG1 lacks mannitol dehydrogenase activity and does not convert fructose to mannitol with concomitant acetate formation (Galle et al. 2010).

The strain-specific ability to produce exopolysaccharides during sourdough fermentation depends on the metabolic activity of the fermentation microbiota (Gänzle et al. 2007), and contributes to the sourdough's ability to influence bread quality (Katina et al. 2009; Galle et al. 2012).

It was the aim of this study to assess the production of EPS, oligosaccharides and organic acids by *Weissella cibaria* in sourdoughs. Protein degradation in gluten-free sourdoughs, as well as the effect of fermentation on dough rheology was also determined.

# 3.3 Materials and Methods

# 3.3.1 Materials

The ingredients used in this study were buckwheat flour (Doves Farm Foods Ltd, UK) (moisture 12.6%), oat flour (E. Flahavan & Son Ltd, Ireland, moisture 10.4%), quinoa flour (Ziegler Naturprodukte, Germany, moisture 12.3%), teff flour (Trouw, The Netherlands, moisture 9.5%), wheat flour (baker's flour, Odlums, Ireland, moisture 12.7%) and sugar (Súicra, Ireland). All other chemicals and microbial media components were purchased from Sigma (Sigma, Arklow, Ireland), unless otherwise specified.

# 3.3.2 Strain and growth conditions

*Weissella cibaria* MG1 was obtained from the culture collection of the cereal science laboratory in University College Cork. *W. cibaria* MG1 was stored in a 35% glycerol stock at -80°C. The strain was routinely maintained on modified deMan-Rogosa-Sharpe agar (mMRS5), supplemented with vitamins and bromocresol green (Meroth et al. 2003), and incubated anaerobically at 30°C for 48 h. For the preparation of working cultures, single colonies were picked from the agar plates, cultured in mMRS5 broth at 30°C for 12h, and sub-cultured for 12h.

# 3.3.3 Sourdough fermentation

Sourdoughs were prepared from each gluten-free flour as described by (Galle et al. 2010). Cells were harvested by centrifugation (2300 x g, 10 min, 4°C), washed and re-suspended in sterile tap water, and added to the sourdough to an initial cell count of 10<sup>8</sup> CFU/ g dough. Sourdoughs were prepared with an equal weight of flour and water. To support EPS production by *W. cibaria*, 10% of the flour was replaced by sucrose. Sourdoughs were fermented in triplicate for 24 h at 30°C.

# 3.3.4 Analysis of EPS and oligosaccharide formation in sourdough

In order to determine the amount and molecular size of EPS, the isolation of water-soluble polysaccharides from flour and sourdough samples was carried out as described previously (Tieking et al. 2003; Galle et al. 2010). Freeze dried samples were reconstituted in distilled water to a final concentration of 2 mg/ mL. Amount and size of EPS was analysed by size exclusion

chromatography (SEC) using a Superdex 200 Column (GE Healthcare, Baie d'Urfe, Canada). Water was used as a solvent at a flow rate of 0.4 mL/ min for chromatography. EPS were detected with a refractive index detector and their molecular weights were estimated regarding similar retention times using two dextrans (LMW dextran, relative molecular weight (M<sub>r</sub>)  $10^{5}-2x10^{5}$ ; HMW dextran, (M<sub>r</sub>)  $5x10^{6}-4x10^{7}$ ), levan (16.9x10<sup>6</sup>) and inulin from chicory (M<sub>r</sub>=  $10^{4}$ ) (all obtained from Sigma, Oakville, Canada) (Galle et al. 2010). EPS concentrations in the final sourdough were calculated as differences in concentration of water-soluble polysaccharides in unfermented flour.

Oligosaccharides in sourdough were analysed with a Carbopac PA20 column (Dionex, Oakville, Canada). Sucrose, glucose, fructose, maltose, panose, isomaltose, isomaltotriose (all obtained from Sigma, Oakville, Canada) were used as external standards. Panose series oligosaccharides (PSO) as reference were produced enzymatically with *Leuconostoc mesenteroides* FUA 3090 as described (Galle et al. 2010).

# 3.3.5 Cell counts, pH, acidity and metabolite formation in sourdough

To determine viable cell counts, samples of sourdough were serially diluted in Ringer solution and plated in triplicate on mMRS5 agar supplemented with 0.05 g/L bromocresol green. The identity of fermentation microbiota with the inoculum was assessed by comparing the colony morphology to the morphology of *W. cibaria* MG1, and by measuring pH, metabolites and total titratable acidity (TTA) before and after fermentation. The pH and TTA of sourdough was determined as described by Katina et al. (2006).

Maltose, glucose, fructose and sucrose levels of flours and sourdoughs were analysed using an Agilent 1260 high performance liquid chromatography system coupled to a Hi-Plex H column (Agilent, Cork, Ireland). Samples for sugar determination were extracted with distilled water, clarified with Carrez I and Carrez II, and diluted in distilled water (1:10). Using a refractive index detector (RID) concentrations of sucrose, maltose, glucose and fructose in flour and sourdough samples were analysed at 65°C and 25°C to discriminate between coeluting maltose and sucrose peaks. Samples were eluted with water at a flow rate of 0.6 mL/ min. Sourdough samples for organic acid analysis were prepared by precipitating proteins with 7% perchloric acid overnight (15h, 4°C). After centrifugation (2000 x g, 20 min) and filtration (0.450  $\mu$ m), the concentration of lactate, acetate and ethanol were quantified using an Agilent 1200 HPLC system coupled to a refractive index detector and a REZEX  $8\mu$  Organic Acid Column (Phenomenex, USA). Samples were eluted with 0.01N H<sub>2</sub>SO<sub>4</sub> at 65°C and a flow of 0.6 mL/ min.

# 3.3.6 Capillary electrophoresis of extracted proteins

To investigate changes in protein size after sourdough fermentation, samples were analysed with capillary electrophoreses using a lab-on-a-chip technique (Bioanalyzer, Agilent Technologies, Palo Alto, CA). Protein extraction was carried out as per user manual (Agilent Technologies). Preliminary, the protein content of each extract was analysed adding 1 mL Bradford reagent (Sigma, Arklow, Ireland) to 20  $\mu$ l sample (previously diluted 1:10 in extraction buffer) (Bradford 1976). Proteins were extracted under reducing conditions using a dithiothreitol-containing buffer (Hager et al. 2012) and subsequently loading an aliquot on a 80 kDa protein chip in an Agilent Bioanalyzer. Protein peaks with an average concentration lower than 20 mg/ L were not considered, since their significance is low in comparison to the detection limit of the method. The peak area of certain molecular size (kDa) polypeptides was set into relation to the total peak area in the extract. The protein extraction yield (%) is to the protein content in unfermented dough.

# 3.3.7 Oscillation rheology

To evaluate the influence of EPS and acid production produced during sourdough fermentation on different gluten-free batters, rheological measurements were performed with a controlled stress and strain rheometer (Anton Paar MCR 301, Ostfildern, Germany). Sourdough samples were produced as described above using *W. cibaria* MG1 with addition of 10% sucrose (SD Wc+) and without sucrose addition (SD Wc-). Flours were sifted (mesh size 0.05) prior to fermentation to standardise particle size. Wheat sourdough was analysed using a parallel plate geometry (PP50/P2-SN13968; gap d=1 mm), consisting of a 50 mm diameter corrugated probe and plate. Excess of sample was removed after loading and a thin layer of paraffin oil was applied to the edges of the sample to prevent loss of moisture. Buckwheat, quinoa and teff sourdough samples were analysed using a 25 mm concentrically cylinder fitted in a 27 mm cup (CC27-SN8085; d=0 mm). All samples were allowed to rest for 5 min prior

to analysis. Tests were performed at 30°C. Initially, amplitude sweeps were performed in the range 0.001 - 100% strain on all samples to determine the linear viscoelastic region. Frequency sweeps were performed in the range 1-50 Hz angular frequency ( $\omega$ ), with 0.05% strain. Data related to complex modulus (G\*) obtained from the frequency sweeps were fitted according to the power law equation G\* ( $\omega$ ) = A<sub>F</sub>· $\omega$  <sup>1/z</sup> for weak gel model (Gabriele et al. 2001). All results are averages of two measurements of three individual preparations. The change of dough strength, A<sub>F</sub>, of the sourdough sample in relation to the initial dough strength of control is given as  $\Delta A_F$  (= A<sub>F</sub> (sample) - A<sub>F</sub> (control)/ A<sub>F</sub> (sample)\*100).

#### 3.3.8 Determination of enzymatic activity and mineral content in flours

The activity of indigenous  $\alpha$ -amylase of the flours was analysed using a commercial kit (Ceralpha method K-CERA 01/12) (Megazyme International, Kildare, Ireland), as per user manual. One IU is equal to one micromole of 4-nitrophenol released from the substrate in one minute under defined assay conditions. The activity of and proteinases was analysed using haemoglobin as substrate (Brijs et al. 1999). One unit of enzyme activity is equal to release of 1 mg L-leucine per hour under defined assay conditions. Experiments were performed in triplicate. Mineral contents were analysed by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) following the method EN ISO 11885 E22.

#### 3.3.9 Statistical analyses

Results are reported as an average with confidence interval. Statistical analyses were performed with Statistica (data analysis software system), version 7.1 on all data using one-way ANOVA. Fisher's least significant differences test was used to describe means at 5% significance level.

#### 3.4 Results

# 3.4.1 EPS and oligosaccharide production during sourdough fermentation

EPS were extracted from the flour, as well as from the 10% sucrose supplemented sourdough after completion of fermentation. The molecular weight of the EPS as analysed using SEC ranged between 10<sup>6</sup> and 10<sup>7</sup> Da (Figure 3-1). Concentrations reached 0.9 g/ kg dry weight sourdough in teff, 3.2 g/ kg in quinoa and 4.2 g/ kg in buckwheat. In wheat sourdough the amount of EPS did not exceed the initial amount of polysaccharides present in flour.

The qualitative analysis of oligosaccharides formed during sourdough fermentation in the presence of sucrose is shown in Figure 3-2. In wheat sourdoughs, relatively large quantities of oligosaccharides of the panose-series (PSO) were detected (Figure 3-2). Buckwheat sourdough contained very low amounts of oligosaccharides. In quinoa and teff sourdoughs, glucooligosaccharides (GlcOS), a mixture of PSO and isomalto-oligosaccharides, were formed during fermentation (Figure 3-2).



Figure 3-1 Size Exclusion Chromatograms of (A) EPS standards (Levan, high molecular dextran, Dex HM, low molecular dextran, Dex LM, and Inulin) and (B) EPS in *W. cibaria* MG1 sourdoughs





# 3.4.2 Cell counts, pH, acidity and metabolite formation in sourdough

A relatively high inoculum of 10<sup>8</sup> CFU/ g dough was used to ensure dominance of *W. cibaria* MG1 in all sourdoughs. Cell counts after 24 h of fermentation were comparable in buckwheat, quinoa, teff, wheat sourdoughs (Table 3-1) and fermentation microbiota were dominated by *W. cibaria* in all samples. The final pH values ranged from 4.1 (wheat) to 4.5 (quinoa) and the highest TTA was observed in quinoa sourdoughs (Table 3-1).

Parameters	Sample	Buckwheat	Quinoa	Teff	Wheat
Cell count	Flour	8.2 ± 0	$8.0 \pm 0$	8.3 ± 0.1	8.8 ± 0.1
(log CFU/g)					
	Wc+ <sup>a</sup>	8.9 ± 0	9.5± 0.1	9.6 ± 0.2	$8.8 \pm 0$
	Wc - <sup>b</sup>	9.5 ± 0	9.4 ± 0.1	9.2 ± 0.2	9.3 ± 0.1
pH level	Flour	6.9 ± 0	$6.8 \pm 0$	6.8 ± 0	6.1 ± 0.1
	Wc+ <sup>a</sup>	$4.4 \pm 0.1$	$4.4 \pm 0$	$4.2 \pm 0$	4.3 ± 0
	Wc- <sup>b</sup>	$4.2 \pm 0.2$	$4.3 \pm 0.2$	$4.1 \pm 0.1$	4.1 ± 0.3
TTA (ml)	Flour	$2.7 \pm 0.1$	$3.2 \pm 0$	$2.0 \pm 0.1$	$1.5 \pm 0.1$
	Wc+ <sup>a</sup>	16.8 ± 1.2	$26.4 \pm 0.6$	$16.2 \pm 0.4$	8.2 ± 0.3
	Wc- <sup>b</sup>	19.5 ± 2.1	30.9 ± 1.2	$18.5 \pm 0.7$	7.6 ± 0.4

Table 3-1 Weissella cibaria MG1 sourdough fermentation analyses

<sup>a</sup>Wc+ with 10% flour replaced by sucrose

<sup>b</sup> Wc- has no added sucrose

Levels of fermentable sugars were lowest in oat flour with only 5 mmol maltose, 7 mmol sucrose, 13 mmol glucose and 14 mmol fructose per kg flour. W. cibaria grew poorly in oat sourdoughs, even at this high inoculum level, and produced less than 30 mmol / L lactic acid. Contaminating microbiota was also observed. Oat sourdoughs were thus not evaluated further. Sucrose levels were highest in teff flour (113 mmol/ kg flour) and twice as high as in quinoa and wheat flour (Table 3-2). Quinoa flour contained highest amounts of maltose (158 mmol), approximately twice as much as buckwheat flour. Wheat and buckwheat flour contained comparable amounts of glucose (25 mmol and 36 mmol), whereas quinoa flour contained highest levels of glucose (203 mmol), namely five times more than buckwheat and eight times more than wheat flour. Fructose levels were similar in quinoa, teff and wheat flour (80, 75 and 73 mmol). In both types of sourdoughs, sucrose levels increased with the exception of teff and wheat sucrose-supplemented sourdoughs remaining similar (wheat Wc+) or decreasing (teff Wc+). In both types of wheat sourdoughs (Wc+/ Wc-) maltose levels increased during fermentation (Table 3-2). In non-sucrose supplemented (Wc-) maltose was not detectable in gluten-free sourdoughs. Greatest increase of fructose levels was observed in sucrose-supplemented sourdoughs. Concentrations of lactate in sourdough ranged from 123 mmol/kg flour (sucrose supplemented wheat sourdough) to 291 mmol/kg flour (nonsupplemented quinoa sourdough). Generally, lactate concentrations were not statistically significant between sucrose-supplemented sourdoughs and controls sourdoughs without added sucrose (Table 3-2). This confirms the pH and TTA findings (Table 3-1).

Substrate/	Sample	Buckwheat	Quinoa	Teff	Wheat
metabolites					
(mmol/ kg flour)					
Sucrose	Flour	73 ± 3	56 ± 27	$113 \pm 11$	51 ± 1
	Wc+a	$178 \pm 24$	74 ± 15	56 ± 6	$54 \pm 18$
	Wc-b	107 ± 5	153 ± 4	126 ± 17	81 ± 2
Maltose	Flour	80 ± 13	$158 \pm 16$	$103 \pm 1$	127 ± 13
	Wc+a	119 ± 1	127 ± 6	$210 \pm 10$	259 ± 33
	Wc-b	0	0	0	491 ± 78
Glucose	Flour	36 ± 2	203 ± 3	56 ± 2	25 ± 1
	Wc+a	283 ± 68	676 ± 15	231 ± 22	85 ± 18
	Wc-b	101 ± 22	$780 \pm 10$	62 ± 5	121 ± 11
Fructose	Flour	56 ± 3	80 ± 22	75 ± 25	73 ± 2
	Wc+a	478 ± 81	843 ± 15	657 ± 24	595 ± 98
	Wc-b	106 ± 13	306 ± 26	96 ± 22	$110 \pm 20$
Lactate	Flour	0	0	0	0
	Wc+a	211 ± 43	195 ± 35	152 ± 29	123 ± 7
	Wc-b	226 ± 47	291 ± 67	223 ± 55	211 ± 39
Acetate	Flour	0	0	0	0
	Wc+a	25 ± 2	35 ± 6	24 ± 5	$10 \pm 0$
	Wc-b	31 ± 7	43 ± 1	36 ± 1	21 ± 2
Ethanol	Flour	0	0	0	0
	Wc+a	98 ± 11	129 ± 22	99 ± 26	86 ± 17
	Wc-b	94 ± 11	$110 \pm 11$	97 ± 7	89 ± 6

Table 3-2 Sugar amounts and metabolites formed before and after fermentation with *W. cibaria*. Results shown are average values  $\pm$  confidence intervals ( $\alpha$ =0.05) of two independent experiments

 $^{\rm a}\,10\%$  flour replaced by sucrose, equal to 278 mmol sucrose per kg flour

 $^{\rm b}$  non-sucrose supplemented sourdough

# 3.4.3 Determination of enzymatic activities and mineral content of flours

The indigenous  $\alpha$ -amylase activity in all flours was analysed in relation to maltose and glucose release during fermentation. Indigenous flour proteolytic activities were assayed to interpret protein degradation in sourdoughs. Wheat and teff flours had the highest amylolytic activity, but no amylase activity could be determined in oat flour (Table 3-3). Quinoa flour showed the greatest proteolytic activity. Mineral contents per 100 g flour dry mass as analysed by ICP-AES increased in the order: oat (5164 ± 113 mg) < wheat (6193 ± 54 mg) < buckwheat (10,0237 ± 125 mg) < teff (12,631 ± 34 mg) < quinoa flour (15,235 ± 218 mg).

Table 3-3  $\alpha$ -Amylase and protease activities of buckwheat, oat, quinoa, teff and wheat flours

Enzyme	Dualruhaat	Oct	Quince	Toff	Wheat
activities	вискупеат	Uat	Quinoa	Tell	wheat
α-Amylase	0.22 ± 0.16 °	ND	$0.17 \pm 0.05$ d	$0.45 \pm 0.05$ b	$0.67 \pm 0.09^{a}$
(IU/g)					
Protease	9.2 ± 1.0 <sup>b</sup>	6.7 ± 1.0 °	$22.0 \pm 2.4^{a}$	$5.0 \pm 0.4$ <sup>d</sup>	$4.1 \pm 0.5$ d
(U/g)					

Different superscript letters in the same row represent values of statistical difference (p < 0.05)

#### 3.4.4 Capillary electrophoreses of extracted proteins

Modifications of the flour and SD protein extracts during fermentation were compared by capillary electrophoresis. Typical peaks obtained from the electropheretogram and the protein extraction yields obtained are summarised in Table 3-4. Storage protein extraction was conducted under reducing conditions, as described above. The proportional ratio of the globulin fraction 43-55 kDa decreased during fermentation in sucrose-supplemented SD. In quinoa flour peak patterns at 23, 31 and 39 kDa were found. After quinoa fermentation, percentage of peaks between 30-41 kDa decreased slightly, whereas peptides with molecular size between 18-29 and 43-55 were drastically reduced and a 50 kDa peak was eliminated through proteolysis. Major peaks in unfermented teff flour were found at 26, 40 and 60 kDa. Extensive reductions of protein peak areas were observed for teff SD between 18 - 29, 30 - 41 and 43 - 55 kDa. As in guinoa SD, the amount of extractable proteins was decreased in teff SD when compared to the flour. Upon fermentation of wheat flour with W. cibaria, peaks between 59-79 kDa were eliminated through proteolysis, whilst the area of peaks between 30-41 kDa increased.

Table 3-4 Relative protein size distribution of different flours and sucrose-supplemented sourdough after 24 h fermentation with *Weissella cibaria* MG1

Cod-D control riotord	Buckwhe	at (%)*	Quinoa (	(%)	Teff (%)		Wheat (%	(9
Frotein range (KDa)	SD	Flour	SD	Flour	SD	Flour	SD	Flour
18-29	26	24	22	41	2	14	~1 1	<1
30-41	6	15	24	38	15	37	6	9
43-55	ഹ	6	2	10		17	67	67
59-79	I	ı		<1	24	6	ı	21
Protein extraction yield (g/kg)	8.6 ± 1.2	37.4 ± 4.2	$0.4 \pm 0$	11.5 ± 2.5	$1.3 \pm 0.1$	9.3 ± 2.1	8.1 ± 0.4	23.3 ± 0.1
* Represents percentag - means no peak detect SD means <i>Weissella cib</i>	ge of total p ed, <i>aria</i> MG1 se	eak areas, ourdough						

# 3.4.5 Oscillation rheology

The effects of SD fermentation on microstructural changes in the system, and thereby dough rheology, were evaluated through rheological frequency sweep analyses (Table 3-5). The weak gel model, as introduced by Gabriele et al. (2001), was applied to determine the resistance to deformation and the network connectivity of the samples over the range of angular frequencies  $\omega$  from 0 to 9.63 Hz. Two parameters were extracted from the power law equation  $G^*(\omega) = A_F \cdot \omega^{1/z}$  (Gabriele et al. 2001), where the strength of the dough towards deformation is represented by  $A_F$ , and the extent of interaction (network connectivity) in the gel is represented by z. Values for  $A_F$ , z and correlation coefficient  $R^2$  are given in Table 3-5. In all dough, the elastic moduli (G') were higher than the viscous moduli (G''), indicating that controls (flour and water), as well as SD samples (whether sucrose-supplemented or not) had a solid, elastic-like behaviour (data not shown).

Generally, the fermentation of all flours with *W. cibaria* lead to a significantly decreased dough strength (parameter  $A_F$ , p<0.05) for SD in comparison to the unfermented control (flour & water), indicating a lower network strength of the SD samples. This results in a decrease (expressed as  $\Delta A_F$ ) of over 90% for buckwheat, quinoa and wheat SD. Fermentation of buckwheat flour resulted in significantly lower values for dough strength in sucrose-supplemented SD than for the non-supplemented SD. Conversely, dough strength  $A_F$  for quinoa sucrose-supplemented SD was slightly higher than for the non-supplemented SD. Changes in the dough strength between sucrose-supplemented and non-supplemented teff SD were not significant. Sucrose-supplemented wheat sourdough showed slightly higher dough strength than non-supplemented sourdough. Network connectivity (z) changed significantly during fermentation for most cereal substrates however, in buckwheat sucrose-supplemented SD and both teff sourdoughs it remained unaffected (p<0.05) (Table 3-5).

Flour	Sample	$A_F$ (Pa·s <sup>1/z</sup> )	ΔA <sub>F</sub> (%)	Z	R <sup>2</sup>
Buckwheat	Flour/water	2170 ± 330 °	_	$4.69 \pm 0.08^{a}$	0.979 ± 0.025
	SD Wc+ <sup>a</sup>	122 ± 83 ª	-94	$4.67 \pm 0.5^{a}$	$0.992 \pm 0.005$
	SD Wc-b	197 ± 75 <sup>b</sup>	-91	$5.98 \pm 0.95^{b}$	0.993 ± 0.003
Quinoa	Flour/water	153 ± 26 °	-	16.41 ± 4.18 <sup>c</sup>	0.934 ± 0.181
	SD Wc + a	12 ± 2 <sup>b</sup>	-92	$5.26 \pm 1.58^{a}$	0.980 ± 0.009
	SD Wc – <sup>b</sup>	8 ± 1.5 ª	-95	$6.66 \pm 0.58^{b}$	0.990 ± 0.004
Teff	Flour/water	20 ± 6 <sup>b</sup>	-	$6.00 \pm 1.12^{a}$	$0.991 \pm 0.004$
	SD Wc + <sup>a</sup>	$4.4 \pm 0.6$ <sup>a</sup>	-78	$6.03 \pm 0.77^{a}$	0.981 ± 0.013
	SD Wc – <sup>b</sup>	5 ± 1.5 ª	-75	$5.90 \pm 0.49^{a}$	$0.979 \pm 0.012$
Wheat	Flour/water	2133 ± 510 °	-	14.36 ± 3.10°	$0.928 \pm 0.137$
	SD Wc + <sup>a</sup>	44 ± 1 <sup>b</sup>	-98	$3.26 \pm 0.22^{a}$	$0.917 \pm 0.212$
	SD Wc – <sup>b</sup>	36 ± 4 ª	-98	$3.55 \pm 0.10^{b}$	$0.986 \pm 0.032$

Table 3-5 Deformation (A<sub>F</sub>) and elasticity (z) rheological measurements of *Weissella* cibaria MG1 sourdoughs (angular frequency,  $\omega = 0 - 9.63$  Hz at target strain,  $\gamma = 0.05$  %)

<sup>a</sup> SD Wc + is with 10% flour replaced by sucrose,

<sup>b</sup> SD Wc – has no added sucrose,

- reference point for calculation of SD Wc +/- results,

Superscript letters represent statistical differences in values for the same flour in the same column

#### 3.5 Discussion

The dextran forming strain *W. cibaria* MG1 grew well in buckwheat, teff and quinoa sourdoughs, in keeping with previous investigations (Moore et al. 2007; Galle et al. 2010). EPS were produced with a molecular weight of  $5 \cdot 10^{6} \cdot 4 \cdot 10^{7}$  Da in sucrose-supplemented sourdoughs, corroborating prior observations with the same strain in sorghum sourdough (Galle et al. 2010; Galle et al. 2011). The strain failed to grow during oat fermentations, likely due to the low concentration of fermentable sugars maltose, sucrose, fructose and sucrose. The EPS yield in sourdough fermentations is determined by the fermentation conditions, properties of the EPS producing strain, the substrate, and the amount of sucrose added (Kaditzky and Vogel 2008; Ruehmkorf et al. 2012a).

W. cibaria produced higher amounts of lactate and had a higher titratable acids in buckwheat, quinoa and teff sourdoughs when compared to wheat sourdoughs. However, the final pH was lowest in wheat sourdough. This observation relates to the higher buffering capacity of buckwheat, quinoa, and teff flours due their higher mineral contents. An increased buffering capacity of sourdough does not alter the final pH, but enables production of higher contents of lactic acid (Gänzle et al. 1998). The accumulation of maltose in wheat sourdough is attributable to the high amylase activity which exceeds degradation by glucoamylase or consumption by *W. cibaria* (Gänzle et al., 2007). In gluten-free sourdoughs, maltose consumption by W. cibaria and cereal enzymes exceeded maltose formation by amylases, comparable with prior observations in sorghum sourdoughs (Galle et al., 2010). This study strongly indicates that the effect of the fermentation substrate is linked to the activity of starch degrading enzymes and the concentration of acceptor carbohydrates, particularly maltose. Maltose acts as a strong acceptor carbohydrate for dextransucrases, and fermentation with W. cibaria in the presence of maltose and sucrose as substrates supported the formation of panose-series oligosaccharides at the expense of dextran formation (Schwab et al. 2008; Katina et al. 2009; Galle et al. 2010). Panose-series oligosaccharides do not influence dough rheology or bread texture but may have a prebiotic effect (Grimoud et al. 2010). Correspondingly, wheat sourdoughs with high concentrations of maltose were characterized by occurrence of oligosaccharides and low levels of EPS. Coinciding, maltose levels were low in buckwheat
sourdough containing oligosaccharides. Higher maltose levels (158 vs. 80 mmol), fructose (80 vs. 56 mmol/ kg) and glucose levels (203 mmol/ kg vs. 36 mmol/ kg) in quinoa flour in comparison to buckwheat flour indicate promoted oligosaccharide production in quinoa sourdough with concurrent EPS formation. Although, sugar levels in teff were higher than in buckwheat flour, EPS formation was higher in the later, indicating its optimal adaption to the microflora from which it was isolated (Moroni et al. 2011). The proportion of EPS produced from supplemented sucrose (1 g EPS from x g sucrose) is expressed by the conversion rate: The higher the value x, the more sucrose is necessary to produce EPS and the lower the conversion rate. In this study, the conversion rates from 50 g sucrose (10% sucrose based on flour) were 1:12 for buckwheat (4.2 g EPS/ kg SD), 1:16 for quinoa (3.2 g EPS/ kg SD) or even 1:45 for teff flours (0.9 g EPS/ kg SD). The lower EPS yield in this study in comparison to previous studies can be attributed to different conditions during fermentation (i.e. inoculum size and the amount of sucrose supplementation).

Protein degradation in gluten-free sourdoughs fermented with *W. cibaria* MG1 differed substantially in this study. Only protein peaks in the range of 18 to 79 kDa were considered in this study as this is the region of cereal and pseudocereal storage protein alterations during fermentation (Lacaze et al. 2007). A decrease in the protein content in quinoa and teff sourdough was observed by capillary electrophoresis. Buckwheat, quinoa and teff have higher crude protein contents than wheat flour (Hager et al. 2012). The main proteins in quinoa, globulins and albumins, are more hydrophilic than wheat gluten (Stikic et al. 2012) and are, therefore, more susceptible to proteolysis (Lorenz and Nyanzi 1989). Quinoa flour also exhibited a high protease activity. However, the extensive proteolysis in quinoa sourdough did not influence dough rheology, indicating that other flour components are primarily responsible for dough strength in quinoa.

The formation of organic acids and the resulting pH drop during fermentation not only activates indigenous proteolytic enzymes, but also imparts a net positive charge to proteins. Thus, intramolecular repulsions augment causing proteins to unfold which then increase in solubility (Galal et al. 1978) consequently resulting in softer dough. In buckwheat and teff SD, EPS production, in combination with acidification, affected the rheological properties of sourdough, leading to a reduction in dough strength. Similar levels of organic acids in both SD trials (sucrose supplemented and non-supplemented SD), indicate, that different dough softening effect after fermentation can be mainly attributed to the dextran produced by *W. cibaria* rather than to the organic acid effects. This is similar to previous findings for sorghum SD (Galle et al. 2012). However, fermentation of wheat flour showed a softening effect, although no EPS production was observed. Gluten degradation induced by gluten-associated proteases and organic acids affected wheat rheological behaviour giving softer dough upon sourdough acidification (Barber et al. 1992; Clarke et al. 2004). Although the amount of EPS formed in quinoa SD was comparable to buckwheat, the EPS-mediated dough softening effect was not detected in the earlier. Increased levels of damaged starch (5.3% of total starch) and dietary fibre (8 g/ 100 g dry weight flour) in quinoa flour compared to buckwheat (damaged starch 3%; dietary fibre 2.5 g/ 100 g flour) and teff (damaged starch 2.4%; dietary fibre 5 g/ 100g flour) flour (Hager et al. 2012), indicate that fortified swelling of starch and dietary fibre in quinoa sourdough might contribute to increased dough strength (Belitz et al. 2008; Delcour and Hoseney 2010).

#### 3.6 Conclusion

Sourdough performance and yield of the exopolysaccharide dextran by *W. cibaria* depended on the substrate used and was highest in buckwheat and quinoa sourdough. The production of dextran was inversely related to oligosaccharide formation, and strongly depended on the concentration of the acceptor carbohydrate maltose. The presence of dextran positively influenced dough rheology imparting a softening effect in buckwheat and teff sourdoughs. Consequently, the heterofermentative lactic acid bacteria *W. cibaria* MG1 is a suitable starter culture for gluten-free flours fermentation.

The use of the highly nutritive gluten-free flours, buckwheat, quinoa and teff, combined with the production of dextran during *W. cibaria* MG1 fermentation could serve to improve the baking characteristics, sensory properties and overall nutritional profile of sourdough-containing gluten-free breads. The impending application in bread making will yield further insight into the strain's functionality and technological contributions.

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# Chapter 4 Influence of dextranproducing *Weissella cibaria* MG1 on baking properties and sensory profile of gluten-free and wheat breads

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#### 4.1 Abstract

Breads based on gluten-free buckwheat, quinoa, sorghum and teff flour were produced with addition of 20% sourdough fermented with exopolysaccharide (EPS) producing Weissella cibaria MG1. Wheat bread was baked as a reference. Dough rheology, bread quality parameters and sensory properties of the sourdough-containing breads were compared to control breads of the respective flour not containing sourdough. The specific volume remained unaffected by sourdough application. In buckwheat, sorghum, teff and wheat sourdough breads, acidification increased crumb porosity compared to control breads. Crumb hardness was significantly reduced in buckwheat (-122%), teff (-29%), guinoa (-21%) and wheat sourdough breads (-122%). The staling rate was significantly reduced in buckwheat, teff and wheat sourdough breads. Water activity of the sourdough-containing bread crumb was not influenced by the presence of exopolysaccharides (EPS). Due to the presence of EPS and influence of acidification, the dough strength, A<sub>F</sub>, as measured by oscillation tests decreased significantly in sourdough-containing buckwheat, sorghum and wheat dough, but increased in sourdough-containing quinoa and teff dough. Microbial shelf-life was neither significantly prolonged for gluten-free sourdough nor for wheat sourdough breads. Scanning electron microscopy of control and sourdough bread crumbs did not show differences in structural starch features. In addition, the aroma of most breads was not improved by sourdough addition.

#### 4.2 Introduction

Cereal products are important staple foods of the human diet (EFIC 2013). However, the digestion of the storage protein gluten (present in wheat and related grains like barley, rye and triticale) releases a peptide from the  $\alpha$ -gliadin fraction which induces a systemic immune-mediated disorder, called coeliac disease, in genetically susceptible persons (Green and Cellier 2007; Fasano and Catassi 2012). Worldwide, 0.6-1.0% of the population is affected by this autoimmune disease which damages the intestinal mucosa through inflammation of the micro-villi and thereby deteriorates the ability to absorb nutrients (Green and Cellier 2007; Fasano and Catassi 2012). Currently, the only available treatment is the complete avoidance of gluten-containing cereals (Arendt et al. 2011). A wide range of gluten-free flours is available as alternative. Breads produced therefrom are often of low nutritional quality and show poor sensory characteristics such as dry crumb, poor mouth feel and off-flavours (Gallagher 2009; Hager et al. 2011). Most gluten-free formulations include gluten-free starches, protein-based ingredients and hydrocolloids which mimic the viscoelastic properties of gluten (Gallagher et al. 2004). Hydrocolloids are ingredients commonly used to improve crumb structure of gluten-free breads, as reviewed by Hager et al. (2013) and Anton and Artfield (2008).

The synthesis of exopolysaccharides (EPS) by lactic acid bacteria has gained increasing interest to improve textural properties of fermented foods in general and bread quality especially. EPS can be divided into homo- (made of one sugar moiety) and heteropolysaccharides (made of two to three different monosaccharides) (Monchois et al. 1999). EPS have the potential to replace hydrocolloids (Di Cagno et al. 2006) and to improve textural properties as well as shelf-life of conventional (Decock and Cappelle 2005; Tieking and Gänzle 2005; Lacaze et al. 2007) and gluten-free breads (Schwab et al. 2008; Galle et al. 2012; Ruehmkorf et al. 2012b). However, the performance in baking applications is determined by the structure of the polymers and is also affected by the flour quality, recipe and parameters used for dough processing and baking. Therefore, strains have to be selected and fermentation conditions to be optimised to maximise *in situ* EPS production while reducing acid production which allows acceptable volume, crumb structure and flavour of breads (Kaditzky et al. 2008). The large variety of EPS-positive strains allows selection

of strains with additional metabolic traits that improve bread flavour, texture and shelf life (Tieking and Gänzle 2005)). Improvement of technological functionality and baking properties of *in situ* formed EPS, and in particular the homopolysaccharides dextran was previously demonstrated in breads made from buckwheat, sorghum and teff flour formulations (Schwab et al. 2008; Galle et al. 2012; Ruehmkorf et al. 2012a). Dextran from *Leuconostoc mesenteroides* is already commercially applied as bread improver (Decock and Cappelle 2005).

*Weissella cibaria* MG1 was chosen due to its ability to produce high amounts of the homopolysaccharide dextran (an  $\alpha$ -1,6-linked glucan) with a molecular weight of  $5x10^6 - 4x10^7$  kDa, but only low amounts of acetate which can deteriorate the crumb structure (Galle et al. 2010) and the organoleptic properties (Hansen and Schieberle 2005). Yields of up to 4.7 g dextran per kg wheat sourdough (Galle et al. 2010) and 4 g per kg buckwheat sourdough (Wolter et al. 2014) have been found previously. Therefore, the influence of sourdough fermented with *Weissella cibaria* MG1 on bread quality and flavour profile of gluten-free breads using a basic recipe based on buckwheat, quinoa, sorghum and teff flour is have been investigated and compared to wheat counterparts as reference.

# 4.3 Materials and Methods

# 4.3.1 Ingredients

The ingredients used in this study were buckwheat flour (Doves Farm Foods Ltd, UK; moisture 12.6%, protein 12.2%, fat 4.2%), quinoa flour (Ziegler Naturprodukte, Germany; moisture 12.3%, protein 13.8%, fat 8.6%), sorghum flour (Twin Valley Mills, Nebraska, USA; moisture 11.1%, protein 4.7%, fat 3.5%), teff flour (Trouw, The Netherlands; moisture 9.5%, protein 12.8%, fat 4.4%), wheat flour (baker's flour, Odlums, Ireland; moisture 12.7%, protein 11.5%, fat 1.8%), yeast (Puratos, Belgium), sugar (Siúcra, Ireland) and salt (Glacia British Salt Limited, UK).

# 4.3.2 Sourdough preparation

Sourdoughs were prepared using *W. cibaria* MG1 as previously described by Wolter, Hager et al. (2013). To ensure exopolysaccharide (EPS) production, 10% flour was replaced with sucrose. Flour, sterile tap water and cell culture solution containing 10<sup>9</sup> CFU LAB/ ml broth were mixed to gain a final inoculum of 10<sup>8</sup> CFU/ ml dough and a dough yield of 190. Fermentations were carried out in triplicates at 30°C for 24 hours.

# 4.3.3 Cell counts, pH and total titratable acidity after sourdough fermentation

Viable cell counts were determined in sourdough by serially diluting samples in triplicate in Ringer solution and plating on modified deMan-Rogosa-Sharpe agar (mMRS5) (Meroth et al. 2003) supplemented with 0.05 g/L bromocresol green. The identity of fermentation microbiota with the inoculum was assessed by comparing the colony morphology to the morphology of *W. cibaria* MG1, and by measuring pH and total titratable acidity (TTA) before and after fermentation. The TTA of sourdough was determined as the amount of 0.1 N sodium hydroxide solution which is necessary to adjust the pH of 10 g sample in 90 ml distilled water to 8.5 as described by Katina et al. (2006).

# 4.3.4 Dough rheology

A controlled stress and strain rheometer (Anton Paar MCR 301, Ostfildern, Germany) was used to evaluate the influence of exopolysaccharides (EPS) and acid production on rheological properties of sourdough samples. All bread batters were prepared without yeast addition to ensure reproducibility of measurements. Sourdough-containing bread batters were prepared replacing 20% w/w of flour by the equivalent amount of fermented flour in the form of sourdough (SD). Bread batters prepared without sourdough served as controls (ctrl). The sourdoughs for rheological trials were prepared using sifted flours (mesh size 0.05 mm). Measurements were carried out as previously described by Galle et al. (2011). For sorghum and wheat samples a parallel plate geometry (PP50/P2-SN13968; gap d=1 mm) was used, consisting of a 50 mm diameter corrugated probe and plate. Excess sample was trimmed off after loading and a thin layer of paraffin oil was applied to the edges of the sample to avoid moisture loss. For buckwheat, guinoa and teff samples a 25 mm cylinder fitted in a 27 mm cup (CC27-SN8085; d=0 mm) was used. Samples were allowed to rest for five minutes prior to analysis. Initially, the linear viscoelastic region was determined for all samples during amplitude sweeps with the strain ( $\gamma$ ) ranging from 0.001 - 100%. Frequency sweeps were performed at 30°C with an angular frequency ( $\omega$ ) ranging from 0 - 9.63 Hz and a target strain ( $\gamma$ ) of 0.05 %. Complex modulus values (G\*) obtained from the frequency sweeps were matched to the power law equation  $G^*(\omega) = A_F \cdot \omega^{1/z}$  for weak gel model (Gabriele et al. 2001). Two parameters were extracted from the power law equation: A<sub>F</sub>, subsequently referred to as the dough strength, and z, the network connectivity (Gabriele et al. 2001). All results are averages of at least two measurements of at least three individual fermentations.

#### 4.3.5 Bread production

Non sourdough-containing breads (control breads) from four different glutenfree flours and wheat flour were produced as previously described by Hager et al. (2012a) using 100% flour, 2% salt, 2% sugar and 3% dry-yeast (weight based on flour, BF). The optimal water addition level (WL) based on flour (BF) was determined through preliminary baking trials for gluten-free flours (85% BF for buckwheat, 95% BF for quinoa, sorghum and teff bread) and with the farinograph method 54-21 (AACC, 2000) for wheat flour (63% BF) (Hager et al. 2012a). Sourdough breads were prepared replacing 20% of flour with the equivalent quantity of flour in the form of sourdough. Gluten-free breads were baked at 190°C for 45 min and wheat breads for 30 min at 220°C top and 235°C bottom heat. Three batch replicas were prepared. Bread loaves were cooled at room temperature for two hours prior to analysis.

#### 4.3.6 Bread characteristics

Bake loss was determined by weight determination of dough before and bread after baking. The influence of various water levels applied in the different gluten-free formulations was taken into account by division of bake loss by water addition level. The moisture of bread crumb on the day of baking (day zero) was determined using the two stage air-oven method 44-15.02 (AACC, 2000). Water activity of the fresh bread crumb was determined using an AquaLab 4TE water activity meter (Decagon Devices Inc., Pullman, Washington, USA). The specific volume of three breads from each baking batch was determined using a laser scanning system (Volscan Profiler, Stable Micro Systems, UK). The instrumental textural crumb evaluation of three slices from three different loaves per batch was conducted according to AACC method 74-09 (AACC, 2000) using a Universal Testing Machine (TA-XT2i texture analyser, Stable Micro Systems, Surrey, UK) on day zero, two and five of storage compressing the slice to 40% of its initial height with a 35 mm probe (buckwheat, sorghum, teff and wheat bread). Due to smaller sample dimension a 12 mm aluminium cylindrical probe was used for quinoa bread. The staling rate was calculated as increase in hardness within five days of storage (staling rate = [hardness (day 5 - day 0)/ days of storage)]. The crumb structure was analysed from three middle slices of three breads per batch in terms of slice area, number of cells, porosity (ratio pore area/slice area) and crumb brightness (mean grey level of pixels, value 0 - 255) using a C-cell Bread Imaging system (Calibre Control International Ltd., UK).

#### 4.3.7 Sensory evaluation

Sensory analyses were performed with a trained panel (n=22) under the conditions described by Hager et al. (2012a) and briefly described below.

#### 4.3.7.1 Aroma profile analyses

Bread loaves were cut in slices (thickness about 2 cm) and the crusts were removed. The samples were presented to the sensory panel, which sniffed the crumbs and described the perceived odour qualities. The panel finally agreed on characteristic odour attributes in a group discussion. Crumb samples were presented again to the panel in a second session and the intensities of the predefined odour attributes were evaluated on a scale from 0 (not detectable) over 1 (weak intensity), 2 (medium intensity) to 3 (high intensity). The results of each attribute were calculated as arithmetic mean. The assessors were trained immediately prior to analysis with aqueous odorant solutions in defined concentrations (factor 100 above the odour threshold), (Buttery et al. 1976; Schuh and Schieberle 2006; Czerny et al. 2008). The odorant solutions reflected the evaluated characteristic odour attributes of the flours: buttery (butane-2,3-dione; 120  $\mu$ g/L), cooked potato-like (3-(methylthio)-propanal); 140  $\mu$ g/L), fatty ((E,E)-deca-2,4-dienal; 7.7  $\mu$ g/L), grassy (hexanal; 1000  $\mu$ g/L), mouldy (geosmin; 2.1  $\mu$ g/L), pea-like (3-isobutyl-2-methoxypyrazine; 3.9  $\mu$ g/L), popcorn-like, roasty (2-acetyl-1-pyrroline; 12  $\mu$ g/L) and oat flakes-like ((E,E,Z)-nona-2,4,6-trienal; 2,6  $\mu$ g/L).

The odorant references were purchased from Sigma-Aldrich (Taufkirchen, Germany; Acros, Geel, Belgium) and AromaLab (Freising, Germany). The attributes "hay-like", "hazelnut-like", "sourdough-like", "wheat bread-like", "yeast dough-like", "soy-sauce-like" and "cooked potato-like" were evaluated based on the experience of the trained assessors.

#### 4.3.7.2 Evaluation of aroma preference

Bread crumb slices were prepared as described above and presented to the panel. The assessors evaluated the preference of the samples on a nine-point-scale from 1 (dislike very much) over 5 (neither like nor dislike) to 9 (like very much). The results were calculated as the arithmetic mean.

#### 4.3.8 Microbial shelf life

The microbial shelf life of breads was determined using the method described by Dal Bello et al. (2007). Briefly, each loaf was sliced transversely in a sterile manner to obtain uniform slices of 25 mm thickness. Each side of the slice was exposed to the air for 5 min, packed in a plastic bag and heat sealed. A tip of a pipette was inserted to ensure comparable aerobic conditions in each bag. Bags were incubated at room temperature and examined for mould growth over a 12-day storage period quantified as the number of slice surfaces, i.e. both front and back of the slice, showing aerial mycelia as a percentage of total bread slices.

# 4.3.9 Scanning electron microscopy

Microscopic crumb structure of sourdough breads was analysed by scanning electron microscopy (SEM). Prior to SEM, bread samples where freeze-dried for approximately 20 h and ground in a mortar. Powdered samples were affixed tape to aluminium stubs with double-sided carbon tape and sputter-coated with a 25 nm layer of gold (BIORAD Polaron Division SEM Coating System). Samples were examined under high vacuum in a field emission scanning electron microscope (JEOL JSM-5510 SEM) with a working distance of 8 mm. Secondary electron images were acquired at an accelerating voltage of 5 kV. For processing of the images SEM Control User Interface, Version 5.21 (JEOL Technics Ltd., Japan) was used.

# 4.3.10 Statistical analyses

Statistical analyses were performed with SigmaPlot (Version 11.0, Systat Software, Inc. 2008) using one-way ANOVA. Fisher's least significant differences test to describe means at 5% significance level.

#### 4.4 Results

# 4.4.1 Cell counts, pH and total titratable acids of sourdoughs, batters and bread crumb

Cell counts after 24 hours of fermentation reached 10<sup>9</sup> CFU/ g sourdough for buckwheat, quinoa, sorghum, teff and wheat and the fermentation microbiota was dominated by *W. cibaria* in all samples. Values for total titratable acids (TTA) of sourdoughs after fermentation with *Weissella cibaria* MG1 increased in the order: wheat (8.2 ml) < sorghum (9.2 ml) < teff (16.2 ml) < buckwheat (16.8 ml) < quinoa sourdough (26.4 ml). Upon incorporation of these sourdoughs into bread batters the pH decreased significantly and increased values for TTA was observed in buckwheat and wheat bread dough Table 4-1.

#### 4.4.2 Dough rheology

The effect of sourdough fermentation on rheological properties of dough was evaluated by performance of oscillation tests. In all samples the elastic modulus (G') was higher than the viscous modulus (G"), indicating that control bread dough as well as sourdough-containing bread dough samples had a solid, elastic like behaviour (data not shown).

The dough strength,  $A_F$ , was significantly decreased upon incorporation of 20% sourdough in comparison to the control dough in buckwheat (-62%), sorghum (-43%) and wheat bread dough (-34%) (Table 4-2). Sourdough-containing quinoa and teff batter showed significantly increased dough strengths (+158% and +70%, respectively). The network connectivity z generally remained unaffected in sourdough containing dough samples. Only in buckwheat dough the addition of sourdough led to a significant increase of the network connectivity.

Table 4-1 Values for pH and total titratable acid (TTA) of *W. cibaria* sourdough before (SD 0h) and after fermentation (SD 24h) of bread without (Ctrl bread) and with SD addition (SD bread)

		Buckwheat	t Quinoa	Sorghum	Teff	Wheat
рН	SD 0h	6.9 ± 0ª	6.7 ± 0 <sup>b</sup>	6.7 ± 0 <sup>b</sup>	6.8 ± 0ª	6.1 ± 0°
	SD 24h	$4.4 \pm 0^{f}$	$4.4 \pm 0^{f}$	$4.2 \pm 0^{h}$	$4.2 \pm 0^{h}$	$4.3 \pm 0^{g}$
	Ctrl bread	$6.3 \pm 0.1^{K}$	6.0 ± 0 <sup>M</sup>	5.9 ± 0 <sup>N</sup>	$6.1 \pm 0^{L}$	6.0 ± 0 <sup>M</sup>
	SD bread	$5.8 \pm 0.1^{\circ}$	$5.6 \pm 0^{w}$	$5.6 \pm 0^{\circ}$	5.5 ± 0×	5.3 ± 0y
TTA (ml)	SD 0h	$2.7 \pm 0^{b}$	3.2 ± 0ª	2.2 ± 0°	$2.0 \pm 0^{d}$	$1.5 \pm 0^{e}$
	SD 24h	$16.8 \pm 1.2^{g}$	$26.4 \pm 0.6^{f}$	$9.2 \pm 0.5^{h}$	$16.2 \pm 0.4^{g}$	8.2 ± 0.3 <sup>i</sup>
	Ctrl bread	$4.4 \pm 0.2^{1}$	$8.9 \pm 0.3^{k}$	$4.0 \pm 0.5^{1}$	$4.3 \pm 0.2^{1}$	3.0 ± 0.2 <sup>m</sup>
	SD bread	5.0 ± 0 <sup>w</sup>	9.8 ± 1.2 <sup>v</sup>	3.3 ± 0.3×	4.2 ± 0.4 <sup>x</sup>	3.6 ± 0.2 <sup>x</sup>
Values give Different si	n as mean ± co	onfidence (α= ints in same	=0.05). column indic	ates statistic	al significand	ą
Different ki	nd of supersci Ttrl hread (k-n	ripts in a rov	v indicates st	atistical sign	nificance with	in SD 0h ( <sup>a-e</sup> ),
	1 nn ran (	יוט טט אווש (	an ( ) ( ) no			

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Table 4-2 Parameters from weak gel model for control bread batter (Ctrl) and sourdough containing bread batter (SD)

		Buckwheat	Quinoa	Sorghum	Teff	Wheat
Dough strength, A <sub>F</sub> , (Pas <sup>1/z</sup> )	Ctrl	437 ± 195 <sup>b</sup>	50 ± 11°	19238 ± 6798ª	30 ± 9ª	11352 ± 3950 <sup>b</sup>
	SD	177 ± 21 <sup>x</sup>	127± 58 <sup>x</sup>	10939 ± 2670 <sup>v</sup>	$51 \pm 8^{\gamma}$	7459 ± 504 <sup>w</sup>
Network connectivity. z	Ctrl	4.95 ± 1.01℃	$7.04 \pm 0.58^{b}$	$11.53 \pm 0.15^{a}$	$7.76 \pm 1.22^{b}$	5.27 ± 1.21 <sup>c</sup>
	SD	$5.70 \pm 0.17^{Y}$	6.61±0.18×	11.35 ± 0.86 <sup>v</sup>	7.37 ± 0.55 <sup>w</sup>	$5.45 \pm 0.12^{z}$
Values given as m	lean ± c	onfidence (α=0.	.05).	-	-	-

Different size of superscripts in same column indicates statistical significance between sourdough and control breads. Different kind of superscripts in a row indicates statistical significance within control (a-e) or sourdough (v-z) breads series (P<0.001)

# 4.4.3 Bread characteristics

Cross-sections of resulting gluten-free and wheat breads are depicted in Figure 4-1. Buckwheat breads showed the darkest crumb, in comparison to the wheat bread crumb which had the brightest crumb. The application of sourdough decreased bake loss significantly in quinoa and sorghum sourdough breads compared to control breads (Table 4-3). Among the gluten-free sourdough breads, the highest moisture content of bread crumb was found in quinoa and teff sourdough breads, confirming the relation to higher water addition level in these formulations during the dough preparation compared to buckwheat and wheat bread. Moisture contents of sourdough breads did not differ significantly except for buckwheat sourdough bread which had slightly increased moisture content.



Figure 4-1 Photographs of bread cross-sections from buckwheat, quinoa, sorghum, teff and wheat breads (from top to bottom) as control (left) and sourdough breads (right)

Among gluten-free breads, sorghum, teff and buckwheat sourdough breads had highest specific volumes, however only half that of wheat sourdough bread (Table 4-3). Sourdough application did not affect the specific volume of glutenfree breads. Only wheat sourdough bread showed significantly increased specific volume (+76%) compared to the control bread. Crumb hardness on the day of baking was significantly reduced in buckwheat (-79%), teff (-29%), quinoa (-21%) and wheat sourdough breads (-122%). In sorghum sourdough bread, crumb hardness was insignificantly increased (+9%) (Table 4-3, Figure 4-2). The staling rate for gluten-free breads was lowest for quinoa sourdough bread (1 N/ day) and highest for sorghum sourdough bread (10 N/ day). In comparison, wheat sourdough bread showed a rate of 1 N/ day. A significant reduction of staling rate was observed in buckwheat (-156%), teff (-50%) and wheat (-268%) sourdough breads (Figure 4-2).

Table 4-3 Baking properties of gluten-free control (Ctrl bread) and sourdough (SD bread) breads in comparison to wheat equivalent

		Buckwheat	Quinoa	Sorghum	Teff	Wheat
Bake loss (%)	Ctrl Bread	$15.8 \pm 0.2^{\circ}$	$15.5 \pm 0.2^{c}$	$19.2 \pm 0.4^{a}$	$13.9 \pm 0.4^{d}$	$17.1 \pm 0.3$
	SD Bread	$15.3 \pm 0.3^{x}$	$14.0 \pm 0.4^{\text{Y}}$	$17.6 \pm 0.7^{W}$	$14.5 \pm 0.4^{y}$	$20.7 \pm 0.4$
Moisture (%)	Ctrl Bread	$50.6 \pm 2.4^{b}$	48.2 ± 2.3 <sup>cd</sup>	44.3 ± 4.5e	$50.3 \pm 1.6^{b}$	$46.3 \pm 1.9$
	SD Bread	$47.0 \pm 0.6^{XY}$	$50.0 \pm 1.6^{\circ}$	43.4 ± 3.9 <sup>v</sup>	48.6 ± 2.6 <sup>vw</sup>	$43.3 \pm 3.2$
Water activity, a <sub>w</sub>	Ctrl Bread	0.971 ± 0.002°	$0.974 \pm 0.001^{b}$	0.980 ± 0.002ª	0.978±0.002ª	$0.967 \pm 0.0$
	SD Bread	0.976 ± 0.011 <sup>v</sup>	0.959 ± 0.027 <sup>v</sup>	$0.981 \pm 0.011^{\circ}$	0.976±0.002v	$0.975 \pm 0.0$
Specific volume (ml/g)	Ctrl Bread	$1.69 \pm 0.05^{d}$	$1.51 \pm 0.04^{f}$	$1.85 \pm 0.10^{\circ}$	1.60 ± 0.02 e	$2.62 \pm 0.1$
	SD Bread	$1.67 \pm 0.06^{\circ}$	$1.44 \pm 0.04^{z}$	$1.84 \pm 0.04^{\circ}$	$1.72 \pm 0.11$ <sup>wx</sup>	$3.39 \pm 0.1$
Hardness (N), day 0	Ctrl Bread	$42.9 \pm 1.9^{ab}$	$31.7 \pm 1.1^{\circ}$	26.3 ± 1.9 <sup>d</sup>	43.1 ± 2.5ª	$8.5 \pm 0.5$
	SD Bread	$24.0 \pm 1.1^{X}$	$26.3 \pm 1.3^{X}$	29.1 ± 2.0 <sup>w</sup>	$33.5 \pm 1.3^{\circ}$	$3.8 \pm 0.4$
Staling rate (N/ day)	Ctrl Bread	8 ± 1 <sup>c</sup>	$1 \pm 1^{e}$	$10 \pm 1^{b}$	13 ± 1ª	5 ± 1 <sup>d</sup>
	SD Bread	3 ± 0 <sup>w</sup>	$1 \pm 1^{x}$	10 ± 2 <sup>v</sup>	9 ± 1 <sup>∨</sup>	$1 \pm 1^{x}$



Figure 4-2 Crumb hardness of gluten-free and wheat control breads confronted to sourdough breads baked over five days of storage

# 4.4.4 Crumb structure

Digital image analysis was used to characterise crumb structure and compare the breads in terms of slice area, number of cells, cell volume, porosity and crumb brightness (Table 4-4, Figure 4-3). The highest slice area for gluten-free breads was found in buckwheat sourdough bread. In general, the slice area was decreased by the application of sourdough in all gluten-free formulations, but differences were only significant in sorghum sourdough bread. In wheat sourdough bread a significant increase (11%) of the slice area was also detected. With the exception of buckwheat sourdough bread, the incorporation of sourdough led to a significantly lower number of cells, but simultaneously to a significant increase in the cell volume indicating a more open structure for the sourdough breads (Table 4-4). Hence, the crumbs of sourdough breads appeared coarser than those of the respective control breads. Highest porosity was found in sorghum sourdough bread. Wheat sourdough bread showed the brightest crumb. Among the gluten-free flours, teff and quinoa sourdough bread showed the brightest crumb, whereas buckwheat and sorghum sourdough bread showed the darkest crumb. As the application of sourdough led to an increase in the cell volumes, and the crumb structure appears more open (Table 4-4, Figure 4-3), lower values for crumb brightness are achieved. This is reflected in significantly higher porosities for buckwheat, sorghum, teff and wheat sourdough breads (Table 4-4).

Table 4-4 Crumb properties of gluten-free control (Ctrl Bread) and sourdough (SD Bread) breads in comparison to wheat bread

			)	0.01 Brittin		
Slice area (mm²) Ct	trl Bread	4794 ± 29 <sup>b</sup>	4006 ± 54°	5014 ± 301 <sup>b</sup>	4401 ± 45 <sup>d</sup>	6846 ±
SD	D Bread	4471 ± 69 <sup>w</sup>	3776 ± 96 <sup>y</sup>	4379 ± 162 <sup>wx</sup>	$4040 \pm 185^{xy}$	7676±6
Number of cells Ct	trl Bread	$1908 \pm 24^{d}$	2668 ± 44°	2788 ± 114°	$3170 \pm 92b$	4907 ±
SD	D Bread	$2068 \pm 224^{X}$	2147 ± 343 <sup>x</sup>	$1888 \pm 115^{X}$	2187 ± 215 <sup>vx</sup>	3311±2
Cell volume (mm <sup>3</sup> ) Ct	trl Bread	$8.2 \pm 0.1$ cd	7.5 ± 1.0 <sup>d</sup>	$15.1 \pm 1.2^{a}$	$6.4 \pm 0.3^{d}$	6.3 ± 0
SD	D Bread	$16.0 \pm 5.6^{WX}$	13.1 ± 8.5×	22.4 ± 5.4 <sup>vw</sup>	$11.0 \pm 2.2^{X}$	$13.8 \pm 1$
Porosity (%) Ct	trl Bread	49.9±0.1 <sup>d</sup>	50.6±0.7 <sup>cd</sup>	$54.6 \pm 0.4^{ab}$	$47.8\pm0.4^{\mathrm{e}}$	51.3±
SD	D Bread	$55.1 \pm 1.5^{W}$	$52.3 \pm 2.9^{xy}$	57.3 ± 1.2 <sup>vw</sup>	$51.6 \pm 1.5^{\gamma}$	55.8±0
Brightness (%) Ct	trl Bread	90.7 ± 0.9 <sup>d</sup>	94.8 ± 1.8°	95.7 ± 3.2°	$103.1 \pm 1.8^{b}$	146.5±
SD	D Bread	$80 \pm 0.5^{\text{Y}}$	95.8 ± 2.4 <sup>w</sup>	80.7 ± 2.0 <sup>D</sup>	$90.8 \pm 1.4^{X}$	134.6±



Figure 4-3 Cross-section images from C-Cell Imaging System corrected by brightness of buckwheat, quinoa, sorghum, teff and wheat breads (from top to bottom) as control (left) and sourdough breads (right)

#### 4.4.5 Sensory evaluation

The aroma qualities of bread crumbs, which were prepared from buckwheat, quinoa, sorghum, teff and wheat flour (reference) and the sourdough containing bread were determined in a first investigation by the evaluation of the overall aroma preference (data not shown). The orthonasal preference of the sourdough reference bread (wheat) scored 7.9 points on the nine-point-scale meaning that it was liked very much. The preference score was even higher than the one, which was determined in control bread crumb (Hager et al., 2012a) indicating that *Weissella cibaria* improves the aroma quality of wheat breads. A lower preference value was found for buckwheat sourdough bread (4.6), which was disliked slightly. Even lower scores were determined for sourdough breads prepared from sorghum, quinoa and teff (3.2, 2.8 and 2.5, respectively) which were disliked moderately.



Figure 4-4 Aroma profile analysis of bread crumbs prepared with *Weissella cibaria* sourdough from different flours

Detailed information on the odour characteristics of the crumbs was obtained by aroma profile analysis. The characteristic odour attributes were evaluated by a trained sensory panel, which then scored the intensities of the attributes. The reference crumb made from wheat flour was characterised with an intense "wheat bread-like" note (Figure 4-4). The buttery note was perceived as an additional aroma contributor and it was scored with a weak to medium intensity. The attributes "fatty", "popcorn-like/ roasty", "sourdough-" and "yeast-like" were also characteristic for wheat bread crumb but their intensity were perceived weakly. It can be concluded that the evaluated attributes are responsible for the high preference.

Buckwheat crumb did not exhibit the characteristic "wheat bread-like" and "buttery" attribute of the reference (Figure 4-4) which is the reason for the distinct lower preference. "Grassy", "mouldy" as well as "pea-", "hay-" and "hazelnut-like" notes were evaluated on a weak intensity level as odour characteristics of buckwheat.

Regarding the remaining three bread crumbs (quinoa, sorghum and teff), which had a low preference scoring, all of them also missed in particular the positive attributes "wheat bread-like" and "buttery" of the reference crumb (Figure 4-4). In quinoa bread crumb, the odour qualities "pea"- and "cooked potato-like" were evaluated with medium intensities as the dominant aroma characteristics (Figure 4-4). Additional perceivable attributes were "grassy" and "mouldy" with weak to medium, and "hay-like" with a weak intensity. Besides the odour qualities "cooked tomato-" and "pea-like" which were characteristic for both sorghum and teff crumb, "hay-like" (sorghum) and "grassy" (teff) notes were determined as further attributes (Figure 4-4). Since all the bread samples had the "pea-like" attribute in common, a negative influence of this attribute on preference can be assumed.

# 4.4.6 Microbial shelf life

Environmental shelf life studies were conducted on gluten-free breads containing sourdough fermented with *Weissella cibaria* MG1. For gluten-free control breads, the first mould growth was observed on day four giving a shelf life of three days. Sourdough addition did not prolong microbial shelf life, since also sourdough containing gluten-free and wheat breads started to show mould growth on day four (data not shown).

#### 4.4.7 Scanning electron microscopy

Micrographs of sourdough containing breads showed similar appearance of starch granules compared to the sourdough non-containing control breads (Figure 4-5). While the gluten network was visible in the micrographs of both control and sourdough wheat breads as a sheet-like structure, this feature was missing in the micrographs of the gluten-free breads.



Figure 4-5 Scanning electron micrographs of crumbs from buckwheat, quinoa, sorghum, teff and wheat breads (from top to bottom); control breads (left) and sourdough breads (right); at 2000x magnification; scale bar represents 10µm

#### 4.5 Discussion

The influence of the exopolysaccharide (EPS) dextran produced *in situ* by *Weissella cibaria* MG1 on bread and its sensory properties as well as on dough rheology of buckwheat, quinoa, sorghum and teff bread was studied. Gluten-free sourdough breads were produced using 20% sourdough and compared to wheat sourdough bread as reference. *In situ* produced microbial exopolysaccharides can have beneficial effects on bread quality (Schwab et al. 2008; Galle et al. 2012; Ruehmkorf et al. 2012b) similar to added hydrocolloids such as for example pectin, carboxymethyl cellulose and xanthan (Lazaridou et al. 2007).

Sourdough fermentation with Weissella cibaria MG1 induced significant changes on flour components resulting in varying modifications of rheological properties in the sourdough-containing bread dough. The influence of hydrocolloids on dough properties can be related to two opposite effects on the starch-gel: On the one hand, swelling of starch granules and leaching out of amylose are reduced which increased rigidity. On the other hand, interparticle contacts can be inhibited and thereby the composite starch network is weakened (Biliaderis et al. 1997). Therefore, at intermediate solid particle concentrations (as in bread dough) rheological properties are dependent on both the volume fraction of starch granules and the rigidity of the particles (Biliaderis et al. 1997). Galle et al. (2012) found that the addition of 20% sourdough which contained dextran with the same molecular size decreased the dough strength of a gluten-free sorghum bread formulation. With regard to the weak gel model these authors suggested that dextran interferes with the structural flour components resulting in less interaction between starch-protein associations in the dough system. In this study, the addition of sourdough to buckwheat and sorghum samples caused a dough softening which can be related to the dextran amounts produced in buckwheat (4.2 g/kg sourdough) (Wolter et al., 2013) and sorghum (1.1 g/ kg sourdough; data not published). Similar EPS amounts were also found in quinoa (3.2 g/ kg) and teff (0.9 g/ kg) sourdoughs (Wolter et al., 2013). However, the addition of these sourdoughs to the bread formulation led to significantly increased dough strengths. Therefore, it can be concluded, that effects of acidification and presence of EPS manifest itself to a different extent depending on the flour matrix. The influence of EPS on rheological behaviour of gluten-free flours is poorly understood and factors, such as starch and protein degradation also have to be considered. While the rheology of wheat dough is mainly influenced by the gluten network, dough rheology in gluten-free systems is much more influenced by starch properties. Compared to intact starch, damaged starch which occurs during dry milling of the grain binds more water (Gallagher et al. 2004) and increases dough consistency by swelling (Belitz et al. 2008). Highest contents of damaged starch were found in quinoa and sorghum (5.4 and 5.2 % dwb) followed by wheat (9.0 % dwb) flour. Buckwheat (3.0 % dwb) and teff (2.4 % dwb) flour showed the lowest values (Hager et al. 2012b). Increased dough strength as observed in quinoa sourdough bread batter could therefore partially be explained with higher damaged starch contents (Gallagher et al. 2004). In wheat sourdough breads, no microbial EPS were produced during fermentation (Wolter et al., 2013). Nevertheless, dough strength decreased. Previously, Clarke et al. (2004) related the softening of a wheat sourdough bread to the presence of organic acids which cause a positive net charge that leads to an unfolding of the protein (Galal et al. 1978). The pH drop during sourdough fermentation also changes the activity of indigenous proteinases present in the flour (Bleukx et al. 1997; Thiele et al. 2002) leading to the weakening of the protein-network by activation of gluten degrading wheat proteinases (optimum activity at pH values  $\leq$  4) (Bleukx et al. 1997; Bleukx and Delcour 2000).

This study showed that EPS production did not generally lead to an improved bread quality, but that the effect depended on the flour matrix used. Since EPS act as hydrocolloids and are able to bind water to their molecular structure (Hoefler 2004), the higher dextran content in quinoa sourdough could explain the lower bake loss of the equivalent sourdough bread. Whereas, the weakening of the gluten-network under acidic conditions (Takeda et al. 2001; Clarke et al. 2004) led to an increased bake loss in wheat sourdough bread in current study. The specific loaf volume is a crucial parameter determining bread quality (Maleki et al. 1980). In the wheat bread systems the incorporation of sourdough caused an increase in loaf volume and can be linked to better gas holding capacity of gluten in the acidified dough (Gobbetti et al. 1995; Katina et al. 2006). Specific loaf volumes of gluten-free sourdough breads are comparable to values found in previous studies (Alvarez-Jubete et al. 2010; Galle et al. 2012) confirming that sourdough generally does not have a significant influence on specific volume of gluten-free breads (Moore et al. 2007). As shown by Galle et al. (2012) for sorghum sourdough bread, our study confirmed that *in situ* dextran production did not improve the specific volume. Controversially, external addition of a purified dextran of higher molecular size (8x10<sup>7</sup> – 24x10<sup>7</sup> Da) produced by *Lactobacillus animalis* increased specific volume and decreased crumb hardness of a buckwheat/rice flour based bread (Ruehmkorf et al. 2012b). This increase in specific volume might also be due to the absence of organic acids in the purified EPS, which if present in sourdough could counteract the positive effect of EPS (Katina 2005).

Furthermore, in the above mentioned study a higher dough yield (dy) of 200 was applied (Ruehmkorf et al. 2012b), which results in softer dough and facilitates oven-spring (compared to a dy of 190 used in this study). Producing soft (low consistency) dough using high water levels previously improved the loaf specific volume of sorghum breads (Schober et al. 2005). However, crumb softening upon sourdough addition was also observed in our study for all breads except for sorghum sourdough bread. The favourable reduction of crumb hardness of buckwheat, quinoa and teff bread can be partially related to *in situ* production of EPS by *Weissella cibaria* MG1 (4.2; 3.2 and 0.9 g/ kg sourdough, respectively). The crumb softening effect of EPS was previously linked to their ability to act as hydrocolloids interfering with the starch-protein-interactions (Galle et al. 2012).

However, in addition to the effect of EPS, acidification also influenced crumb hardness in sourdough breads. Although EPS amounts found in sorghum and teff sourdoughs were comparable, crumb hardness of teff but not of sorghum sourdough bread was decreased in comparison to the non-acidified control breads. These findings are in keeping with previous results, where even high amounts of EPS (8.0 g/ kg SD) (Galle et al. 2010) did not significantly change the crumb hardness of fresh sorghum sourdough bread (Galle et al. 2012). Previously, crumb hardness in a gluten-free formulation was significantly reduced after the application of isolated homopolysaccharides (Ruehmkorf et al. 2012b).

In our study during fermentation no microbial EPS have been produced in wheat sourdough (Wolter et al., 2013), and therefore the reduced crumb

hardness of wheat sourdough breads can be attributed to the softening effect caused by acidification (Clarke et al. 2004). The application of sourdough decreased the staling rate in buckwheat, teff and in wheat sourdough bread. The staling rate in the wheat system is mainly determined by crumb firming caused by recrystallization of amylopectin, water redistribution between crumb and crust, and the gluten network (Maleki et al. 1980). Gluten slows down the movement of water (Sciarini et al. 2010) and thereby reduces the staling rate. Therefore, the lack of a continuous protein network in gluten-free breads leads to quicker staling. This is confirmed by staling rates in the present study, being nearly twice as high in gluten-free control (sorghum and teff) than in wheat control breads. The reduction of staling rate upon sourdough application (in buckwheat and teff bread) confirms reduced staling rate in EPS-containing sorghum sourdough bread as found in a previous study (Galle et al. 2012).

Consumers' choice of bread is greatly influenced by the crumb structure (Cauvain, 2007). The type of bread determines the expectation on crumb appearance. Usually, gluten-free breads show a dry, dense crumb structure (Gallagher and Gormley 2002) resulting from the incapability to incorporate and retain gas due to the lack of a gluten network (Gallagher et al. 2004). These crumb characteristics were also observed in the current study. The application of sourdough improved gluten-free bread crumbs and led to a coarser, more open structure indicated by the lower number of cells in combination with an increased cell volume and lower crumb brightness. The intensity of reflected light depends on the cellular structure of the bread crumb (Scanlon and Zghal 2001). Therefore, regions with a finer structure reflect more light, whereas regions with a coarser structure reflect less light.

The overall mean porosity (48–57 %) was higher than that previously reported [38%; (Gallagher et al. 2003); 33%, (Crowley et al. 2000) and 46%, (Sapirstein et al. 1994)]. This effect of sourdough on crumb porosity however, was also reported for biologically and chemically acidified gluten-free sourdough breads based on starch and rice flour (Moore et al. 2007). The sorghum crumb system was not strong enough to support a cohesive crumb structure and showed a collapsed top which confirms previous findings, that the protein/ starch phases in the crumb of sorghum bread lacks strength to support the weight of the batter during proofing and baking (Renzetti and Arendt 2009). In a previous

study by Ruehmkorf et al. (2012b) external addition of purified microbial dextran, which did not contain organic acids, did not influence crumb porosity in comparison to a hydrocolloid-free control. The increase of crumb porosity observed in the current study can therefore be attributed to changes in batter system caused by acidification.

The aroma properties of the sourdough bread crumbs showed some difference to breads, which were prepared from the gluten-free flours by yeast fermentation without any sourdough addition (Hager et al. 2012a). The superior aroma of the reference wheat bread was once more confirmed in this study. The addition of buckwheat sourdough to the final bread dough influenced the preference positively most probably due to the hazelnut, cooked-potato- and sourdough-like note, which was detectable in the sourdough bread crumb. The opposite was observed for sorghum bread, which was evaluated as inferior in comparison to yeast sorghum bread. This observation is most likely the result of the generated cooked tomato-like aroma during sourdough fermentation. *W. cibaria* MG1 sourdough did not increase the very low preference of teff and quinoa breads and the aroma profiles of yeast and sourdough crumbs were almost identical. In particular, the negative and intense pea-like and mouldy attributes in quinoa bread were not reduced by the lactobacilli.

It can be assumed that either the level of sourdough addition is not sufficient to influence the overall aroma positively or the microorganism is not able to eliminate the odorants causing this negative bread aroma. Investigations on the aroma potential of lactic acid bacteria however have demonstrated that specific strains are able to generate individual aroma profiles and odorant compositions due to their metabolic properties (Czerny et al. 2005). Therefore, the application of other lactobacilli strains can be promising regarding aroma improvement of gluten-free breads.

In the present study, the microbial shelf-life of gluten-free and wheat bread was not prolonged by application of sourdough. Previously, improved mould-free shelf-life of maize flour and maize starch based breads was achieved by application of mixed cultures of lactic acid bacteria (Sanni et al. 1998; Edema et al. 2005). The delay of mould growth in gluten-free breads was previously associated with the presence of lactic and acetic acid (Roecken 1996; Corsetti et al. 1998). Hager et al. (2012a) explained the earlier appearance of mould on gluten-free breads with the higher water level (85 or 95%) compared to the wheat formulation (63%). However, this study contradicts the previous explanation to some extent, since mould growth commenced on the same day of storage for gluten-free and wheat bread. The rate of mould growth on buckwheat and quinoa sourdough breads was delayed possibly due to higher amounts of total titratable acids (TTA) in the crumb compared to the gluten-free control breads. In general, prolonged microbial shelf life can be related to a reduced water activity and higher acidity in food systems (Belitz et al., 2008). Although, hydrocolloids and exopolysaccharides possess the ability to surround their molecular structure with "organised" water molecules (Hoefler 2004), this does not lead to a reduction in water activity, since the reduction of free available water is mostly achieved by addition of low molecular weight molecules such as glycerine, fructose or salt (Hoefler 2004; Belitz et al. 2008). Therefore, the incorporation of EPS-containing sourdough did not change the water activity in gluten-free or wheat sourdough breads.

Micrographs of sourdough containing breads did not show any difference between control breads and sourdough-containing breads in terms of general appearance of starch granules. Bread represents a limited water system and therefore starch cannot gelatinise completely during the baking process (Gallagher 2009). Even the high water addition levels in gluten-free formulation did not lead to a complete gelatinisation of starch granules. Intact granules can still be observed, which are able to influence rheological properties.

#### 4.6 Conclusion

The production of exopolysaccharides (EPS), the change of starch properties and the degradation of proteins present in flour during fermentation of buckwheat, sorghum and teff flour with Weissella cibaria MG1 resulted in batter softening and influenced rheological dough behaviour and baking properties. Application of dextran containing sourdough did not influence specific volume of gluten-free breads, and decreased crumb hardness of all fresh gluten-free and the reference wheat bread. Furthermore, W. cibaria MG1 sourdough reduced crumb hardness after five days of storage and therefore decreased the staling rate in buckwheat, teff and wheat bread due to increased cell volume porosity augmented in all breads upon sourdough application. No extension of shelf-life life was found. Overall, the use of guinoa and buckwheat flour in combination with sourdough application resulted in agreeable breads with good crumb structure and could serve to supplement bland and nutrient-poor commercial gluten-free breads. However, the improvement of gluten-free breads by W.cibaria MG1 was much less pronounced than in wheat bread. The aroma of most gluten-free bread crumbs was not improved by the lactobacilli.

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# Chapter 5 Impact of *Lactobacillus plantarum* FST1.7 as sourdough starter on baking properties of gluten-free breads

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#### 5.1 Abstract

Sourdoughs were produced from buckwheat, oat, quinoa, sorghum, teff and wheat flours using the heterofermentative lactic acid bacterium Lactobacillus plantarum FST1.7 and added to a basic bread formulation (20% addition level). Dough rheology, textural (crumb hardness, specific volume) and structural bread characteristics (crumb porosity, cell volume, brightness) of sourdoughcontaining breads were compared to non-sourdough containing breads (control). Changes in protein profiles as analysed with capillary electrophoresis were observed in all sourdoughs. Furthermore, sourdough addition led to decreased dough strength resulting in softer dough. No influences on specific volume and hardness on day of baking were found for gluten-free sourdough breads. The staling rate was reduced in buckwheat (from  $8 \pm 2$  to  $6 \pm 2$  N/ day) and teff sourdough bread ( $13 \pm 1$  to  $10 \pm 4$  N/ day), however not significantly in comparison to the control breads. On the contrary, in wheat sourdough bread the staling rate was significantly reduced  $(2 \pm 1 \text{ N/day})$  in comparison to control bread  $(5 \pm 1 \text{ N/ day})$ . Sourdough addition increased the cell volume significantly in sorghum (+61%), teff (+92%) and wheat sourdough breads (+78%). Therefore, crumb porosity was significantly increased in all gluten-free and wheat sourdough breads. Shelf life for sourdough breads was one (teff and oat), two (buckwheat, quinoa and sorghum) and three days (wheat) and was not prolonged by sourdough addition. The inferior aroma of breads prepared from the gluten-free flours was also not increased by sourdough addition.

#### 5.2 Introduction

Coeliac disease is an autoimmune disease which is triggered upon ingestion of gluten and related proteins from barley, rye and triticale. It can affect the mucosa of the small intestine in genetically susceptible persons. These patients suffer from self-perpetuating mucosal inflammation which is characterized by the progressive loss of the absorptive villi. (Green and Cellier 2007; Fasano and Catassi 2012) A life-long gluten-free diet is the only effective treatment for coeliac patients. (Arendt et al. 2011) The application of gluten-free flours presents a promising alternative, but also a technological challenge due to poor baking performance (related to the lack of gluten), low nutritional quality and poor sensory characteristics. In addition, they possess only a short microbial shelf life (Gallagher 2009; Hager et al. 2011).

The addition of sourdough previously served to improve flavour, texture, shelf life and nutritional properties (Gänzle et al. 2007) of conventional bread due to the synthesis of aroma compounds (Czerny and Schieberle 2002; Hansen and Schieberle 2005) enzymes and antifungal compounds during fermentation. (Ryan et al. 2008; Poutanen et al. 2009) Also, fermentation of gluten-free flours has previously been shown to improve overall bread quality (Schober et al. 2007; Wolter et al. 2014a) and crumb hardness. (Dal Bello et al. 2007) The facultatively heterofermentative strain *Lactobacillus plantarum* was one of the key species during fermentation besides *L. sanfranciscensis* and *L. pontis* in wheat (Gänzle et al. 2007). In addition, *L. plantarum* was also among the dominant lactic acid biota in gluten-free sourdoughs from rice, amaranth, quinoa (Vogelmann et al. 2009) or buckwheat and teff flour (Moroni et al. 2011a). Previously, the strain improved staling rate and crumb hardness of a brown rice, buckwheat based, gluten-free formulation with an addition level of 33% sourdough and inoculum size of  $10^8$  CFU/g (Moore et al. 2007).

Breads made from different gluten-free flours exhibit an undesirable aroma. (Hager et al. 2012a) However, a study demonstrated that selected lactic acid bacterial strains are able to generate very specific aroma profiles and odorant compositions, respectively (Czerny et al. 2005) and it seems to be a promising approach to use the individual metabolic properties of lactic acid bacteria in order to increase aroma quality of gluten-free breads.

However, no studies were performed so far using the strain to ferment single flours and investigate the influence on a basic gluten-free formulation. Therefore, this study investigates the suitability of *Lactobacillus plantarum* FST1.7 as starter culture for gluten-free sourdough fermentation of five different flours (buckwheat, oat, quinoa, sorghum and teff) in comparison to wheat flour. Furthermore, the influence of gluten-free *L. plantarum* FST1.7 sourdoughs on dough rheology, protein degradation as well as shelf life and structural and textural crumb characteristics of sourdough-containing breads is examined.

# 5.3 Materials and Methods

# 5.3.1 Materials

The ingredients used in this study were buckwheat flour (Doves Farm Foods Ltd, UK) (moisture 12.6%), oat flour (E. Flahavan & Son Ltd, Ireland, moisture 10.4%), quinoa flour (Ziegler Naturprodukte, Germany, moisture 12.3%), sorghum flour (Twin Valley Mills, Nebraska, moisture 11.1%), teff flour (Trouw, The Netherlands, moisture 9.5%), wheat flour (baker's flour, Odlums, Ireland, moisture 12.7%), sugar (Siucra, Ireland) and salt (Glacia British Salt Limited, UK).

### 5.3.2 Strains and growth conditions

*L. plantarum* FST1.7 was previously isolated from malted barley (Dal Bello et al. 2007) and obtained from the culture collection of the cereal science laboratory in University College Cork. Working cultures of *L. plantarum* FST1.7 were prepared from glycerol/ water (35% v/v) stock solution stored at -80°C as described by Galle et al. (Galle et al. 2011). The strain was routinely maintained on modified deMan-Rogosa-Sharpe agar (mMRS5) (Meroth et al. 2003) and incubated anaerobically at 30°C for 48 h. For preparation of working cultures single colonies were picked from the agar plates and cultured in mMRS5 broth at 30°C and sub-cultured for 24h.

#### 5.3.3 Sourdough fermentation

Sourdough were prepared as previously described by Wolter et al. (2014b). Briefly, cells were harvested by centrifugation (2300 x g, 10 min, 4°C), washed and re-suspended in sterile tap water, and added to the sourdough to an initial cell count of 10<sup>8</sup> CFU/g dough. Fermentations were performed in triplicates using 250 g of flour, 200 ml of sterile tap water and 50 ml of cellular suspension (dough yield dy=200) at 30°C and for 24 hours.

# 5.3.4 Cell counts, pH, TTA and metabolite formation in sourdough

For determination of viable cell counts, samples of sourdough were serially diluted in Ringer solution and plated in triplicate on mMRS5 agar supplemented with 0.05 g/ L bromocresol green. The identity of fermentation microbiota with the inoculum was assessed by comparing the colony morphology, and by measuring pH, metabolites and total titratable acidity (TTA) before and after

fermentation. The pH and TTA of sourdough were determined suspending 10 g of sample in 90 ml distilled water (Arbeitsgemeinschaft Getreideforschung e.V. 1994). Glucose and fructose levels of flours and sourdoughs as well as lactic and acetic acid of the sourdough samples were analysed using an Agilent 1260 high performance liquid chromatography system coupled to a Hi-Plex H column (Agilent, Cork, Ireland). Samples for sugar and organic acid determination were extracted with distilled water, clarified with Carrez I ( $3.6\% \text{ w/v } \text{K}_4[\text{Fe}(\text{CN})_6]$ ) and Carrez II ( $7.2\% \text{ w/v } \text{Zn}(\text{CH}_2\text{COO})_2 \cdot 2\text{H}_2\text{O}$ ), and diluted with distilled water (1:10). Glucose and fructose concentrations in flour and sourdough samples were analysed using a refractive index detector. Organic acids were detected using a diode array detector ( $\lambda$ =210 nm). Samples for sugar and acid determination were eluted with water or 0.004 N sulphuric acid, respectively, at a flow rate of 0.6 mL/ min and 25°C.

#### 5.3.5 Capillary electrophoreses of extracted proteins

To investigate the influence of sourdough fermentation on changes in protein profile, samples were subjected to capillary electrophoreses, using a lab-on-achip technique ("Bioanalyzer", Agilent Technologies, Palo Alto, CA). Proteins were extracted under reducing conditions from 40 mg sample using 400 µl extraction buffer (pH 8.8) containing 0.1 M Tris, 2 M urea, 15 % glycerol and 0.1 M dithiothreitol (DTT) for 5 min in an ultrasonic water bath. After centrifugation (10 min at 5000 x g) the supernatant of each protein extract was used for analysis of molecular size distribution (Hager et al. 2012b). A sample aliquot of 4 µl was mixed with 2 µl Agilent sample buffer and loaded on an 80 kDa protein chip in an Agilent Bioanalyzer. Protein peaks with an average concentration lower than 20 ng/  $\mu$ l were not considered, since their significance was low in comparison to the detection limit of the method. The peak area of certain molecular size (kDa) polypeptides was set into relation to the total peak area in the extract. The protein content of flours was determined according to the AACC method 46-12 and calculated with a factor of 6.25 (buckwheat, oat, quinoa, sorghum, teff) and 5.7 for wheat. The protein content after extraction was analysed adding 1 ml Bradford reagent (Sigma, Arklow, Ireland) to 20 µl sample (previously diluted 1:10 in extraction buffer) (Bradford 1976). The protein extraction yield (%) was calculated as the amount of proteins extracted

from sample under reducing conditions to the initial amount of protein in samples as determined by Kjeldahl.

#### 5.3.6 Rheology

To evaluate the influence of sourdough fermentation and acid production on rheological dough properties, oscillatory measurements were performed with a controlled stress and strain rheometer (Anton Paar MCR 301, Ostfildern, Germany). Sourdough samples were prepared as described above and fermented for 24 h at 30°C. Flours were sifted (mesh size 0.05 mm) prior to fermentation to standardise particle size. However, due to the high fat content sifting of oat flour was not convenient. Nevertheless, the performance of the unsifted flour showed a good repeatability during the amplitude sweep. Yeast-free bread batters prepared without sourdough were used as controls (Ctrl bread). Depending on dough consistency, measurements of sourdough samples (before and after fermentation) and yeast-free bread batters with 20% sourdough addition (SD bread) were carried out using a parallel plate geometry (PP50/P2-SN13968; gap d=1 mm) (sorghum and wheat samples) consisting of a 50 mm diameter corrugated probe and plate. Excess of sample was removed after loading and a thin layer of paraffin oil was applied to the edges of the sample to prevent loss of moisture. Buckwheat, oat, quinoa and teff samples were analysed using a 25 mm concentrically cylinder fitted in a 27 mm cup (CC27-SN8085; d=0 mm). Samples were allowed to rest for 5 min prior to analysis. Tests were performed at 30°C. Initially, amplitude sweeps were performed in the range 0.001 - 100% strain ( $\gamma$ ) on all samples to determine the linear viscoelastic region. Frequency sweeps were performed in the range of angular frequency ( $\omega$ ) 1-50 Hz, with 0.05% strain on all samples. Data relative to complex modulus (G\*) obtained from the frequency sweeps were fitted according to the power law equation  $G^*(\omega) = A_F^* \omega^{1/z}$  for weak gel model as applied for bread dough by Gabriele et al. (2001). Parameters extracted from the power law equation  $G^*(\omega) = A_F \cdot \omega^{1/z}$ , were dough strength  $A_F$  and the network connectivity z. All results are averages of two measurements of three individual preparations.

# 5.3.7 Bread preparation

Control breads (without sourdough) from four different gluten-free flours and wheat flour were produced as previously described by Hager et al. (2012a) using 100% flour, 2% salt, 2% sugar and 3% dry-yeast (based on flour, BF). The optimal water addition level (WL) based on flour (BF) was determined through preliminary baking trials for gluten-free flours (85% BF for buckwheat; 95% BF for oat, quinoa, sorghum and teff bread) and with the farinograph method 54-21 (AACC 2000) for wheat flour (63% BF) (Hager et al. 2012a). Sourdough breads were prepared accordingly replacing 20% of flour with the equivalent quantity of fermented flour in the form of sourdough. Three replicates were prepared. Bread loaves were cooled at room temperature for two hours prior to analysis.

# 5.3.8 Bread characteristics

Bake loss was determined by weight determination of dough before and of the bread after baking. The influence of various water levels applied in the different gluten-free formulations was taken into account by division of bake loss by water addition level. The moisture of bread crumb on the day of baking (day zero) was determined using the two stage air-oven method 44-15.02 (AACC 2000). Water activity of the fresh bread crumb was determined using an AquaLab 4TE water activity meter (Decagon Devices Inc., Pullman, Washington, USA). The specific volume of three breads from each baking batch was determined using a laser scanning system (Volscan Profiler, Stable Micro Systems, UK). The instrumental textural crumb evaluation of three slices from three different loafs per batch was conducted according to AACC method 74-09 (AACC 2000) using a Universal Testing Machine (TA-XT2i texture analyser, Stable Micro Systems, Surrey, UK) on day zero, two and five of storage compressing the slice to 40% of its initial height with a 35 mm (buckwheat, sorghum, teff and wheat bread) or 12 mm (quinoa bread) aluminium cylindrical probe. The staling rate was calculated as increase in hardness within five days of storage (staling rate = [hardness (day 5 - day 0)/ days of storage]. The crumb structure was analysed from three middle slices of three breads per batch in terms of slice area, cell volume, crumb porosity (ratio pore area/ slice area) and crumb brightness (mean grey level of pixels, value 0 - 255) using a C-cell Bread Imaging system (Calibre Control International Ltd., UK).

### 5.3.9 Sensory evaluation

Sensory analyses were performed with a trained panel (n=22) and under the conditions described by Hager et al. (2012a) and Wolter et al. (2014a) and briefly described below.

# 5.3.9.1 Aroma profile analyses

Bread loaves were cut in slices (thickness about 2 cm) and the crusts were removed. The samples were presented to the sensory panel, which sniffed the crumbs and described the perceived odour qualities. The panel finally agreed on characteristic odour attributes in a group discussion. Crumb samples were presented again to the panel in a second session and the intensities of the predefined odour attributes were evaluated on a scale from 0 (not detectable) over 1 (weak intensity), 2 (medium intensity) to 3 (high intensity). The results of each attribute were calculated as arithmetic mean. The assessors were trained immediately prior to analysis with aqueous odorant solutions in defined concentrations (factor 100 above the odour threshold) (Schuh and Schieberle 2006; Czerny et al. 2008). The odorant solutions reflected the evaluated characteristic odour attributes of the flours: buttery (butane-2,3-dione; 120 μg/L), cooked potato-like (3-(methylthio)-propanal); 140 μg/L), fatty ((E,E)deca-2,4-dienal; 7.7 µg/L), grassy (hexanal; 1000 µg/L), mouldy (geosmin; 2.1  $\mu$ g/L), pea-like (3-isobutyl-2-methoxypyrazine; 3.9  $\mu$ g/L), oat flakes-like ((E,E,Z)-nona-2,4,6-trienal; 2,6  $\mu$ g/L), vinegar-like (acetic acid, 18 g/L) and vomit-like (butanoic acid, 770,000 µg/L). The odorant references were purchased from Sigma-Aldrich (Taufkirchen, Germany; Acros, Geel, Belgium) and AromaLab (Freising, Germany). The attributes "hay-like", "hazelnut-like", "sourdough-like", "wheat bread-like" and "yeast dough-like" were evaluated based on the experience of the trained assessors.

# 5.3.9.2 Evaluation of aroma preference

Bread crumb slices were prepared as described above and presented to the panel. The assessors evaluated the preference of the samples on a nine-point-scale from 1 (dislike very much) over 5 (neither like nor dislike) to 9 (like very much). The results were calculated as the arithmetic mean.

# 5.3.10 Microbial shelf life

The microbial shelf life of breads was determined using the method described by Dal Bello et al. (2007). Each loaf was sliced transversely in a sterile manner to obtain uniform slices of 25 mm thickness. Each side of the slice was exposed to the air for 5 min, packed in a plastic bag and heat sealed. A tip of a pipette was inserted to ensure comparable aerobic conditions in each bag. Bags were incubated at room temperature and examined over a 12-day storage period. Mould growth was quantified as the number of slice surfaces, i.e. both front and back of the slice, showing aerial mycelia as a percentage of total bread slices.

### 5.3.11 Statistical evaluation

Statistical analyses were performed with SigmaPlot (Version 11.0, Systat Software, Inc.) on all data using one-way ANOVA. Holm-Sidak Test was used to describe means at 5% significance level.

#### 5.4 Results

# 5.4.1 Sourdough acidification, analysis of sugars and fermentation products

Microbial cell counts reached 10<sup>9</sup> CFU/ g sourdough after 24 h of fermentation (data not shown). Values for pH and total titratable acid (TTA) of sourdough and bread crumb are given in Table 5-1. pH values of sourdough before fermentation (SD 0h) ranged from 6.1 in wheat to 6.9 in buckwheat flour. Sourdoughs fermented with *Lactobacillus plantarum* resulted in pH changes between 4.4 (oat) and 3.6 (sorghum) sourdough. Highest content of total titratable acids (TTA) were found for quinoa (35.3 ml), buckwheat (26.0 ml) and teff sourdough (24.5 ml). Accordingly, incorporation of those sourdoughs into bread batters resulted in lower pH and significantly higher values for TTA than for their controls. In comparison, oat, sorghum and wheat sourdough breads showed lower TTA values. Differences in bread pH among the flours were little. The TTA values show that flours under study have different buffering capacities and sourdoughs possess different organic acid profiles.

The amount glucose and fructose present as fermentable sugars in flours together with the amounts of fermentation metabolites are given in Table 5-2 as dry weight basis flour (dwb). Highest glucose concentrations (119 mmol/ kg) were found in quinoa flour. In comparison, wheat flour contained only 20 mmol glucose/ kg flour dwb. Fructose contents were generally lower ranging from 12 mmol/ kg (oat flour) to 78 mmol/ kg (quinoa flour). Glucose levels increased in all flours with exception of buckwheat and oat flour. After fermentation, highest lactate amounts were found in quinoa (521 mmol), buckwheat sourdough (475 mmol) and teff (493 mmol) per kg flour dwb. Acetate levels were very similar between the sourdoughs (62-68 mmol per kg flour dwb). Concentrations of ethanol were 10 to 40 times lower than lactate levels. High lactate levels in buckwheat, quinoa and teff sourdough support their high TTA results. Similar TTA amounts for sorghum and wheat sourdough are reflected in similar lactate amounts. And finally, lowest TTA amounts for oat sourdough correlate with lowest organic acids amounts.

Table 5-1 Values for pH and total titratable acid (TTA) of *Lactobacillus plantarum* FST1.7 sourdough before (SD 0h) and after fermentation (SD 24h), and of bread without (Ctrl bread) and with SD addition (SD bread)

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	Samples	Buckwheat	Uat	Дилоа	Sorgnum	Теп	wneat
Нd	SD 0h	6.9 ± 0 <sup>A</sup>	6.3 ± 0 <sup>D</sup>	6.7 ± 0 <sup>c</sup>	6.7 ± 0 <sup>c</sup>	6.8 ± 0 <sup>B</sup>	$6.1 \pm 0^{E}$
	SD 24h	3.9 ± 0 <sup>h</sup>	$4.4 \pm 0.2^{g}$	3.9 ± 0 <sup>h</sup>	<b>3.6 ± 0.1</b> <sup>j</sup>	<b>3.8</b> ± 0 <sup>i</sup>	3.8 ± 0 <sup>i</sup>
	<b>Ctrl bread</b>	$6.3 \pm 0.1^{K}$	$5.7 \pm 0^{0}$	6.0 ± 0 <sup>M</sup>	$5.9 \pm 0^{N}$	6.1 ± 0 <sup>L</sup>	6.0 ± 0 <sup>M</sup>
	SD bread	$5.4 \pm 0^{n}$	$5.3 \pm 0.1^{n_0}$	5.3 ± 0°	5.6 ± 0 <sup>m</sup>	5.1 ± 0p	5.2 ± 0.1 op
TTA (ml)	SD 0h	2.7 ± 0 <sup>b</sup>	$1.3 \pm 0^{f}$	3.2 ± 0ª	2.2 ± 0c	2.0 ± 0 <sup>d</sup>	$1.5 \pm 0^{e}$
	SD 24h	$26.0 \pm 0.8^{H}$	$4.8 \pm 0.1^{\mathrm{K}}$	$35.3 \pm 1.6^{G}$	$15.5 \pm 0.6^{1}$	24.5 ± 1.0 <sup>H</sup>	$12.8 \pm 0.5^{1}$
	<b>Ctrl bread</b>	$4.4 \pm 0.2^{1}$	$0.9 \pm 0.1^{n}$	$8.9 \pm 0.3^{k}$	$4.0 \pm 0.5^{1}$	$4.3 \pm 0.2^{1}$	$3.0 \pm 0.2^{m}$
	SD bread	$7.3 \pm 0.8^{T}$	$1.5 \pm 0.1^{\circ}$	$11.3 \pm 0.6^{S}$	<b>4.5</b> ±0.5 <sup>u</sup>	$6.5 \pm 1.3^{t}$	$3.9 \pm 0.1^{0}$

Different kind of superscripts in a row indicates statistical significance within SD 0h ( $^{a-1}$ ), SD 24h ( $^{g-1}$ ), Ctrl bread ( $^{m-r}$ ) and SD bread (s-x) Different size of superscripts in a row indicate statistical significance between SD 0h and SD 24, and Ctrl bread and SD bread, respectively Table 5-2 Amounts for fermentable sugars and organic acids from flours and *Lactobacillus plantarum* sourdoughs (SD)

Substrate/ metabolites [mmol/ kg flour]	Sample	Buckwheat	Oat	Quinoa	Sorghum	Teff	Wheat
Glucose	Flour	$40 \pm 3$	$12 \pm 2$	$119 \pm 3$	$18 \pm 2$	59±5	$20 \pm 1$
	SD	39 ± 5	3 ± 0	187 ± 5	$155 \pm 17$	$110 \pm 9$	38±4
Fructose	Flour	45 ± 8	20±3	78±1	24±2	73 ± 10	74 ± 0
	SD	39 ± 0	$31 \pm 1$	$101 \pm 18$	29±2	83±5	nd
Lactate	SD	475 ± 50	$103 \pm 4$	521 ± 99	330±36	493 ± 31	340 ± 4
Acetate	SD	62 ± 2	68 ± 11	67 ± 0	64±5	64±2	62 ± 2
Ethanol	SD	31 ± 2	$13 \pm 0$	$11 \pm 2$	nd	$12 \pm 2$	45 ± 7

#### 5.4.2 Capillary electrophoreses of extracted proteins

Capillary electrophoresis on protein extract of flours and sourdough was conducted to compare protein modifications during fermentation. Conclusion about degradation due to fermentation and microbiological activity can be drawn by the percentage of degradation of certain peaks. Peak areas of proteins in the range of 19 and 79 kDa were gained from the electropheretograms of controls and sourdoughs extracts and are summarized in Table 5-3. The electropheretogram of buckwheat flour comprised typical peaks at 22, 31, 45 and 55 kDa. The proportional ratio of the fraction 43-55 kDa was decreased from 9% to 0% by fermentation with Lactobacillus plantarum. Oat sourdough electropheretograms showed peaks patterns at 26 and 28 kDa, as well as at 49 kDa. Regarding the electropheretogram after fermentation, only a slight increase of the proportion of smaller peptides (18-29 kDa) was found in oat sourdough. Proteins in quinoa flour contributed typical banding patterns at 23, 31 and 39 kDa. After fermentation, percentage of peaks between 18-29 and 43-55 kDa were reduced (18-29 kDa: 41% to 3%) or slightly decreased (30-41 kDa: 38% to 27%) in quinoa sourdough. The 50 kDa peak present in the electropheretogram of quinoa flour (10% of total peak area) could not be detected in the sourdough anymore. The amount of extractable protein was decreased (from 414 to 146 ng/ $\mu$ l extract) in the quinoa sourdough. The percentages of major protein peaks found in sorghum flour were 18-29 (19%), 30-41 (3%) and 59-79 kDa (13%). After fermentation percentages increased (18-29 kDa: 46%; 30-41 kDa: 3%) in sorghum sourdough. A new peak occurred in the range 43-55 kDa (10%), whereas the peaks between 59-79 kDa were not detectable anymore. Major bands in unfermented teff flour were found at 26, 40 and 60 kDa. Reductions of proportional peak area were found between 18-29, 30-41 and 43-55 kDa in teff sourdough. The extractable protein content was reduced to a quarter (139 ng/ $\mu$ l) of the original content (556 ng/ $\mu$ l). Upon fermentation of wheat sourdough peaks between 59-79 kDa were not detectable anymore, while the peak ratio between 30-41 kDa increased from 6% to 15%. Concentration of extractable proteins in wheat sourdough decreased after fermentation from 825 to 661 ng/  $\mu$ l extract.

Table 5-3 Protein size distribution (percentage peak area) of different gluten-free flours after fermentation with *Lactobacillus plantarum* FST1.7 as determined with capillary electrophoresis of four replicates

Molecular mass range	Buckw	heat	Oat		Quinoa		Sorghum		Teff		Wheat	
(kDA)	SD	Ctrl	SD	Ctrl	SD	Ctrl	SD	Ctrl	SD	Ctrl	SD	Ctrl
18-29	24%	24%	39%	33%	3%	41%	46%	19%	4%	14%	3%	<1%
30-41	18%	15%	·	·	27%	38%	23%	3%	17%	37%	15%	6%
43-55		%6	49%	51%	·	10%	10%		2%	17%	41%	67%
59-79		·		·	ı	<1	ı	13%	4%	%6		21%
Protein (ng/µ extract)	ll 379±5	1 687± 8	2 730 ± 2	3 1147 ±	31 146 ± 40	414 ± 169	380 ± 90	221±91	l 139 ± 1	2556 ± 15	661±89	) 825 ± 180

### 5.4.3 Rheology

The effects of sourdough fermentation on microstructural changes in the system and the related influence on dough rheology were evaluated by performance of rheological frequency sweeps. The weak gel model was applied over a range of angular frequencies  $\omega$  from 0 to 9.63 Hz. Values for dough strength A<sub>F</sub>, network connectivity z and correlation coefficient  $R^2$  are given in Table 5-4. In all samples the elastic modulus (G') was higher than the viscous modulus (G''), indicating that controls (unfermented sourdough, SD 0h, bread control dough, Ctrl bread) and fermented sourdough (SD 24h) samples had a solid, elastic like behaviour (data not shown). The fermentation of all flours generally led to a significant decrease of dough strength  $A_F$  (p<0.95) for sourdoughs in comparison to the unfermented flours (SD 0h), indicating a lower dough strength of the sourdough. Strongest reduction of A<sub>F</sub> was found in buckwheat, quinoa and wheat sourdough (by  $\sim$ 95%) in comparison to the control (SD 0h). Decrease of dough strength upon incorporation of 20% sourdough into the control bread batters was less pronounced compared to pure sourdough. However,  $A_F$  still decreased in buckwheat (-58%), oat (-97%), quinoa (-52%), teff (-38%) and wheat (-34%) sourdough-containing bread dough. Dough strength for sorghum bread dough containing sourdough increased significantly. The parameter z as indicator for the network connectivity remained unaffected in most sourdoughs. The only significant changes were on one hand a decrease in guinoa and wheat sourdoughs or a significant increase on the other hand occurring in buckwheat sourdough. Also, for sourdoughcontaining bread dough the network connectivity z remained mostly unaffected by sourdough addition. Only in buckwheat sourdough bread (z=6.57) compared to the control bread (z=4.95) and in teff sourdough bread (z=7.06) in comparison the control bread (z=7.76) a significant decrease was found.

Table 5-4 Rheology Parameters dough strength $A_F$ and network connectivity z
for Lactobacillus plantarum sourdoughs (SD 0h and SD 24h) and
bread dough (control, Ctrl bread and sourdough bread, SD bread).
Angular frequency $\omega$ = 0-9.63 Hz at target strain $\gamma$ = 0.05 %

Flour	Samples	Dough strength	Network	Correlation
		A <sub>F</sub>	connectivity z	coefficient R <sup>2</sup>
Buckwheat	SD 0h	2167 ± 330 <sup>a</sup>	$4.69 \pm 0.08^{a}$	0.979 ± 0.025
	SD 24h	93 ± 30 <sup>d</sup>	$6.57 \pm 0.54^{b}$	0.995 ± 0.002
	Ctrl bread	437 ± 195 <sup>b</sup>	$4.95 \pm 1.01^{a}$	0.985 ± 0.015
	SD bread	255 ± 47°	6.11 ± 0.18 <sup>c</sup>	0.992 ± 0.002
Oat	SD 0h	75 ± 11 <sup>b</sup>	$6.09 \pm 0.31^{a}$	0.988 ± 0.013
	SD 24h	61.5 ± 18.2 <sup>b</sup>	$8.69 \pm 0.85^{a}$	0.886 ± 0.094
	Ctrl bread	$1671 \pm 555^{a}$	$5.75 \pm 0.27^{a}$	0.993 ± 0.002
	SD bread	53.1 ± 15.2 <sup>b</sup>	$6.20 \pm 0.14^{b}$	0.957 ± 0.045
Quinoa	SD 0h	201 ± 79 <sup>a</sup>	16.41 ± 4.18 <sup>c</sup>	0.934 ± 0.181
	SD 24h	11.7 ± 2.7°	$6.29 \pm 0.67^{b}$	0.971 ± 0.038
	Ctrl bread	$50.2 \pm 10.7^{b}$	$7.04 \pm 0.58^{a}$	0.990 ± 0.005
	SD bread	24.3 ± 6.4 <sup>c</sup>	$6.07 \pm 0.53^{a}$	0.962 ± 0.032
Sorghum	SD 0h	20488 ± 10942 <sup>b</sup>	$10.16 \pm 2.47$ ab	0.989 ± 0.029
	SD 24h	4671 ± 1255°	$8.54 \pm 0.10^{a}$	0.997 ± 0.002
	Ctrl bread	19238 ± 6798 <sup>b</sup>	$11.53 \pm 0.15^{a}$	$0.998 \pm 0.001$
	SD bread	90956 ± 22539ª	$11.32 \pm 0.20^{a}$	0.999 ± 0.00002
Teff	SD 0h	$19.9 \pm 5.8^{b}$	$6.00 \pm 1.12^{a}$	0.991 ± 0.004
	SD 24h	12.2 ± 2.5 <sup>b</sup>	$6.52 \pm 0.72^{a}$	$0.988 \pm 0.010$
	Ctrl bread	$30.1 \pm 9.4^{a}$	$7.76 \pm 1.22^{b}$	0.992 ± 0.007
	SD bread	$18.6 \pm 3.4^{a}$	$7.06 \pm 0.40^{a}$	0.989 ± 0.007
Wheat	SD 0h	2133 ± 510°	$14.36 \pm 3.10^{d}$	0.928 ± 0.137
	SD 24h	75.5 ± 14.3 <sup>b</sup>	3.95 ± 0.26 <sup>c</sup>	0.999 ± 0.001
	Ctrl bread	11352 ± 3950 <sup>b</sup>	$5.27 \pm 1.21^{a}$	0.995 ± 0.003
	SD bread	$7544 \pm 1827^{a}$	$5.03 \pm 0.45^{a}$	0.999 ± 0.001

Different superscripts <sup>a-d</sup> in column indicate significant difference (P<0.05) within one flour type.

#### 5.4.4 Bread characteristics

Values for loaf characteristics bake loss, specific volume, hardness and staling rate are given in Table 5-5. The addition of sourdough led to a significant decrease of bake loss compared to control breads for all gluten-free breads with the exception of teff bread (no significant difference). In wheat sourdough bread bake loss was significantly increased. The specific volume upon sourdough addition remained unaffected in gluten-free breads, with exception of oat sourdough bread (significant decrease). Crumb hardness on day of baking was reduced in most breads after addition of *L. plantarum* sourdough Table 5-5 with the exception of oat (no significant change) and sorghum (significant increase) sourdough breads. Crumb hardness was decreased by 12%, 11% and 7% in buckwheat, quinoa and teff sourdough bread, respectively, in relation to control

breads. In wheat bread the hardness was reduced (48% in comparison to control bread). The lowest staling rate over five days of storage occurred for quinoa (1N/ day) and the highest for sorghum sourdough bread (11N/ day). The staling rate for sourdough breads baked with *Lactobacillus plantarum* FST1.7 was reduced for buckwheat (28%), teff (27%) and wheat (156%) sourdough breads.

Structural characteristics of control and sourdough breads regarding slice area, cell volume, porosity and crumb brightness as characterised by digital image analysis are given in Table 5-6 and Figure 5-2. The slice area for sourdough breads was, apart from wheat sourdough bread, highest for oat and sorghum sourdough bread. Lowest values were found for guinoa sourdough bread. In general, the application of sourdough slightly decreased the slice area compared to control breads, with the exception of oat and wheat sourdough bread where the slice area was increased. Overall, differences between sourdough and control breads were not significant. The application of sourdough led to significant increase in porosity for all gluten-free and wheat breads compared to their control breads. The increase in porosity was highest for teff (+7.6%) bread followed by sorghum sourdough (+6.5%) and buckwheat (4.5%) sourdough bread. The increase in cell volume upon sourdough application resulted in a coarser structure (see Table 5-6) leading to lower values for crumb brightness. The value is lower for products with a darker crumb and with larger or deeper cells (Calibre Control International Ltd. 2012). Wheat sourdough bread showed the brightest crumb, followed by oat bread among the gluten-free flours whereas buckwheat bread showed the darkest crumb (Table 5-6). Associated with increasing cell volume and crumb porosity, sourdough addition reduced crumb brightness for all sourdough breads significantly with exception of quinoa bread (not significant decrease).

Table 5-5 Loaf characteristics of control and *Lactobacillus plantarum* sourdough breads (SD bread)

Flours	Bake Ic	(%) ssc	Specific volu	ume (ml/g)	Hardne	ss (N) <sup>1</sup>	Staling ra	te (N/ day)
	SD bread	Control	SD bread	Control	SD bread	Control	SD bread	Control
Buckwheat	14.7 ± 0.2∘	$15.8 \pm 0.1^{\circ}$	1.60 ± 0.03p	1.69 ± 0.05°	38.3 ± 2.3 <sup>m</sup>	$42.9 \pm 1.9^{ab}$	6 ± 2 <sup>no</sup>	8 ± 2°
Oat	$15.5 \pm 0.4^{n}$	$17.5 \pm 0.3^{B}$	2.07 ± 0.09 <sup>n</sup>	$2.40 \pm 0.08^{A}$	4.8 ± 0.4 <sup>p</sup>	$4.5 \pm 0.3^{\mathrm{e}}$	$3 \pm 1^{\circ}$	3 ± 1 <sup>d</sup>
Quinoa	$13.7 \pm 0.3$ p	$15.5 \pm 0.2^{\circ}$	1.36 ± 0.05ª	$1.51 \pm 0.04^{d}$	29.5 ± 0.9∘	31.7 ± 1.1°	2 ± 0°	$1 \pm 0^{e}$
Sorghum	$16.3 \pm 0.4^{n}$	$19.2 \pm 0.4^{\rm A}$	$1.84 \pm 0.04^{\circ}$	$1.85 \pm 0.10^{b}$	29.4 ± 1.9∘	26.3±1.9 <sup>d</sup>	$11 \pm 2^{m}$	$10 \pm 1^{\mathrm{b}}$
Teff	14.3 ± 0.2°	$13.9 \pm 0.4^{d}$	1.61 ± 0.05 <sup>p</sup>	1.60 ± 0.02°	38.9 ± 1.2 <sup>mn</sup>	$43.1 \pm 2.5^{a}$	$10 \pm 4^{mn}$	$13 \pm 1^{a}$
Wheat	$21.0 \pm 0.5^{M}$	$17.1 \pm 0.3^{b}$	2.79 ± 0.20 <sup>m</sup>	$2.62 \pm 0.18^{a}$	5.7 ± 0.7p	$8.5 \pm 0.5^{D}$	$2 \pm 1^{\circ}$	$5 \pm 1^{D}$
<sup>1</sup> Hardness on Different kind	day of baking ( of superscript:	(day zero) s indicate sign	ificant differen	ces between cor	trol ( <sup>a-f</sup> ) or sou	rrdough bread	S (m-r)	

ilantarum	
Lactobacillus p	
after	
and	
before	is.
bread	analys
wheat	l image
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Crumb	sourdo
5-6	
Table	

FloursSD breadControlSD breadControlSD breadControlSIBuckwheat $4340 \pm 76^{\circ}$ $4794 \pm 29^{\circ}$ $10.3 \pm 2.5n$ $8.2 \pm 0.1^{\circ}$ $52.3 \pm 1.1^{P}$ $49.9 \pm 0.1^{d}$ $8!$ Dat $5643 \pm 176n$ $4995 \pm 133b$ $20.0 \pm 4.5n$ $14.2 \pm 2.1^{ab}$ $56.2 \pm 1.2N$ $53.9 \pm 1.2b$ $9!$ Oat $3612 \pm 50p$ $4006 \pm 54c$ $10.4 \pm 3.9n$ $7.5 \pm 1.0^{cd}$ $52.1 \pm 1.90p$ $50.6 \pm 0.7^{cd}$ $9!$ Quinoa $3612 \pm 50p$ $4006 \pm 54c$ $10.4 \pm 3.0n$ $7.5 \pm 1.0^{cd}$ $52.1 \pm 1.90p$ $50.6 \pm 0.7^{cd}$ $9!$ Quinoa $3612 \pm 50p$ $4006 \pm 54c$ $10.4 \pm 3.0n$ $7.5 \pm 1.0^{cd}$ $52.1 \pm 1.90p$ $50.6 \pm 0.7^{cd}$ $9!$ Purpo $4791 \pm 1139o$ $5014 \pm 301b$ $24.2 \pm 5.1M$ $15.1 \pm 1.2a$ $58.4 \pm 1.0M$ $54.6 \pm 0.4ab$ $8!$ Verb $7007 \pm 327m$ $6846 \pm 73a$ $11.2 \pm 0.8N$ $6.3 \pm 0.3d$ $51.7 \pm 1.8P$ $47.8 \pm 0.4e$ $9!$ Wheat $7007 \pm 327m$ $6846 \pm 73a$ $11.2 \pm 0.8N$ $6.3 \pm 0.3d$ $51.7 \pm 1.8P$ $47.8 \pm 0.4e$ $9!$ Values are arithmetic means $\pm$ confidence interval from three different baking batches. $54.6 \pm 0.4ab$ $8!$ $11.7 \pm 0.8N$ $11.2 \pm 0.8N$ $54.6 \pm 0.60$ $51.3 \pm 0c$ $11.2$ Different superscripts indicate significant differences between controls $(a^{-1})$ or sourdough breads $(m^{-1})$ . $11.6 \pm 0.80$ $11.2 \pm 0.8N$ <		Slice ar	ea (mm²)	Cell vol	ume (mm³)	Crumb po	orosity (%)	Crumb brigh	tness (pixel) <sup>1</sup>
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Oat $5643 \pm 176^{\text{n}}$ $4995 \pm 133^{\text{b}}$ $20.0 \pm 4.5^{\text{m}}$ $14.2 \pm 2.1^{\text{ab}}$ $56.2 \pm 1.2^{\text{N}}$ $53.9 \pm 1.2^{\text{b}}$ $9^{\text{m}}$ Quinoa $3612 \pm 50^{\text{p}}$ $4006 \pm 54^{\text{c}}$ $10.4 \pm 3.9^{\text{m}}$ $7.5 \pm 1.0^{\text{cd}}$ $52.1 \pm 1.9^{\text{op}}$ $50.6 \pm 0.7^{\text{cd}}$ $9^{\text{m}}$ Quinoa $3612 \pm 50^{\text{p}}$ $4006 \pm 54^{\text{c}}$ $10.4 \pm 3.01^{\text{h}}$ $24.2 \pm 5.1^{\text{m}}$ $15.1 \pm 1.2^{\text{a}}$ $58.4 \pm 1.0^{\text{m}}$ $54.6 \pm 0.4^{\text{ab}}$ $8^{\text{m}}$ Feff $4116 \pm 80^{\circ}$ $4401 \pm 45^{\text{d}}$ $12.2 \pm 3.2^{\text{N}}$ $6.4 \pm 0.3^{\text{d}}$ $51.7 \pm 1.8^{\text{p}}$ $47.8 \pm 0.4^{\text{e}}$ $9^{\text{m}}$ Wheat $7007 \pm 327^{\text{m}}$ $6846 \pm 73^{\text{a}}$ $11.2 \pm 0.8^{\text{N}}$ $6.3 \pm 0.3^{\text{d}}$ $54.6 \pm 0.6^{\circ}$ $51.3 \pm 0^{\text{c}}$ $12^{\text{m}}$ Values are arithmetic means $\pm$ confidence interval from three different baking batches. $54.6 \pm 0.60^{\circ}$ $51.3 \pm 0^{\text{c}}$ $12^{\text{m}}$ Values are arithmetic means $\pm$ confidence interval from three different baking batches. $51.6 \pm 0.60^{\circ}$ $51.3 \pm 0^{\text{c}}$ $12^{\text{m}}$ Values are arithmetic means $\pm$ confidence interval from three different baking batches. $54.6 \pm 0.60^{\circ}$ $51.3 \pm 0^{\text{c}}$ $12^{\text{m}}$ Values are arithmetic means $\pm$ confidence interval from three different baking batches. $14^{\text{m}}$ $14^{\text{m}}$ $14^{\text{m}}$ Values are arithmetic means $\pm$ confidence interval firm three differences between controls (a^{-1}) or sourdough breads (m^{-1}).Different size of superscripts shows significant differences between controls deeper cells (C-cell Manual, Callit L	Buckwheat	4340 ± 76°	4794 ± 29 <sup>b</sup>	$10.3 \pm 2.5^{n}$	8.2 ± 0.1 <sup>c</sup>	$52.3 \pm 1.1^{P}$	49.9±0.1 <sup>d</sup>	85.6 ± 2.4°	90.7 ± 0.9 <sup>D</sup>
Quinoa $3612 \pm 50^{\text{p}}$ $4006 \pm 54^{\text{c}}$ $10.4 \pm 3.9^{\text{n}}$ $7.5 \pm 1.0^{\text{cd}}$ $52.1 \pm 1.9^{\text{op}}$ $50.6 \pm 0.7^{\text{cd}}$ $9^{\text{cd}}$ Sorghum $4791 \pm 139^{\text{o}}$ $5014 \pm 301^{\text{b}}$ $24.2 \pm 5.1^{\text{M}}$ $15.1 \pm 1.2^{\text{a}}$ $58.4 \pm 1.0^{\text{M}}$ $54.6 \pm 0.4^{\text{ab}}$ $8^{\text{c}}$ Teff $4116 \pm 80^{\text{o}}$ $4401 \pm 45^{\text{d}}$ $12.2 \pm 3.2^{\text{N}}$ $6.4 \pm 0.3^{\text{d}}$ $51.7 \pm 1.8^{\text{P}}$ $47.8 \pm 0.4^{\text{e}}$ $9^{\text{c}}$ Wheat $7007 \pm 327^{\text{m}}$ $6846 \pm 73^{\text{a}}$ $11.2 \pm 0.8^{\text{N}}$ $6.3 \pm 0.3^{\text{d}}$ $54.6 \pm 0.6^{\text{o}}$ $51.3 \pm 0^{\text{c}}$ $11.2^{\text{c}}$ Wheat $7007 \pm 327^{\text{m}}$ $6846 \pm 73^{\text{a}}$ $11.2 \pm 0.8^{\text{N}}$ $6.3 \pm 0.3^{\text{d}}$ $54.6 \pm 0.6^{\text{o}}$ $51.3 \pm 0^{\text{c}}$ $11.2^{\text{c}}$ Wheat $7007 \pm 327^{\text{m}}$ $6846 \pm 73^{\text{a}}$ $11.2 \pm 0.8^{\text{N}}$ $6.3 \pm 0.3^{\text{d}}$ $54.6 \pm 0.6^{\text{o}}$ $70^{\text{c}}$ Wheat $7007 \pm 327^{\text{m}}$ $6846 \pm 73^{\text{a}}$ $11.2 \pm 0.8^{\text{N}}$ $6.3 \pm 0.3^{\text{d}}$ $54.6 \pm 0.6^{\text{o}}$ $70^{\text{c}}$ Wheat $7007 \pm 327^{\text{m}}$ $6846 \pm 73^{\text{a}}$ $11.2 \pm 0.8^{\text{N}}$ $6.3 \pm 0.3^{\text{d}}$ $54.6 \pm 0.6^{\text{o}}$ $70^{\text{c}}$ Values are arithmetic means $\pm$ confidence interval from three different baking batches. $11.2^{\text{c}}$ $11.2^{\text{c}}$ $11.2^{\text{c}}$ $11.2^{\text{c}}$ $11.2^{\text{c}}$ Values tsuperscripts indicate significant differences between controls (a^{-1}) or sourdough breads (p^{-1}). $11.2^{\text{c}}$ $11.2^{\text{c}}$ $11.2^{\text{c}}$ Different superscripts showe	Oat	$5643 \pm 176^{n}$	$4995 \pm 133^{b}$	$20.0 \pm 4.5^{m}$	$14.2 \pm 2.1^{ab}$	$56.2 \pm 1.2^{N}$	$53.9 \pm 1.2^{b}$	$95.8 \pm 1.4^{n}$	$104.9 \pm 0.9^{B}$
Sorghum $4791 \pm 139^{\circ}$ $5014 \pm 301^{\circ}$ $24.2 \pm 5.1^{\circ}$ $15.1 \pm 1.2^{\circ}$ $58.4 \pm 1.0^{\circ}$ $54.6 \pm 0.4^{\circ}^{\circ}$ $82.7^{\circ}^{\circ}$ Teff $4116 \pm 80^{\circ}$ $4401 \pm 45^{\circ}$ $12.2 \pm 3.2^{\circ}$ $6.4 \pm 0.3^{\circ}$ $51.7 \pm 1.8^{\circ}$ $47.8 \pm 0.4^{\circ}$ $9(-6)^{\circ}$ Wheat $7007 \pm 327^{\circ}$ $6846 \pm 73^{\circ}$ $11.2 \pm 0.8^{\circ}$ $6.3 \pm 0.3^{\circ}$ $54.6 \pm 0.6^{\circ}$ $51.3 \pm 0^{\circ}$ $11.2^{\circ}$ Wheat $7007 \pm 327^{\circ}$ $6846 \pm 73^{\circ}$ $11.2 \pm 0.8^{\circ}$ $6.3 \pm 0.3^{\circ}$ $54.6 \pm 0.6^{\circ}$ $51.3 \pm 0^{\circ}$ $11.2^{\circ}$ Values are arithmetic means $\pm$ confidence interval from three different baking batches.Different superscripts indicate significant differences between controls (a-f) or sourdough breads (m-r).Different superscripts indicate significant differences between controls (a-f) or sourdough breads (P-0.6^{\circ}).The value is lower for products with a darker crumb and with larger or deeper cells (C-cell Manual, Calit Ltd.)Ltd.)	Quinoa	$3612 \pm 50^{p}$	$4006 \pm 54^{c}$	$10.4 \pm 3.9^{n}$	7.5 ± 1.0 <sup>cd</sup>	52.1 ± 1.9 <sup>0P</sup>	50.6±0.7 <sup>cd</sup>	$91.3 \pm 2.6^{\circ}$	$94.8 \pm 1.8^{\circ}$
Teff $4116 \pm 80^{\circ}$ $4401 \pm 45^{d}$ $12.2 \pm 3.2^{N}$ $6.4 \pm 0.3^{d}$ $51.7 \pm 1.8^{P}$ $47.8 \pm 0.4^{\circ}$ $9($ Wheat $7007 \pm 327^{m}$ $6846 \pm 73^{a}$ $11.2 \pm 0.8^{N}$ $6.3 \pm 0.3^{d}$ $54.6 \pm 0.6^{\circ}$ $51.3 \pm 0^{\circ}$ $1.2^{\circ}$ Walues are arithmetic means $\pm$ confidence interval from three different baking batches.Different superscripts indicate significant differences between controls (a-f) or sourdough breads (m-r).Different size of superscripts shows significant differences between controls (a-f) or sourdough breads (P-0.0.1^{\circ}).The value is lower for products with a darker crumb and with larger or deeper cells (C-cell Manual, Calit Ltd.)	Sorghum	$4791 \pm 139^{\circ}$	$5014 \pm 301^{b}$	$24.2 \pm 5.1^{M}$	$15.1 \pm 1.2^{a}$	$58.4 \pm 1.0^{M}$	$54.6 \pm 0.4^{\mathrm{ab}}$	82.4 ± 2.7p	95.7 ± 3.2°
Wheat $7007 \pm 327^{m}$ $6846 \pm 73^{a}$ $11.2 \pm 0.8^{N}$ $6.3 \pm 0.3^{d}$ $54.6 \pm 0.6^{0}$ $51.3 \pm 0^{c}$ $1.2^{c}$ Values are arithmetic means $\pm$ confidence interval from three different baking batches.Different superscripts indicate significant differences between controls (a-f) or sourdough breads (m-r).Different size of superscripts shows significant differences between control and sourdough breads (P<0.0.1.1).The value is lower for products with a darker crumb and with larger or deeper cells (C-cell Manual, Calit Ltd.)	Teff	$4116 \pm 80^{\circ}$	4401 ± 45 <sup>d</sup>	$12.2 \pm 3.2^{N}$	$6.4 \pm 0.3^{d}$	$51.7 \pm 1.8^{p}$	$47.8 \pm 0.4^{e}$	90.8 ± 2.8₀	$103.1 \pm 1.8^{B}$
Values are arithmetic means $\pm$ confidence interval from three different baking batches. Different superscripts indicate significant differences between controls (a-f) or sourdough breads (m-r). Different size of superscripts shows significant differences between control and sourdough breads (P<0.(1The value is lower for products with a darker crumb and with larger or deeper cells (C-cell Manual, Calit Ltd.)	Wheat	7007 ± 327 <sup>m</sup>	6846 ± 73ª	$11.2 \pm 0.8^{N}$	$6.3 \pm 0.3^{d}$	$54.6 \pm 0.6^{\circ}$	$51.3 \pm 0^{\circ}$	$129.2 \pm 3.8^{m}$	$146.5 \pm 0.9^{A}$
	Values are a Different su Different siz 1The value is Ltd.)	rithmetic means perscripts indica e of superscripts s lower for produ	± confidence int te significant dif shows significa icts with a darke	erval from thr ferences betw nt differences er crumb and v	ee different ba een controls (ª between contr vith larger or d	king batches. ſ) or sourdoug] ol and sourdou eeper cells (C-	h breads ( <sup>m-r</sup> ). Igh breads (P< cell Manual, Cà	:0.001). alibre Control l	



Figure 5-1 Cross-section of bread of buckwheat, oat, quinoa, sorghum, teff and wheat breads (from top to bottom) as control and *Lactobacillus plantarum* sourdough breads (from left to right)



Figure 5-2 Cross-section images of buckwheat, oat, quinoa, sorghum, teff and wheat breads (from top to bottom) as control (right) and *Lactobacillus plantarum* sourdough breads (left) corrected by brightness

#### 5.4.5 Sensory evaluation

The aroma preference of the sourdough bread crumb was investigated in a first sensory test. A high preference was evaluated for wheat bread (score: 7.4 on the nine-point scale). Among the gluten-free breads, oat was the only cereal with an indifferent scoring (5.7). Moderate and high disliking was observed for the remaining breads within a score range from 3.5 (teff) and 2.2 (quinoa).

Aroma profile analysis provided detailed information on the aroma characteristics of the sourdough crumbs. Wheat bread (Figure 5-3) exhibited the typical wheat bread aroma with a high intensity. The descriptors buttery, fatty and yeast-like were perceived additionally with weak to medium intensities. These attributes can be correlated with the high aroma preference.

The aroma of buckwheat crumb was described differently. The typical aroma of wheat bread was not perceivable but fatty, mouldy, pea-, hay- and hazelnut-like on a weak intensity level were characteristic for buckwheat bread (Figure 5-3). Oat bread showed in contrast an aroma profile, which showed some similarities wheat bread. The descriptor wheat bread-like was also detectable in oat bread but on a weaker level than in wheat bread. The buttery and yeast-like note was a little bit decreased in oat and the fatty intensity was comparable (Figure 5-3). The only additional attribute found in oat was the oat flakes-like note (weak intensity). A very different picture was found after analysing quinoa bread. With the exception of fatty, none of the characteristic wheat bread attributes were evaluated in quinoa bread (Figure 5-3). Pea-, hay- and cooked potato-like as well as grassy and mouldy attributes were found as characteristic and dominant descriptors of quinoa crumb. The crumb aroma of sorghum sourdough bread was characterised with fatty, cooked potato-, vomit- and sourdough-like notes, which were only detected with weak intensities (Figure 5-3). The typical wheat bread-like and buttery attributes of wheat bread were missing in sorghum bread. These positive wheat bread descriptors was also absent in teff bread (Figure 5-3). It was dominated by cooked potato-, sourdough- and vinegar-like notes on a weak to medium intensity level and completed by fatty, grassy, metallic, pea- and vomit-like descriptors, which were perceived only weakly.



Figure 5-3 Aroma profile analysis of bread crumbs prepared with *Lactobacillus* plantarum FST 1.7 sourdough made from different flours

#### 5.4.6 Microbial shelf life

Microbial shelf life studies were conducted on gluten-free sourdough breads fermented with *Lactobacillus plantarum* FST1.7 (data not shown). With the exception of wheat bread, the first mould growth was observed on day three for buckwheat, quinoa and sorghum bread and day two for teff and oat sourdough bread, giving the breads a shelf life of two or one day, respectively. Wheat bread showed mould growth from day four on, resulting in a shelf life of three days. In comparison to the other gluten-free sourdough breads, quinoa sourdough bread had the lowest increase of mould spoilage per day.

#### 5.5 Discussion

This study reports a comprehensive investigation of the suitability of *Lactobacillus plantarum* FST1.7 as sourdough starter for five different glutenfree flours. Fermentable sugars in quinoa and teff favoured the growth of the strain in the respective sourdoughs. The type of flours decisively influences sourdough fermentation. It affects the amount and quality of carbohydrates as primary fermentation substrates, nitrogen sources and growth factors such as vitamins, minerals and the buffering capacity (Hammes et al. 2005).

During fermentation, higher amounts of organic acids were produced in buckwheat, quinoa and teff sourdough. Yet, pH values for those sourdoughs did not differ greatly from values in wheat sourdough with lower amounts of organic acids. This shows the influence of flour components on buffering capacity during gluten-free sourdough fermentation. Initial titratable acidities were 1.5 - 2 folds higher in buckwheat and quinoa flour, suggesting that the content of buffering compounds is higher in the gluten-free flours than in wheat flour. High mineral contents in flours increase the buffering capacity of the sourdough. Hence, the final fermentation pH is not altered but lactic acid fermentation is enabled to proceed longer and results in a higher content of lactic acid (Salovaara and Valjakka 1987; Roecken 1995; Gänzle et al. 1998). analysed mineral contents were highest in buckwheat Previously (10,237 mg/ kg flour dwb), quinoa (15,235 mg/ kg dwb) and teff (12,631 mg/ kg)dwb) flour compared to wheat flour (6193 mg/ kg dwb) (Hager et al. 2012b). Therefore, excellent growth performances in buckwheat, quinoa and teff sourdoughs can be attributed to generally higher buffering capacities in the gluten-free flours in comparison to wheat flour.

Heterofermentative bacteria as *L. plantarum* FST1.7 produce lactic acid, carbondioxide acetic acid, and/or ethanol (Corsetti and Settanni 2007) from glucose using glycolysis (phosphogluconate/ phosphoketolase pathway) for hexose fermentation. The presence of co-substrates enables the regeneration of reduced cofactors (Gänzle et al. 2007). Generally, in most heterofermentative lactobacilli the preferred use of fructose as electron acceptor is observed (von Weymarn et al. 2002). The reduction of fructose to mannitol by mannitol dehydrogenase (and thereby acting as an electron acceptor) favours the production of acetic acid against ethanol given that an extra ATP is obtained.

L. plantarum preferentially ferments glucose and fructose (Gobbetti 1998; Siezen and Vlieg 2011). During sourdough fermentation with Lactobacillus plantarum FST1.7 both metabolites, lactate and acetate, were produced indicating the use of fructose which is present in the flours. L. plantarum FST1.7 produced high amounts of lactate proving that the strain is able to utilize a broad variety of sugars (Boekhorst et al. 2004). This confirms that it is a high acid producer as previously stated (De Vuyst and Neysens 2005; Dal Bello et al. 2007). In our study, the overall best fermentation performances of *L. plantarum* FST1.7 in terms of cell growth and acidification were found in buckwheat, quinoa and teff sourdoughs, linked to higher levels of fermentable sugars. This confirms the dominance of this strains in gluten-free sourdoughs as investigated by Vogelmann et al. (Vogelmann et al. 2009). However, acidification properties in oat and wheat sourdoughs in our study were lower in comparison to sourdoughs of the previously mentioned study. This might be due to application of refreshment steps and longer fermentation time (~14 days instead of one day) favouring adaptability of lactic acid bacteria to the sourdough microflora.

Summarizing, the strain *Lactobacillus plantarum* FST1.7 is a suitable sourdough starter for gluten-free flour fermentation.

The degradation of protein upon sourdough fermentation was detected by capillary electrophoresis. This is in agreement with previous findings for bread batter containing 20% fermented buckwheat flour (Moroni et al. 2011b). In general, proteolytic events occurring in wheat or rye sourdoughs affect the overall quality of the bread and have been comprehensively illustrated by Gänzle et al. (2008). It was discussed that primary proteolysis in wheat and rye is exerted by indigenous enzymes, which are activated by the low pH (Vermeulen et al. 2005). Since no coding genes for extracellular protease were found for *L. plantarum* species (Kleerebezem et al. 2003) which is required for polypeptide utilization, the primary breakdown of proteins can explain the activation of indigenous proteases present in the flours. Higher proteolysis occurring during quinoa sourdough fermentation can be explained by higher proteinase activity in quinoa flour in comparison to the other gluten-free flours and wheat (Wolter et al. 2014b). Rheological properties of the sourdoughs and bread batters were influenced by changes of structural components upon fermentation. Due to organic acid production and proteolysis induced by sourdough fermentation dough softening of sourdough samples occurred. This is in accordance with dough softening previously found during investigations for wheat bread prepared with sourdough (Angioloni et al. 2006). Also, fermentation of amaranth flour with *L. plantarum* decreased the elastic part by 53% resulting in softer dough (Houben et al. 2010). In the current study, dough softening accounted for facilitated expansion of gas cells which led to increased cell volume and increased porosity of the bread crumb.

The specific volume upon sourdough addition was not improved. This confirms previous findings by Dal Bello et al. (2007) where the specific volume for Lactobacillus plantarum sourdough wheat bread was not different to the nonacidified control bread. A trend of delayed staling after five days of storage upon sourdough addition was visible in some gluten-free breads (buckwheat and teff bread) in our study. This confirms a previous study, in which addition of L. plantarum FST1.7 sourdough delayed staling of wheat bread formulations (1.96 N/ day) (Dal Bello et al. 2007). This however, was only significant compared to chemically acidified bread (3.5 N/ day) (Dal Bello et al. 2007), whereas no delay of staling was found in comparison to the non-sourdough containing control breads (1.97 N/ day) (calculated from Dal Bello et al. (2007). No improvements regarding microbial shelf life were observed since sourdough-containing, gluten-free breads showed mould growth after the same storage time as their control breads. On the contrary, the microbial shelf life of wheat sourdough bread was prolonged by a day in a previous studies using Lactobacillus plantarum FST1.7 (Dal Bello et al. 2007). Quinoa and sorghum sourdough bread showed the lowest increase of microbial shelf life due to the fact of higher amount of total titratable acids. In addition, the inhibition of mould growth is determined by components of the flour matrix and water content of bread formulation. The water addition level is higher in gluten-free breads which could serve to explain higher increase of mould growth.

The aroma quality of the gluten-free sourdough breads was in part very low. Oat bread was the only exception showing a higher preference but its aroma was also inferior to the reference. Aroma profile analysis of the breads clearly showed that the absence or a much lower intensity wheat bread-like note, which was found as the characteristic and positive odour characteristic of wheat bread, was responsible for the low odour quality in the gluten-free breads. Several in part very characteristic odour attributes were found in gluten-free breads, e.g. pea-like (buckwheat, quinoa, teff), cooked potato-like (quinoa, teff), vomit-like (sorghum, teff) and mouldy (buckwheat, quinoa). These undesirable notes and the odorants causing these notes, respectively, can be considered to cause an additional negative impact on the aroma quality of the sourdough breads.

A previous study showed that many breads prepared from the investigated gluten-free flours by yeast fermentation also exhibited the negative aroma descriptors, e.g. pea-like in quinoa and cooked potato-like in teff (Hager et al. 2012a). Addition of *L. plantarum* FST1.7 sourdough could consequently not improve the crumb aroma quality.

#### 5.6 Conclusion

The result of the present study showed that chosen gluten-free flours are able to serve as substrates for Lactobacillus plantarum FST1.7. In terms of acidification strongest performance was shown by L. plantarum fermenting quinoa and buckwheat flour. Due to the excellent performance during the sourdough fermentation and benevolent nutritional composition buckwheat and quinoa flour (high levels of lysine and methionine (Lorenz and Nyanzi 1989)) could therefore serve as sourdough substrate to enrich gluten-free bread. However, no improvement of microbial shelf life was determined. The sensory quality of gluten-free bread still needs to be improved. Most likely, the added amount of sourdough (20%) was not sufficient to cause degradation of flour odorants with negative aroma, and/or generation of positive odorants during fermentation. The type of flour in addition to type of lactic acid bacteria are also important factors influencing aroma profiles of sourdough breads (Thiele et al. 2002; Hansen and Schieberle 2005). The chemical structures of positive wheat aroma compounds are known (Czerny and Schieberle 2002). Potent lactic acid bacteria able to modulate aroma profile by production of chemical compounds are already applied in other foods, for example beer, wine and dairy products (Urbach 1995; Krieger 1997). Therefore, further screening of lactic acid bacteria and yeasts as a future strategy is necessary to find most suitable combinations of lactic acid bacteria and yeasts able to produce odour active compounds associated with the positive "malty, buttery" bread aroma (Czerny and Schieberle 2002; Czerny et al. 2005). Alternatively, germination and roasting of gluten-free grains (Mäkinen et al. 2013) could create favourable roasty and nutty flavour in the breads on the one hand and increase amino acids release through proteolysis serving as flavour precursors on the other hand (Thiele et al. 2002).

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# Chapter 6 In vitro starch digestibility and predicted glycaemic indices of buckwheat, oat, quinoa, sorghum, teff and commercial gluten-free bread

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## 6.1 Abstract

The in vitro starch digestibility of five gluten-free breads (from buckwheat, oat, quinoa, sorghum or teff flour) was analysed using a multi-enzyme dialysis system. Hydrolysis indices (HI) and predicted glycaemic indices (pGI) were calculated from the area under the curve (AUC; g RSR/100g TAC\*min) of reducing sugars released (RSR), and related to that of white wheat bread. Total available carbohydrates (TAC; mg/4 g bread "as eaten") were highest in sorghum (1634 mg) and oat bread (1384 mg). The AUC was highest for quinoa (3260 g RSR), followed by buckwheat (2377 g RSR) and teff bread (2026 g RSR). Quinoa bread showed highest predicted GI (95). GIs of buckwheat (GI 80), teff (74), sorghum (72) and oat (71) breads were significantly lower. Significantly higher gelatinisation temperatures in teff (71°C) and sorghum flour (69°C) as determined by differential scanning calorimetry (DSC) correlated with lower pGIs (74 and 72). Larger granule diameters in oat (3-10 µm) and sorghum (6-18  $\mu$ m) in comparison to quinoa (1.3  $\mu$ m) and buckwheat flour (3-7  $\mu$ m) as assessed with scanning electron microscopy resulted in lower specific surface area of starch granules. The data is in agreement with predictions that smaller starch granules result in a higher GI.

#### 6.2 Introduction

The ingestion of gluten (the storage protein in wheat) and related proteins in barley (hordein) and rye (secaline) triggers a systemic immune-mediated disorder, called coeliac disease, in genetically susceptible individuals. Worldwide, 0.6-1.0% of the population is affected by this inflammation of the small intestine which damages the micro-villi and leads to different silent presentations such as osteoporosis and iron-deficiency anaemia (Fasano and Catassi 2012). The only effective treatment is the life-long adherence to a gluten-free diet, which eventually results in mucosal recovery (Green and Cellier 2007). An increased risk of autoimmune disorders occurs for patients with coeliac disease in comparison to the general population. High incidences of type I (insulin-dependent) diabetes mellitus in coeliac disease patients have been reported. The frequent simultaneous occurrence of insulin-dependent diabetes mellitus and coeliac disease is linked to the involvement of similar human leucocyte antigens (HLA DQ2, DQ8) (Viljamaa et al. 2005). Therefore, the maintenance of a good glycaemic control is an important task for coeliac disease patients (Berti et al. 2004).

The postprandial glycaemic effect of foods is related to the rate of carbohydrate digestion and reliably characterized by the glycaemic index (GI), a model which enables the comparison of a variety of starchy foods (Jenkins et al. 2002). The GI is defined as the incremental area under the curve (AUC) of the blood glucose concentration occurring upon ingestion of a carbohydrate-containing food relative to reference food (glucose, GI glucose=100, or white wheat bread, GI white wheat bread=100) (Jenkins et al. 1981). Foods can be distinguished into those with low (<55, legumes, nuts, dairy products and pasta), intermediate (55-70, muesli, certain breads) and high GI (>70, whole meal barley flour bread, white wheat bread) (Atkinson et al. 2008). Breads fall into intermediate (58; whole meal rye bread) and high GI categories (136; white plain baguette) (compared to GI wheat bread = 100) (Atkinson et al. 2008). Foods with high GI cause a rapid and large release of glucose, whereas foods with low GI contain slowly digested carbohydrates and cause slower and lower increase of the blood glucose level (Brand-Miller et al. 2009). The glycaemic response has been related to the rate of digestion and absorption of carbohydrate-containing foods with help of *in vitro* methods, which are mimicking *in vivo* digestion processes.

The use of a restricted system in this study, i.e. employing dialysis tubing, relates the procedure to absorption processes rather than to digestion alone, since the measured concentration of reducing sugars in the dialysate represents the carbohydrates that have passed through the membrane. Previously, the rate of *in vitro* amylolysis during a multi-enzymes dialysis system corresponded well with the *in vivo* rate of starch uptake as judged from the postprandial blood glucose response (Singh et al. 2010).

The glycaemic response depends on indigenous factors related to the food matrix (starch susceptibility, protein and lipid content) as well as on the macroscopic structure of the food (botanical integrity of ingredients, physical texture). Starch susceptibility is determined by its native structure, physical encapsulation, crystallinity, degree of gelatinisation and retrogradation of the starch granules, as well as by the proportion of damaged granules (Fardet et al. 2006).

Atkinson's "International Tables of Glycemic Index" also contain *in vivo* GIs of three gluten-free breads: gluten-free white bread (57), gluten-free buckwheat bread (103) and gluten-free multigrain bread (113) (Atkinson et al. 2008). Nevertheless, information about starch digestibility and glycaemic response for the majority of gluten-free foods is scant. Furthermore, studies on the GI of gluten-free foods have been conducted on composite recipes (Matos Segura and Rosell 2011; Capriles and Areas 2013) making it difficult to estimate the influence of the individual gluten-free starch on blood glucose response.

Therefore, this study aims to assess *in vitro* GIs for basic gluten-free bread formulations and thereby to evaluate the influence of starch-properties of buckwheat, oat, quinoa, sorghum or teff flour on *in vitro* starch digestibility. The hydrolysis indices (HI) gained after *in vitro* enzymatic digestions were used to estimate the glycaemic indices (GI) and to calculate the glycaemic load (GL) taking into account the influence of total available carbohydrate amounts available in a defined food portion on the glycaemic responds. Furthermore, starch morphology and gelatinisation temperatures were determined to relate typical features to the GI of the respective bread.

## 6.3 Experimental

# 6.3.1 Bread production

The following ingredients were used for bread production: buckwheat flour (Doves Farm Foods Ltd, UK), oat flour (E. Flahavan & Son Ltd, Ireland), quinoa flour (Ziegler Naturprodukte, Germany), sorghum flour (Twin Valley Mills, Nebraska), teff flour (Trouw, The Netherlands), wheat flour (baker's flour, Odlums, Ireland), yeast (Puratos, Belgium), sugar (Siúcra, Ireland) and salt (Glacia British Salt Limited, UK). Breads were produced as previously described by Hager et al. (2012a) using 100% flour, 2% salt, 2% sugar and 3% dry-yeast (based on flour, BF). Optimal water addition level (WL) for gluten-free flours was determined through preliminary baking trials (buckwheat: 85% BF; oat, quinoa, sorghum and teff bread: 95% BF) and with the farinograph method (AACC 2000) for wheat flour (63% BF). Bulk fermentation of wheat dough was carried out for 15 min at 30°C and 85% relative humidity (RH). The gluten-free bread batters and the wheat dough were proofed in tins for 30 and 75 min, respectively (30°C, 85% RH) and baked in a deck-oven (gluten-free breads for 45 min at 190°C; wheat bread for 30 min at 220°C top and 235°C bottom heat). Breads were cooled for two hours at room temperature and frozen (-18°C) until analysis. Three batch replicas were prepared. Commercial bread (soft white loaf, ingredients: water, corn starch, tapioca starch, potato starch, dried egg white, white rice flour, buckwheat flour, rice bran, thickening agent: xanthan gum; yeast; cellulose; sourdough: fermented quinoa and rice flour); psyllium; salt; rapeseed oil; pea protein; agar agar; potassium sorbate; thickening agent; guar gum; hydroxypropyl methylcellulose; flour treatment agent: ascorbic acid, raising agent: sodium bicarbonate) was analysed as a gluten-free reference.

# 6.3.2 Determination of total available carbohydrates

Amounts of total available carbohydrates (TAC, mg/ 4 g fresh bread) were determined spectrophotometrically ( $\lambda$ =510 nm) as the sum of free available and starch derived sugars in freeze-dried, ground samples of gluten-free and wheat bread crumbs using a total starch assay kit (K-TSTA, Megazyme, Ireland).

# 6.3.3 Reducing sugars released and *in vitro* starch digestibility

*In vitro* starch digestibility was analysed as previously described by Brennan and Tudorica (2008). Dialysis tubing, chemicals and enzymes were obtained

from Sigma Aldrich, Arklow, Ireland. Triplicate samples (4 ± 0.001g of homogenized gluten-free and wheat bread crumb "as eaten") were dissolved in sodium potassium phosphate buffer (pH 6.9). After adjustment of pH to 1.5, samples were incubated for 30 min at 37°C with 5 mL pepsin solution (EC 3.4.23.1; porcine gastric mucosa, 115 U/ mL in dist. water). The pH was then adjusted to 6.9 and 7 mL  $\alpha$ -amylase solution (EC 3.2.1.1; porcine pancreatic; 16 U/ mL in Tris HCl buffer) were added. The samples together with glass beads were transferred into dialysis tubing (cut off size 10-11 kDa) and placed into a beaker containing potassium phosphate buffer (pH 6.9). During four hours of incubation at 37°C tubings were inverted every 15 minutes. An aliquot of dialysate was taken every 30 min for quantification of reducing sugars and replaced with the same amount of fresh buffer. Amounts of reducing sugars in the dialysate were determined spectrophotometrically ( $\lambda$ =540 nm) after reaction with 3,5-dinitrosalicylic acid reagent (DNS) (2 M sodium hydroxide, 0.04 M 3,5-dinitrosalicylic acid, 1.1 M potassium sodium tartrate in distilled water). A maltose standard (1 g/ L) was used for the calculation. Amounts of reducing sugars released, RSR (%), were calculated as maltose equivalents (in g) as percentage of the total available carbohydrates (TAC) in 4 g sample [RSR = (A sample \*500\*0.95)/ (A maltose standard \*TAC)\*100; where A sample is sample absorbance, A maltose standard is absorbance of maltose standard, TAC is total available carbohydrates (mg in 4 g sample), 500 is total volume (mL) in dialysis beaker, 0.95 is conversion factor from maltose to starch]. The amount of RSR (g/ 100g TAC) was plotted against the digestion time (min) and the area under the hydrolysis curve (AUC, g/ 100g TAC\*min) was calculated geometrically for 180 min using the trapezoidal method described by Wolever and Jenkins (1986). The hydrolysis index (HI) was calculated from AUC of gluten-free samples as a percentage of the corresponding area of the reference white wheat bread (HI=AUC sample/ AUC wheat bread\*100).

#### 6.3.4 Predicted glycaemic index and glycaemic load

The predicted GI was calculated using the equation: GI=0.549\*HI+39.71 (Capriles and Areas 2013) with wheat bread as the standard food (GI wheat bread=100). To obtain the GI in relation to glucose as standard food (GI glucose=100), obtained predicted GI values were multiplied with 0.7 (Wolever et al. 2008). The glycaemic load (GL) was calculated for 50 g portion of bread from

the glucose related GI according to  $GL = [GI_{glucose=100} \cdot TAC)/(100)$  taking into account the total available carbohydrates of each sample (Atkinson et al. 2008).

## 6.3.5 Differential scanning calorimetry

Gelatinisation temperatures of starch granules in the different flours (not in the commercial gluten-free bread) were determined by differential scanning calorimetry (DSC) as described by (Hager et al. 2013). Triplicate measurements were carried out on a DSC821<sup>e</sup> (Mettler Toledo, Switzerland). Briefly, flour samples (3-5 mg) were weighed directly into aluminium pans and 10  $\mu$ L of water were added. An empty container was used as reference. Samples were heated from 25°C to 105°C at a rate of 5°C/ min and onset temperatures (T<sub>o</sub>), peak temperature (T<sub>P</sub>) as well as end temperature (T<sub>E</sub>) were recorded.

## 6.3.6 Scanning electron microscopy

The size of starch granules was analysed by scanning electron microscopy (SEM). Prior to SEM, flour samples were dried in an air-oven for one hour at 103°C. Samples were affixed with double-sided carbon tape to aluminium stubs and coated with a 25 nm layer of sputtered gold (BIORAD Polaron Division SEM Coating System). Samples were examined under high vacuum in a field emission scanning electron microscope (JEOL JSM-5510 SEM) with a working distance of 8 mm. Secondary electron images were acquired at an accelerating voltage of 5 kV. For processing of the images the SEM Control User Interface, Version 5.21 (JEOL Technics Ltd., Japan) was used. Representative micrographs were taken of each starch type at magnifications between x2.000 and x2.500. The starch granule diameter was measured by averaging the largest dimension of ten starch granules from three micrographs with image analysis software "ImageJ 1.46r" (National Institutes of Health, USA).

## 6.3.7 Statistical analyses

Results are reported as average with confidence interval. Statistical analyses were performed with SigmaPlot (Systat Software Inc.; version 11, UK) on all data using one-way ANOVA. Fisher's least significant differences test was used to describe means at 5% significance level.

#### 6.4 Results

#### 6.4.1 Determination of total available carbohydrates

Values for total available carbohydrates in 4 g fresh bread increased in the order: quinoa < buckwheat < teff < oat < sorghum (Table 6-1). The commercial gluten-free bread contained  $1109 \pm 12$  mg total available carbohydrates. Wheat bread contained  $1543 \pm 12$  mg of available carbohydrates per 4g fresh bread.

#### 6.4.2 Reducing sugars released and *in vitro* starch digestibility

The plot of amounts of reducing sugars released (RSR) into the dialysate versus dialysis time gives different appearance of hydrolysis curves for gluten-free breads (Figure 6-1). Curve progressions of RSR were highest for wheat and quinoa bread, followed by buckwheat, oat, sorghum and teff breads. The RSR curve of the commercial gluten-free bread was comparable to that of oat, sorghum and teff bread. The area under the hydrolysis curve of reducing sugars released (AUC) after 180 min for quinoa was not significantly different from wheat bread but higher than all other gluten-free breads. The AUC of buckwheat, oat, sorghum and teff breads were not significantly different (Table 6-1). The hydrolysis index (HI) of the gluten-free breads increased in the order: oat, sorghum < teff < buckwheat < quinoa. The commercial gluten-free bread had a significantly lower HI.



Figure 6-1 Reducing sugars released from 4 g fresh samples during hydrolysis of buckwheat, oat, quinoa, sorghum, teff, commercial gluten-free and wheat bread. Error bars represent confidence interval ( $\alpha$ =0.05)

Parameters	Buckwheat	0at	Quinoa	Sorghum	Teff	CGF	Wheat
TAC (mg/ 4g bread)	$1171 \pm 17^{e}$	1384 ± 21∘	$1033 \pm 13^{f}$	$1634 \pm 17^{a}$	$1296 \pm 13^{d}$	1109 ± 12 <sup>f</sup>	$1543 \pm 12^{b}$
AUC (g/ 100g TAC*min)	2377 ± 76 <sup>b</sup>	$1884 \pm 48^{\circ}$	3260 ± 119ª	1934 ± 63 <sup>bc</sup>	2026 ± 64 <sup>bc</sup>	2009 ± 76 <sup>bc</sup>	3266 ± 114ª
HI	73 ± 2 <sup>b</sup>	58 ± 1°	$100 \pm 4^{a}$	59 ± 2°	62 ± 2°	54 ± 2 <sup>d</sup>	
pGI wheat bread=100	80 ± 1 <sup>b</sup>	71 ± 1 <sup>cd</sup>	95 ± 2ª	72 ± 1 <sup>c</sup>	74 ± 1°	69 ± 1 <sup>d</sup>	100a
pGL 50g portion	8d	9c	9c	$10^{\mathrm{b}}$	8 <sup>d</sup>	7e	<b>1</b> 3ª
Values given as mean ± confi	dence (α=0.05).	Different sup	erscripts in san	ie row indicate	statistical differ	ence (P<0.001).	

# 6.4.3 Predicted glycaemic index and glycaemic load

The predicted glycaemic index (pGI) was calculated from hydrolysis index (HI) using the equation as presented by Capriles and Areas (2013) in relation to wheat bread (GI <sub>wheat bread</sub> =100). Values ranged from 71 ± 1 for oat to 95 ± 2 for quinoa bread (Table 6-1). The predicted GIs were calculated relative to glucose (GI <sub>glucose</sub>=100) to derive the predicted glycaemic load (Table 6-1) for a 50 g portion of bread. The pGL increased in the order: buckwheat, teff < oat, quinoa < sorghum. Although the GI <sub>glucose</sub> was the same for wheat and quinoa bread, wheat bread had a significantly higher pGL (13) due to its significantly higher total available carbohydrate content. The pGL of the commercial bread formulation was significantly lower than those of all other gluten-free breads.

# 6.4.4 Differential scanning calorimetry

Gelatinisation temperatures of the flour raw materials showed significant differences (Table 6-2). Buckwheat, teff and sorghum flours had significantly highest onset temperatures  $T_0$  (59°C, 66°C, and 64°C, respectively) than quinoa flour ( $T_0$  52°C), oat flour ( $T_0$  51°C) and wheat flour ( $T_0$  55°C). Peak temperature  $T_P$  was highest for teff flour ( $T_P$  71°C) and lowest for oat flour ( $T_P$  56°C). Buckwheat, teff and sorghum flours showed highest end temperatures  $T_E$  ( $T_E$ : 72°C, 77°C, and 73°C, respectively).

Table 6-2 Temperatures at onset, peak and end of gelatinisati	on measured with
differential scanning calorimetry	

Flours	Ten	nperatures	(°C)
	Onset, T <sub>0</sub>	Peak, T <sub>P</sub>	End, T <sub>E</sub>
Buckwheat	$59 \pm 0.07^{b}$	$66 \pm 0.47^{\circ}$	72 ± 1.59 <sup>b</sup>
Oat	$51 \pm 0.46^{d}$	$56 \pm 0.42^{f}$	$62 \pm 1.02^{d}$
Quinoa	$52 \pm 1.15^{d}$	58 ± 0.25 <sup>e</sup>	$64 \pm 0.04^{cd}$
Sorghum	$64 \pm 0.62^{a}$	$69 \pm 0.02^{b}$	$73 \pm 0.54^{b}$
Teff	$66 \pm 1.46^{a}$	$71 \pm 0.43^{a}$	77 ± 1.69 <sup>a</sup>
Wheat	55 ± 0.96°	$61 \pm 0.60^{d}$	66 ± 0.05 <sup>c</sup>

Values given as mean  $\pm$  confidence ( $\alpha$ =0.05). Different superscripts in same column indicate statistical difference (P<0.005).

# 6.4.5 Scanning electron micrographs

Scanning electron micrographs of buckwheat, oat, quinoa, sorghum, teff and wheat flour showed various features of the starch granules (Figure 6-2, a-f). In

oat flour starch granules exhibited different sizes (from 3 to 10  $\mu$ m diameter) and various morphologies (spherical or slightly lenticular) (Figure 6-2, b). Quinoa flour contained uniformly shaped and sized starch granules (1.3  $\mu$ m) (Figure 6-2, c). In sorghum flour, individual granules showed a broader size spectrum (6-18  $\mu$ m) and a larger average granule diameter (10 ± 1.2  $\mu$ m) (Figure 6-2, d). In comparison, starch granules in buckwheat (Figure 6-2, a) and teff flour (Figure 6-2, e) were only half in average diameter (5 ± 0.6  $\mu$ m). Both granule types occurred in aggregates and singly with the same range of diameter (3-8  $\mu$ m), possessing polyhedral (teff) or additionally spherical shape (buckwheat). Wheat starch granules were bimodal showing two sizes of granules (6 ± 0.5 and 15 ± 2.2  $\mu$ m) (Figure 6-2, f). The average diameter of starch granules increased in the order: quinoa (1.3  $\mu$ m) < buckwheat (5  $\mu$ m), teff (5  $\mu$ m) < oat (6  $\mu$ m) < sorghum flour (10  $\mu$ m).



Figure 6-2 Scanning electron micrographs of flours from a) buckwheat, b) oat, c) quinoa , d) sorghum, e) teff and f) wheat. Scale bar represents  $10 \ \mu m$ .

#### 6.5 Discussion

**6.5.1 Predicted glycaemic index and glycaemic load of gluten-free breads** *In vitro* enzymatic digestibility of breads baked from buckwheat, oat, quinoa, sorghum and teff flour was analysed in order to estimate their pGIs. Apart from quinoa bread, all gluten-free breads showed significantly lower pGI values than the reference food (white wheat bread). Quinoa bread had a significantly higher pGI than oat, sorghum, teff and buckwheat bread. Nevertheless, according to the above mentioned GI classification all gluten-free breads fall into the category "high GI" (GI>70). This is in accordance with previously reported *in vitro* GIs: gluten-free bread based on rice flour, potato starch, egg and whole milk powder (93 ± 0.4) (Capriles and Areas 2013) and commercial corn starch-based gluten-free breads (83-91) (Matos Segura and Rosell 2011). Foods with a relatively high GI can still lead to a low insulin secretion *in vivo* due to their low carbohydrate content. This explains the lower predicted GL of quinoa in comparison to wheat bread, since the carbohydrate content of quinoa bread was significantly lower than that of wheat bread.

## 6.5.2 Factors influencing starch digestibility

Among the mechanisms governing the glycaemic response, the rate of starch digestion plays a principal role and is controlled by a combination of factors: a) size of the starch granules, b) extent of damage or gelatinisation, c) their composition and structure (Tester et al. 2004), d) physical encapsulation as well as e) protein and lipid content of the matrix (Singh et al. 2010).

## 6.5.2.1 Gelatinisation temperature

The high GI in breads, in general, is due to the fact that starch gelatinisation and loss of crystallinity during the baking process make the starch granule more susceptible to enzymatic attack by  $\alpha$ -amylase (Fardet et al. 2006). An earlier onset of gelatinisation of quinoa, wheat and buckwheat starches could facilitate susceptibility for  $\alpha$ -amylase attack. This is supported by significantly higher pGI values found for quinoa, wheat and buckwheat breads in this study. While significantly higher peak gelatinisation temperatures (T<sub>P</sub>) for starches in sorghum and teff flour in comparison to buckwheat, oat and wheat flour correlated with lower pGIs for teff and sorghum bread. However, this does not sufficiently explain the lower pGI found for oat bread since the gelatinisation temperatures of oat flour was significantly lower than those of wheat and buckwheat flours. Furthermore, it has to be kept in mind that the conditions during DSC analysis of flours are different to conditions during bread baking, since an excess of water is present during DSC analysis, while bread represents a limited-water system. The use of various starches in the commercial glutenfree formulation makes it difficult to conclude about the influence of gelatinisation temperature on the pGI of the commercial gluten-free bread.

#### 6.5.2.2 Starch granule size

The rate of starch digestibility is increased and enzymatic attack is facilitated for smaller starch granules, since their specific surface area is larger (Tester et al. 2004). From all gluten-free flours under study, quinoa flour possessed significantly smaller starch granules Starch sizes assessed with SEM are in accordance with previously reported result for quinoa (between 1 and 2  $\mu$ m) (Repo-Carrasco et al. 2003), buckwheat (3-9 µm) (Qian and Kuhn 1999), teff (2-6  $\mu$ m) (Bultosa et al. 2002) and sorghum starch (~20  $\mu$ m) (Delcour and Hoseney 2010). Oat starch is represented by granule sizes between 3-10 µm (Delcour and Hoseney 2010). Wheat starch granules are bimodal, showing two sizes of granules (B-type, 2-10 µm and A-type, 20-35 µm) (Delcour and Hoseney 2010). Thus, the specific surface area of quinoa starch granules is larger than those of oat, sorghum and wheat starches making it more susceptible to hydrolysis by  $\alpha$ amylase than wheat starch (Tester et al. 2004). This might explains the significantly higher pGI for quinoa bread in comparison to pGI of oat and sorghum bread but not in comparison to wheat bread. Consequently, starch size alone is not sufficient to explain the different GIs. Damaged starch is enzymatically more susceptible than native starch (Tester et al. 2004). In addition to higher starch surface area in quinoa flour, the higher content of damaged starch (5.4% dwb) in comparison to buckwheat (3.0% dwb) and teff flour (2.4% dwb) (Hager et al. 2012b), can serve to explain higher pGI of the quinoa bread in comparison to teff and buckwheat bread. The relatively high proportion of large starch granules in the commercial gluten-free formulation, i.e. potato starch ( $\sim$ 30 µm) (Dhital et al. 2011), tapioca starch (5-30 µm) (Rao and Tattiyakul 1999) and maize starch ( $\sim 20 \mu m$ ) (Delcour and Hoseney 2010) might induce the low pGI of this sample.

# 6.5.2.3 Composition of starch

Starches high in amylose possess reduced enzyme availability which derives from the double helical and hence more compact structure of amylose upon recrystallization (Dhital et al. 2011). In comparison, this process is hampered for the amylopectin molecule which is sterically hindered (Fardet et al. 2006). In addition, amylopectin is the larger molecule and therefore offers a larger surface area per molecule than amylose making it a preferable substrate for amylolytic attack (Singh et al. 2010). In this study, the amylose content of gluten-free flours correlated negatively with pGI ( $R^2$  =-0.964). The low amylose content of quinoa starch (5.3% dwb) (Hager et al. 2012b) contributed to the high pGI of quinoa bread, whereas the significantly higher content in oat, sorghum and teff starches (20.5-22.8% dwb) led to significantly lower pGI.

Also the formation of amylose-lipid complexes impedes enzymatic susceptibility (Singh et al. 2010). Higher lipid contents in oat (7.5% dwb), buckwheat (4.9% dwb), teff (4.4% dwb) and sorghum flour (3.9% dwb) together with higher amylose contents of these starches (oat: 22.8%; buckwheat: 18.3%; teff: 21.8%; sorghum: 20.5%) (Hager et al. 2012b) could therefore result in the formation of amylose-lipid complexes hindering the enzymatic access. This formation of complexed amylose might be impeded in quinoa and wheat bread, because of lower amylose content in quinoa starch on one hand and lower fat content in wheat flour (2.1%) (Hager et al. 2012b) on the other hand. Similarly, starch susceptibility for the commercial gluten-free bread might be reduced by the formation of amylose-fat complexes originating from 1.5% fat contained in the commercial formulation (i.e. rapeseed oil).

# 6.5.2.4 Dietary fibre

The presence of dietary fibre (DF) can impede enzymatic attack by increasing viscosity (Sasaki and Kohyama 2012). Previously, the presence of the soluble fibre  $\beta$ -glucan reduced digestibility of starch and coincided with its increased *in vitro* extract viscosity which resulted in reduced carbohydrate absorption from the gut (Rao and Tattiyakul 1999). Nevertheless, the influence of fibre-enriched flour additionally depended on the proportion of amylose in the starch (Sasaki and Kohyama 2012). Namely, xanthan and guar gum depressed starch digestibility of corn rice starch that contained high proportion of amylose (Sasaki and Kohyama 2012). Similarly, lowest peak human glucose responses

were found after a high- $\beta$ -glucan barley meal that contained high-amylose (Alminger and Eklund-Jonsson 2008). Concluding, the amylose content in quinoa starch in this study might be too low to support the capability of dietary fibre in quinoa flour (8.1%) to lower the GI. The higher GI in buckwheat in comparison to oat, sorghum and teff bread might result from lower content of dietary fibre in the earlier (2.5%) compared to 5% DF in the latter. Similarly, the presence of dietary fibre and thickening agents (rice bran, psyllium, cellulose, xanthan and guar gum) might have the same effect on starch gelatinisation and digestibility in the commercial gluten-free bread leading to a lower GI by increasing the viscosity.

#### 6.6 Conclusions

An *in vitro* assay was applied to evaluate starch digestibility and to calculate the predicted glycaemic index (GI) of five gluten-free breads based on a basic formulation as well as of commercial gluten-free bread containing a mixture of starches, proteins, oil and fibres. Present *in vitro* results deliver relevant, yet not definitive information about starch digestibility, since it is also influenced *in vivo* by metabolic factors. Metabolic factors such as the rate of gastric emptying, gut hormone profiles, glucose diffusion-absorption through the intestinal mucosa, limited starch accessibility to  $\alpha$ -amylase also significantly affect glycaemia *in vivo* (Berti et al. 2004; Fardet et al. 2006). These human digestive processes cannot be completely mimicked by *in vitro* assays (Berti et al. 2004; Fardet et al. 2006). The results show that starch digestibility is lower in gluten-free breads from buckwheat, oat, sorghum and teff flour, compared to quinoa bread. The lowest GI was predicted for the commercial gluten-free bread. Some gluten-free breads possessed lower glycaemic load than white wheat bread and therefore could comply with glucose control diets.

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# Chapter 7 Influence of sourdough on *in vitro* starch digestibility and predicted glycaemic indices of gluten-free breads

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## 7.1 Abstract

Gluten-free flours (buckwheat, quinoa, sorghum and teff) were fermented using obligate heterofermentative Weissella cibaria MG1 and facultative heterofermentative Lactobacillus plantarum FST1.7 strain. Starch hydrolysis of sourdough-containing and control breads (without sourdough) was analyzed in vitro using enzymatic digestion followed by dialysis (10-11 kDa) mimicking intestinal and pancreatic digestion. Hydrolysis indices as well as predicted glycaemic indices (pGI) were calculated from reducing sugars released into the dialysate. Amounts of resistant starch were determined (% of total starch) by enzymatic digestion. Upon sourdough addition fermented with Weissella cibaria (Wc) or *Lactobacillus plantarum* (Lp), respectively, the resistant starch ratio significantly decreased in buckwheat (Wc 1.28%, Lp 1.44%) and in teff (Wc 0.87%, Lp 0.98%) sourdough breads in comparison to their controls (ctrl 2.01%) and 1.92%, respectively). However, no correlation was found to starch hydrolysis. Predicted GIs were reduced upon sourdough addition in comparison to control breads in wheat (ctrl 100; Wc 85; Lp 76). Still, this was not the case in most gluten-free breads with the exception of sorghum (ctrl 72; Lp 69) and teff sourdough breads (ctrl 74; Lp 68). On the contrary, increased pGIs were found in quinoa (ctrl 95; Wc 106; Lp 103) and buckwheat sourdough breads (ctrl 80; Wc 89; Lp 86).

## 7.2 Introduction

Coeliac disease is the most common food intolerance affecting ~0.6-1% of the world population (Fasano et al. 2003). The ingestion of the wheat storage protein gluten and proteins from related grains (barley, rye and triticale) triggers an auto-immune disease leading to inflammation of the intestinal mucosa. The damage of the micro-villi leads to malabsorption of nutrients which is connected with comprehensive symptoms as osteoporosis and iron-deficiency anemia (Fasano and Catassi 2012). High incidences of type I (insulin-dependent) diabetes mellitus in coeliac disease patients have been reported (Cronin and Shanahan 1997). The frequent simultaneous occurrence of insulin-dependent diabetes mellitus and coeliac disease is linked to the involvement of similar human leukocyte antigens (Viljamaa et al. 2005). The maintenance of a good glycaemic control is therefore an important task for coeliac disease patients (Berti et al. 2004).

The glycaemic index is a model which enables comparison of carbohydratecontaining food in terms of their blood glucose increasing behavior (Jenkins et al. 2002). GI categories comprise foods with low (<55, legumes, nuts, dairy products and pasta), intermediate (55-70, muesli, certain breads) and high GI values (>70, whole meal barley flour bread, white wheat bread) (Atkinson et al. 2008). Glycaemic indices of breads are generally high (GI> 70) due to the fact that starch gelatinization during the baking process increases its enzymatic susceptibility (Haralampu 2000).

Enzymatic starch digestibility depends on its native structure, physical encapsulation, crystallinity, proportion of damaged granules and degree of gelatinization and retrogradation of the starch granules (Fardet et al. 2006).

Using a multi-enzyme dialysis system, good correlations were found between *in vitro* starch digestibility and *in vivo* glycaemic index for cereal products (R<sup>2</sup>= 0.894) (Goñi et al. 1997), and for gluten-free breads (Berti et al. 2004; Capriles and Areas 2013). For gluten-free breads, intermediate to high GI values were found in gluten-free white bread (57), gluten-free buckwheat bread (103) and gluten-free multigrain bread (113) (Atkinson et al. 2008; Matos Segura and Rosell 2011; Capriles and Areas 2013).

For wheat bread postprandial glucose responses were reduced by presence of organic acids produced during sourdough fermentation (De Angelis et al. 2007;

De Angelis et al. 2009; Scazzina et al. 2009; Lappi et al. 2010; Borczak et al. 2011). Both, gastric emptying and *in vitro* starch digestibility in a buffered system were also decreased (HI = 86 or 81, respectively) for white wheat bread (100) containing biologically generated or added lactic acid (1.6g/ 100g bread dwb) (Liljeberg et al. 1995). However, biological acidification of breads lowered starch hydrolysis more effectively than chemical acidification (De Angelis et al. 2007). The effect was linked to the decreased pH upon production of organic acids and subsequent inhibition of hydrolytic enzymes *in vivo* (Liljeberg et al. 1995).

The inclusion of lactic acid into a barley flour/ water mixture prior to heat treatment reduced the rate of starch digestion (HI 81) significantly by promoting interactions between starch and gluten during starch gelatinization in comparison to addition after heat treatment (HI 89) (Östman et al. 2002). Alternatively, sourdough or lactic acid addition might promote retrogradation of starch (Liljeberg et al. 1996) and thereby increase formation of resistant starch (RS) (Scazzina et al. 2009).

Resistant starch is defined as the sum of starch and starch degradation products not absorbed in the small intestine of healthy individuals (Champ et al. 1994). Increased contents of resistant starch were previously linked with reduced starch digestibility *in vivo* for white wheat bread (Brighenti et al. 1998; De Angelis et al. 2007). Common flour-based breads contain <2% resistant starch based on starch (Englyst et al. 1992; Akerberg et al. 1998). Previously, a sourdough bread which contained high amounts of RS (7.7%) significantly reduced the blood glucose response in comparison to a control bread with 6.1% RS (Scazzina et al. 2009). The presence of organic acids possibly facilitates the formation of RS, through debranching of amylopectin moieties during baking (Brighenti et al. 1998). As previously shown, debranched amylopectin may form a high level of RS on heat treatment (Berry 1986).

The formation of resistant starch also seems to be related to the amylose content (Liljeberg et al. 1996), water availability and starch-lipid interaction (Brighenti et al. 1998). Resistant starch can surround starch granules and thereby limit the degree of gelatinization or form a physical barrier to enzymatic attack by  $\alpha$ -amylases (EC 3.2.1.1) (Liljeberg et al. 1996). Also, the

highly ordered molecular structure of resistant starch renders it un-susceptible (Östman et al. 2002).

Similarly for gluten-free breads, a decreased glycaemic response was found *in vivo* upon sourdough addition. Most effective addition levels were 15 and 22% which reduced the GI (52-54) in comparison to a non-acidified control bread (GI 68) (Novotni et al. 2012) and was linked to the presence of organic acids. However, the starch digestibility was investigated *in vivo* and on complex gluten-free samples which were partially frozen.

Little research has been done on *in vitro* starch digestibility of basic gluten-free formulations. For that purpose, breads from four different gluten-free flours (buckwheat, quinoa, sorghum and teff) were produced adding 20% sourdough fermented with two common lactic acid bacteria strains. The obligately heterofermentative strain *Weissella cibaria* MG1 (Wc) was selected as low organic acids producer, whereas the facultatively heterofermentative strain *Lactobacillus plantarum* FST1.7 (Lp) was selected as strong acidifier. Using a restricted multi-enzyme system starch digestibility was analyzed *in vitro*. In this study the influence of sourdough application on starch properties and its potential to reduce the predicted glycaemic index (pGI) of simple gluten-free recipes is investigated.

## 7.3 Experimental

# 7.3.1 Materials

Ingredients used in this study were flours from buckwheat (Doves Farm Foods Ltd, UK), quinoa (Ziegler Naturprodukte, Germany), sorghum (Twin Valley Mills, Nebraska, USA), teff (Trouw, The Netherlands) and wheat (baker's flour, Odlums, Ireland; moisture), as well as yeast (Puratos, Belgium), sugar (Siúcra, Ireland) and salt (Glacia British Salt Limited, UK).

# 7.3.2 Strains and growth conditions

*Weissella cibaria* MG1 and *Lactobacillus plantarum* FST1.7 were obtained from the culture collection of the cereal science laboratory in University College Cork. Strains were routinely maintained on modified deMan-Rogosa-Sharpe agar (mMRS5), supplemented with vitamins and bromocresol green (Meroth et al. 2003), and incubated anaerobically at 30°C for 48 h. For the preparation of working cultures, single colonies were picked from the agar plates, cultured in mMRS5 broth at 30°C for 12h, and sub-cultured for 24h.

# 7.3.3 Sourdough production

Sourdoughs were prepared from each gluten-free flour as described by Galle et al. (2010). Briefly, cells were harvested by centrifugation (2300 x g, 10 min, 4°C), washed and re-suspended in sterile tap water, and added to the sourdough to an initial cell count of 10<sup>8</sup> CFU/ g dough. Sourdoughs were prepared with an equal weight of flour and water (dough yield 200) and were fermented in triplicates for 24 h at 30°C.

# 7.3.4 Bread production

Breads were produced as previously described by Hager et al. (2012b) using 100% flour, 2% salt, 2% sugar and 3% dry-yeast (based on flour, BF). Water addition levels (WL) for gluten-free flours were used as specified by Hager et al. (2012b) (buckwheat: 85% BF; quinoa, sorghum and teff bread: 95% BF) and wheat flour (63% BF). Bulk fermentation of wheat dough was carried out for 15 min at 30°C and 85% relative humidity (RH). Gluten-free bread batters and wheat dough were proofed in tins for 30 and 75 min, respectively (30°C, 85% RH) and baked in a deck-oven (gluten-free breads for 45 min at 190°C; wheat bread for 30 min at 220°C top and 235°C bottom heat). Breads were cooled for

two hours at room temperature and frozen (-18°C) until analysis. Three batch replicas were prepared.

## 7.3.5 pH, total titratable acids and organic acids

The pH and total titratable acids (TTA) of bread crumb was determined was determined suspending 10 g sample in 90 ml distilled water. TTA were determined by adjusting to pH 8.5 titrating with 0.1 N sodium hydroxide solution and re-adjusting after 3 min (Arbeitsgemeinschaft Getreideforschung e.V. 1994). For determination of lactate and acetate from two fermentations, proteins were precipitated with 7% perchloric acid in fresh sourdough samples (1:2 w/v) overnight (15h, 4°C). After centrifugation (2000 x *g*, 20 min) and filtration (0.450  $\mu$ m), concentrations of lactate and acetate were quantified using an Agilent 1200 HPLC system coupled to a refractive index detector and a REZEX 8 $\mu$  Organic Acid Column (Phenomenex, USA). Samples were eluted with 0.01N H<sub>2</sub>SO<sub>4</sub> at 65°C and a flow of 0.6 mL/ min.

## 7.3.6 Resistant starch

Amount of resistant starch (as proportions of total starch) "as is basis" in control and sourdough breads was determined spectrophotometrically ( $\lambda = 510 \text{ nm}$ ) as glucose equivalents using the AACC method 32-40.01 (K-RSTAR enzyme kit, Megazyme, Bray, Ireland). This *in vitro* method mainly recovers retrograded amylose fraction (Goñi et al. 1996).

## 7.3.7 Total available carbohydrates

Amounts of total available carbohydrates (TAC, mg/ 4 g fresh bread) were determined spectrophotometrically ( $\lambda$ =510 nm) in freeze-dried, ground samples of gluten-free and wheat bread crumbs according to AACC Method 76-13.01 as the sum of free available and starch derived sugars using a total starch assay kit (K-TSTA, Megazyme, Bray, Ireland).

## 7.3.8 Reducing sugars released and *in vitro* starch digestibility

*In vitro* starch digestibility was analyzed mimicking hydrolysis reactions in the human intestine as previously described by Brennan and Tudorica (2008). Tubings, chemicals and enzymes were obtained from Sigma Aldrich, Arklow. Briefly, triplicate samples ( $4 \pm 0.001g$ ) of homogenized gluten-free and wheat sourdough bread crumb "as eaten" were incubated for 30 min at 37°C with 5 ml

pepsin solution (EC 3.4.23.1; porcine gastric mucosa, 115 U/ mL in water). Followed by pH adjustment (pH 6.9) and addition of 7 ml  $\alpha$ -amylase solution (EC 3.2.1.1; porcine pancreatic; 16 U/ mL in Tris·HCl buffer). Samples together with glass beads were transferred into dialysis tubings (cut-off size 10-11 kDa) and placed into a beaker containing potassium phosphate buffer. Samples together with glass beads were transferred into dialysis tubing (cut off size 10-11 kDa) and dialyzed for four hours at 37°C in potassium phosphate buffer (pH 6.9). Dialysis tubings were inverted every 15 minutes and an aliquot of dialysate was taken every 30 min for quantification of reducing sugars and replaced with the same amount of fresh buffer. Amounts of reducing sugars in the dialysate were determined spectrophotometrically ( $\lambda$ =540 nm) after reaction with 3.5dinitrosalicylic acid reagent (DNS) (2M sodium hydroxide, 0.04 M 3,5dinitrosalicylic acid, 1.1 M potassium sodium tartrate in distilled water) (Miller 1959). A maltose standard (1 g/ L) was used for the calculation. Amounts of reducing sugars released, RSR (%), were calculated as maltose equivalents (ME, in g) as percentage of the total available carbohydrates (TAC) in 4 g sample following Equation 1.

$$RSR = \frac{A_{sample} \times 500 \times 0.95}{A_{maltose \ standard} \times TAC} \times 100$$
 Equation 1

A<sub>sample</sub> is sample absorbance, A<sub>maltose standard</sub> is absorbance of maltose standard, TAC is total available carbohydrates (mg in 4g sample), 500 is total volume (mL) in dialysis beaker and 0.95 is conversion factor from maltose to starch. The amount of RSR (g/ 100g TAC) was plotted against the digestion time (min) and the area under the hydrolysis curve (AUC, g/ 100g TAC\*min) was calculated geometrically for 180 min using the trapezoidal method described by Wolever and Jenkins (1986). The hydrolysis index (HI) was calculated from AUC of gluten-free samples as a percentage of the corresponding area of the reference (white wheat bread) (HI = AUC<sub>sample</sub>/ AUC<sub>wheat bread</sub>\*100).

#### 7.3.9 Predicted glycaemic index and glycaemic load

The predicted GI (pGI) was calculated with the equation pGI = 0.549\*HI+39.71 used by Capriles et al. (2013) for gluten-free bread, deduced from Goni et al. (1997). Wheat bread was the reference food ( $pGI_{wheat bread} = 100$ ). The predicted glycaemic index in relation to glucose as standard food ( $pGI_{glucose}=100$ ) was

calculated from the obtained pGI<sub>wheat bread</sub> values by multiplication with the factor 0.7 (Wolever et al. 2008). The predicted glycaemic load (pGL) was calculated for a 50 g bread portion from the glucose related GI according to  $pGL = [pGI_{glucose=100} \cdot TAC)/(100)$  taking into account the total available carbohydrates of each sample (Atkinson et al. 2008).

#### 7.3.10 Statistical analyses

Results are reported as averages with confidence interval. Statistical analyses were performed with SigmaPlot (Systat Software Inc.; version 11, UK) on all data using one-way ANOVA. Holm-Sidak Test was used to describe means at 5% significance level.

#### 7.4 Results

## 7.4.1 pH, total titratable acids and organic acids

In order to investigate acidification properties for control and both sourdough type breads, the pH and total titratable acids (TTA) were measured. Addition of sourdoughs from *W. cibaria* MG1 (Wc) or *L. plantarum* FST1.7 (Lp) to the bread formulation resulted in significantly lower pH values and significantly higher TTA in comparison to the control breads (Table 7-1). pH values for breads containing Lp sourdoughs were significantly lower than for Wc sourdoughs. With the exception of quinoa and wheat sourdough bread (no significance), the addition of Lp resulted in significantly higher TTA in comparison to Wc containing sourdough breads (P<0.001). The amounts of lactic and acetic acid were determined in the sourdoughs and flours using HPLC, and are given as g acid per 100 g bread dry weight basis (dwb) (Table 7-1). No organic acids were higher for Lp sourdoughs breads in comparison to Wc sourdough breads ranging. Amounts of acetic acid were ten times lower in both sourdoughs and therefore can be neglected in the sourdough breads.

## 7.4.2 Resistant starch

Amounts of resistant starch (RS) as percentage of total starch (%TS dwb) were determined in control and sourdough breads to estimate the influence of sourdough fermentation on formation of resistant starch and thereby on *in vitro* starch digestibility (Table 7-1). Highest amounts of RS were found in buckwheat and teff control breads. Only for buckwheat and teff breads, both sourdough incorporations led to significantly decreased RS levels. However, in sorghum Lp bread amount of RS was significantly increased.

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Parameter	Bread	Buckwheat	Quinoa	Sorghum	Teff	Wheat
hd	Control	$6.3\pm0.1^{k\#}$	$6.0\pm0^{m^*}$	$5.9 \pm 0^{n*}$	$6.1 \pm 0^{1*}$	$6.0\pm0^{\mathrm{m}*}$
	Wc	$5.8\pm0.1^{\circ}$	$5.6\pm0^{ m w}$	$5.6\pm0^{ m w}$	$5.5 \pm 0^{\mathrm{x}}$	$5.3\pm0^{\mathrm{y}}$
	Lp	$5.4 \pm 0.1^{\text{s}}$	$5.3 \pm 0.1^{\text{s}}$	$4.9\pm0^{ m v}$	$5.1 \pm 0^{\rm u}$	$5.2\pm0^{t}$
TTA (mL)	Control	$4.4\pm0.2^{ m n}$	$8.9\pm0.3^{k\ast}$	$4.0\pm0.5^{n^{*\#}}$	$4.3\pm0.2^{\mathrm{n}}$	$3.0 \pm 0.2^{m^*}$
	Wc	$5.0\pm0^{w\#}$	$9.8 \pm 1.2^{\circ}$	$3.3 \pm 0.3^{x\#}$	$4.2 \pm 0.4^{\mathrm{x}}$	$3.6\pm0.2^{\mathrm{x}}$
	Lp	$7.3\pm0.8^{t^*}$	$11.3 \pm 0.6^{\mathrm{s}}$	$4.5\pm0.5^{u^*}$	$6.5\pm1.3^{\rm th}$	$3.9\pm0.1^{\circ}$
Lactic acid (g/ 100g) <sup>1</sup>	Wc	$0.38\pm0.08$	$0.35\pm0.06$	$0.21\pm0.03$	$0.27\pm0.05$	$0.22 \pm 0.0$
	Lp	$0.58\pm0.02$	$0.73 \pm 0.15$	$0.46\pm0.12$	$0.67\pm0.13$	$0.27 \pm 0.0$
Acetic acid (g/ 100g) <sup>1</sup>	Wc	$0.03 \pm 0$	$0.04\pm0.01$	$0.02 \pm 0$	$0.03\pm0.01$	$0.01 \pm 0$
	Lp	$0.01 \pm 0$	$0.04 \pm 0$	$0.02 \pm 0$	$0.04\pm0$	$0.01 \pm 0$
Resistant starch (%TS dwb)	Control	$2.01 \pm 0.43^{a*}$	$0.96\pm0.05^{\mathrm{b}}$	$0.62\pm0.13^{\circ}$	$1.92\pm0.17^{a^*}$	$0.75 \pm 0.03$
	Wc	$1.28\pm0.09^{\rm m}$	$1.09\pm0.06^{\rm n}$	$0.67\pm0.10^{p}$	$0.87\pm0.15^{\rm o}$	$0.78 \pm 0.16$
	Lp	$1.44 \pm 0.16^{\mathrm{s}}$	$0.93\pm0.18^{t}$	$0.87\pm0.01^{u\ast}$	$0.98\pm0.05^{\rm t}$	$0.79 \pm 0^{v}$

# 7.4.3 Total available carbohydrates

The amount of total available carbohydrates (TAC) per four gram bread "as is" basis was determined enzymatically as sum of starch derived and free available sugars. Significantly highest values were found in sorghum control and both sourdough breads, followed by wheat control and sourdough breads. Lowest amounts of TAC were determined in quinoa sourdough breads (Table 7-2).

## 7.4.4 Reducing sugars released and *in vitro* starch digestibility

The *in vitro* starch hydrolysis was evaluated by plotting the amount of reducing sugars released into the dialysate (% RSR) versus the dialysis time (in min). The area under the curve (AUC) was calculated for each sample (Table 7-2) from the corresponding hydrolysis curves. Highest amounts of RSR and therefore highest AUC were found in quinoa sourdough breads. Releasing only half as much reducing sugars, AUC for sorghum and teff Lp breads twice as low. The AUC was significantly increased after Wc and Lp sourdough breads in comparison to the control. The addition of Lp sourdough decreased the AUC in sorghum and teff sourdough breads significantly. Likewise, for wheat Wc and Lp sourdough breads AUC was decreased significantly in comparison to the control.

	Sample	Buckwheat	Quinoa	Sorghum	Teff	Wheat
TAC (mg/ 4g bread) (	Ctrl	1171 ± 17 <sup>d#</sup>	1033 ± 13⁰	$1634 \pm 17^{a^*}$	1296 ± 13°	$1543 \pm 12^{b}$
4	Wc	$1342 \pm 44^{0*}$	959 ± 1 <sup>p#</sup>	$1674 \pm 12^{m\#}$	$1308 \pm 26^{\circ}$	$1501 \pm 13^{n\#}$
Π	<b>cp</b>	1285 ± 7 <sup>v</sup>	$1007 \pm 9^{w}$	$1653 \pm 9^{s#*}$	1336 ± 7 <sup>u*</sup>	$1541 \pm 3^{t}$
AUC (%RSR*min) <sup>1</sup> (	Ctrl	2377 ± 76 <sup>b#</sup>	3260 ± 119ª#	$1934 \pm 63^{bc#}$	$2026 \pm 64^{bc#}$	3266 ± 114
4	Wc	$2945 \pm 186^{n}$	3952 ± 190 <sup>m</sup>	$2480 \pm 62^{n^*}$	$2634 \pm 126^{n*}$	2676 ± 100 <sup>n</sup>
Π	<b>cp</b>	$2729 \pm 140^{t}$	3792 ± 127 <sup>s</sup>	$1758 \pm 42^{\circ}$	$1682 \pm 34^{v}$	2132 ± 71 <sup>u</sup>
HI (	Ctrl	73 ± 2 <sup>b#</sup>	$100 \pm 4^{a\#}$	59 ± 2°	62 ± 2 <sup>c*</sup>	
2	Wc	$90 \pm 6^n$	121 ± 6 <sup>m</sup>	76±2°#	$81 \pm 4^{n#}$	82 ± 3 <sup>n#</sup>
Π	<b>d</b> J	$84 \pm 4^{t}$	$116 \pm 4^{s}$	$54 \pm 1^{\vee}$	$51 \pm 1^{w}$	65 ± 1 <sup>u</sup>
pGI wheat bread	Ctrl	$80 \pm 1^{c^*}$	95 ± 2 <sup>b#</sup>	$72 \pm 1^{d}$	$74 \pm 1^{d}$	100 <sup>a#</sup>
2	Wc	89 ± 3 <sup>n</sup>	$106 \pm 3^{m}$	$81 \pm 1^{0\#}$	84 ± 2 <sup>n#</sup>	$85 \pm 2^{n^*}$
Π	<b>d</b> J	$86 \pm 2^{t}$	$103 \pm 2^{s}$	$69 \pm 1^{v*}$	$68 \pm 1^{v*}$	76±1 <sup>u</sup>
pGL <sup>2</sup> C	Ctrl	8d	9c	<b>10</b> <sup>b</sup>	8d	13ª#
4	Wc	<b>11</b> n#	90	<b>12</b> <sup>m#</sup>	9 <sup>0#</sup>	$11^{n*}$
Π	<b>d</b> J	$10^{s*}$	9t	$10^{\mathrm{s}}$	8 <sup>u</sup>	$10^{\rm s}$

Table 7-2 Values for total available carbohydrates (TAC), area under curve (AUC), hydrolysis index (HI), predicted

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## 7.4.5 Predicted glycaemic index and glycaemic load

Values for predicted glycaemic indices (pGIs) were calculated using the equation as presented by Capriles et al. (2013) related to white wheat bread (Gl<sub>wheat bread</sub> =100) (Table 7-2). The addition of Wc sourdough led to significantly increased pGI in all gluten-free breads. Equally, incorporation of Lp sourdough led to significantly higher pGIs for buckwheat and quinoa sourdough breads in comparison to their control breads. Conversely, pGIs for Lp sourdoughcontaining sorghum and teff breads were reduced significantly compared to their controls and also in comparison to Wc breads. Sorghum and teff Lp breads hence fall into the intermediate category (GI 58-70). The pGIs of all other sourdough breads belonged to the high GI category (GI>70). Both sourdoughs reduced the pGI of white wheat bread significantly (Wc by 15 units and Lp by 24). Considering the total available carbohydrates of a 50 g portion of fresh bread, the predicted glycaemic load (pGL) was calculated multiplying the glucose related GI (data not shown) with TAC for each sourdough bread. With the exception of quinoa sourdough breads, pGL values for Lp breads were significantly lower than for Wc breads. Highest GL was found in buckwheat, sorghum and wheat Wc bread due to a high pGI (buckwheat and wheat bread) in combination with high TAC (sorghum bread). Therefore, although the pGI for teff Lp bread was similar to the GI for sorghum Lp bread, the pGL for teff Lp bread was lower due to significantly lower TAC in teff in comparison to sorghum Lp bread. Although quinoa Lp bread had the highest pGI due to lowest TAC the pGL is comparable to teff.

#### 7.5 Discussion

The rate of enzymatic starch digestion was analyzed *in vitro* to predict the glycaemic index for non-sourdough containing and sourdough-containing breads made from buckwheat, quinoa, sorghum, teff and wheat flour. Berti et al. (2004) evaluated starch digestibility using a similar *in vitro* dialysis system and concluded that glycaemic responses for gluten-free foods tend to be higher than for gluten-containing products.

The reduced *in vitro* starch hydrolysis and predicted GI upon sourdough addition in wheat bread are in accordance with previous *in vitro* results for sourdough-containing wheat bread (Liljeberg et al. 1995; De Angelis et al. 2007; De Angelis et al. 2009). Frequently, low GI foods contained higher amounts of resistant starch (RS) (Liljeberg and Björck 1994; Björck et al. 2000; De Angelis et al. 2007; Scazzina et al. 2009) which was related to a decreased *in vitro* starch hydrolysis for sourdough breads (RS content 3.4-5.0%) in comparison to a control white wheat bread (RS 1.4%) (De Angelis et al. 2007). Amounts of resistant starch in our study were below 2% of total starch (TS) in gluten-free and below 1% TS in wheat sourdough breads confirming amounts found in literature for processed rice and finger millet (Mangala et al. 1999) and for common wheat breads (Holm and Björck 1992; Akerberg et al. 1998).

However, decreased hydrolysis indices (HI) for wheat sourdough breads did not correlate with resistant starch levels in our study. Previously, the formation of resistant starch was positively linked with content of lactic acid in sourdough and amylose contents in flour (Liljeberg et al. 1996). However, lowest amounts of amylose in quinoa flour (5.3% dwb) (Hager et al. 2012a) and highest acidity in quinoa sourdough breads did not correlated with resistant starch levels. The only significant increase of resistant starch was found in sorghum sourdough bread fermented with *Lactobacillus plantarum* (Lp) possibly explaining the significantly decreased HI of sorghum Lp bread. Significantly decreased amounts of resistant starch in buckwheat and teff breads upon sourdough fermentation with both strains correlated with increased HI in buckwheat sourdough breads and teff Wc bread but not with HI in teff Lp bread (significantly decreased). Overall, *in vitro* starch digestibility did not correlate with amounts of resistant starch in our study (Wc R<sup>2</sup> = 0.583 and Lp R<sup>2</sup> = 0.239).

For samples containing  $\leq 1\%$  RS, amounts can be considered negligible and without nutritional implications (Goñi et al. 1996).

Previously, amounts of organic acids and especially lactic acid have been related to decreased glycaemic response *in vivo* due to reduced gastric emptying (Scazzina et al. 2009). In agreement, a dose-dependent effect of organic acids on *in vitro* starch hydrolysis index for wheat sourdough bread was found (De Angelis et al. 2007; De Angelis et al. 2009). In our study, within one type of sourdough no correlations between amounts of lactic acids in sourdough breads and the *in vitro* hydrolysis were found (Wc R<sup>2</sup>=0.459 and Lp R<sup>2</sup>=0.233). However, comparing the two sourdough strains, hydrolysis indices correlated negatively with lactic acid produced.

Sourdough with a commercial starter added to a gluten-free formulation decreased the glycaemic response *in vivo*, but was less effective in gluten-free than in wheat sourdough bread (Novotni et al. 2012). This however, was explained with lower concentrations of organic acids in gluten-free than in wheat sourdough. Decreased *in vitro* HI for sourdough containing wheat breads was related to pH decrease by formation of organic acids and thereby to inhibition of  $\alpha$ -amylase during the hydrolysis (Liljeberg et al. 1995; De Angelis et al. 2007; De Angelis et al. 2009). Conversely, in our study amounts of organic acids were higher in gluten-free sourdough breads than in wheat bread due to higher buffering capacity and higher acidification in gluten-free sourdoughs. Therefore, the correlation between organic acids and HI does not apply for gluten-free sourdough breads in our study.

The presence of lactic acid during heat treatment lowered the predicted GI, but only in the presence of gluten (Östman et al. 2002) indicating that the creation of a barrier caused by heat-treatment in the presence of lactic acid leads to reduced starch availability in wheat sourdough bread (Björck et al. 2000). This could explain why the presence of lactic acid in gluten-free sourdoughs did not lead to decreased HI.

On the contrary, the presence of organic acids in gluten-free sourdoughs increased the *in vitro* HI in comparison to non-acidified controls in our study. As previously shown, the buffering capacity is higher in gluten-free than in wheat flour (Wolter et al. 2014). This is indicated by smaller amounts of TTA and a stronger pH decrease upon sourdough addition in wheat than in gluten-free

sourdough breads. Within gluten-free sourdoughs the buffering capacity of sourdoughs strongly correlated with hydrolysis indices ( $R^2$  in Wc =0.898 and Lp= 0.700). The pH in gluten-free sourdoughs might still be sufficient for  $\alpha$ -amylase to proceeds with degradation of starch and formation of monosaccharides and thus increase the hydrolysis index.

This might explain why HIs of gluten-free breads after sourdough addition are higher than for their controls. The significantly higher HI of quinoa breads can also be explained with higher amylopectin proportion in quinoa than in the other gluten-free flours. Amylopectin is more accessible to  $\alpha$ -amylase than amylose due to its branched structure (Fardet et al. 2006).

Finally, food structure might have an impact on starch hydrolysis (Fardet et al. 2006; Hager et al. 2013). Sourdough fermentation results in dough softening (Wolter et al. 2014) and gives increased cell volume and hence higher crumb porosity in sourdoughs. This renders starch which is more accessible during the digestive process than in non-acidified control breads and increases the rate of starch hydrolysis.

In general, results in literature are ambivalent regarding the influence of sourdoughs on hydrolysis indices and glycaemic indices and little research has been done on the impact of sourdough on gluten-free breads. Therefore, it is difficult to consider the influence of factors in gluten-free sourdoughs. Decreased glycaemic indices can be obtained by *Lactobacillus plantarum* FST1.7 sourdough addition to sorghum, teff and wheat bread. However, this was not the case when *Weissella cibaria* MG1 sourdough was added to the bread recipe. Results suggest that the increase of hydrolysis indices in gluten-free breads were related to mechanism other than presence of organic acids and formation of resistant starch. Our results contribute to the availability of information on predicted glycaemic indices of gluten-free breads upon addition of sourdough.

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# Chapter 8 General Discussion

#### 8.1 Sourdough performance of Weissella cibaria in gluten-free flours

The addition of sourdough has improved flavour, texture, shelf life and nutritional properties (Gänzle et al. 2007) of conventional wheat bread due to the synthesis of aroma compounds (Czerny and Schieberle 2002; Hansen and Schieberle 2005) and antifungal compounds during bacterial fermentation (Ryan et al. 2008; Poutanen et al. 2009). Therefore the application of sourdough is a suitable solution to improve some of the qualitative problems experienced in gluten-free breads (Dal Bello et al. 2007; Schober et al. 2007; Wolter et al. 2014a).

In particular exopolysaccharide-producing lactic acid bacteria have gained interest. Exopolysaccharides (EPS) can potentially act as hydrocolloids (Schwab et al. 2008), hence improving gluten-free bread quality (Schwab et al. 2008; Galle et al. 2012; Ruehmkorf et al. 2012b). Depending on the monosaccharides present, EPS can be divided into homopolysaccharides (HoPS), (one type; glucose or fructose), and into heteropolysaccharides (3-8 multiple, repeated moieties) (De Vuyst et al. 2001; van Hijum et al. 2006).

HoPS-producing lactic acid bacteria are used in conventional bread making and have improved texture and dough rheology (Decock and Cappelle 2005; Lacaze et al. 2007). *Weissella cibaria* MG1 has been described by Galle et al. (2010; 2012) as a suitable starter to improve bread quality. This has been associated with the potential to synthesise substantial amounts of the HoPS dextran from sucrose with concomitant low acetate production in wheat and sorghum sourdoughs. However, the ability to form dextran during fermentation of other gluten-free flours has not been investigated yet.

Therefore, the first part of this thesis assessed the suitability of gluten-free flours (buckwheat, oat, quinoa and teff) to serve as substrate for fermentation with *Weissella cibaria* MG1. *W. cibaria* produced high amounts of lactate and higher amounts of total titratable acids (TTA) in buckwheat (TTA 16.8 ml), quinoa (26.4 ml) and teff sourdoughs (16.2 ml) compared to wheat sourdough (8.2 ml).

This observation relates to the higher buffering capacity of gluten-free flours in comparison to wheat flour due their higher mineral contents. An increased buffering capacity of sourdough does not alter the final pH, but enables production of higher amounts of lactic acid (Gänzle et al. 1998).

The mineral content in oat flour (5164 mg/kg flour dwb) was lower than in all other flours. Therefore, TTA and amounts of lactate and acetate were lowest in oat sourdough fermented with *Weissella cibaria* MG1 in comparison to other gluten-free sourdoughs. The strain failed to grow during oat fermentation, likely due to the low concentration of fermentable sugars and the low buffering capacity of the flour. Therefore, oat flour was excluded from following trials.

Dextran with a molecular weight of  $5x10^6 - 4x10^7$  Da was produced with up to 4.2 g/kg and 3.2 g/kg in sucrose-supplemented buckwheat and quinoa sourdoughs. The dextran conversion rate from sucrose (g dextran : g sucrose) was 1:12 for buckwheat and 1:16 for quinoa sourdough. In contrast, prior observations with the same strain found higher conversion rate of 1:9 in sorghum sourdough.

The EPS yield in sourdough fermentations is determined by the fermentation conditions, properties of the EPS producing strain, the substrate and the amount of sucrose added (Kaditzky and Vogel 2008; Ruehmkorf et al. 2012a). Therefore, the lower conversion rate can be attributed to lower metabolic activity due to a higher inoculum size and lower amount of sucrose supplementation in comparison to previous study (Galle et al. 2010; Galle et al. 2011).

Maltose acting as a strong acceptor carbohydrate for EPS-forming dextransucrases in the presence of sucrose (Monchois et al. 1999), supported the formation of panose-series oligosaccharides (POS) at the expense of dextran formation in accordance with previous fermentations (Schwab et al. 2008; Katina et al. 2009; Galle et al. 2010). Panose-series oligosaccharides do not influence dough rheology or bread texture but may have a prebiotic effect (Grimoud et al. 2010). Correspondingly, wheat sourdough with higher concentrations of maltose (127 mmol/kg flour) was characterized by occurrence of POS and low levels of dextran. Coinciding, lower maltose levels in buckwheat flour (80 mmol/kg) correlated with POS found in the sourdough. Higher maltose, fructose and glucose levels in quinoa flour (158; 80 and 203 mmol/kg) in comparison to buckwheat flour (80; 56 and 36 mmol/kg flour) indicate promoted POS production in quinoa sourdough with concurrent dextran formation.

Although, sugar levels in teff were higher (103; 75 and 56 mmol/ kg) than in buckwheat flour, dextran formation was still higher in buckwheat than in teff

sourdough. This indicates the strains optimal adaption to the microflora of buckwheat flour from which it was isolated (Moroni et al. 2011).

A decrease in the protein content in quinoa and teff *Weissella cibaria* MG1 sourdough was observed by capillary electrophoresis. The main proteins in quinoa, globulins and albumins, are more hydrophilic than wheat gluten (Stikic et al. 2012) and therefore, more susceptible to proteolysis (Lorenz and Nyanzi 1989). In addition, quinoa flour exhibited a high indigenous protease activity. However, the extensive proteolysis in quinoa sourdough did not influence dough rheology, indicating that other flour components are primarily responsible for the dough strength.

Sucrose-supplemented fermentation with Wc decreased dough strength in buckwheat, quinoa and wheat sourdough (>90%) in comparisons to a nonsucrose supplemented sourdough. The formation of organic acids and the resulting pH drop during fermentation not only activated indigenous proteolytic enzymes, but also imparted a net positive charge to proteins. Thus, intramolecular repulsion augmented causing proteins to unfold which then increase in solubility (Galal et al. 1978) consequently resulting in softer dough.

This study indicates that *Weissella cibaria* MG1 is a suitable starter culture for buckwheat, quinoa and teff fermentation. Depending on the substrate, substantial amounts of dextran and organic acids were produced which can serve to enhance texture, structure and sensory profile of gluten-free breads.

#### 8.2 Influence of *Weissella cibaria* sourdough on gluten-free bread quality

Consequently, the influence of dextran-producing *Weissella cibaria* MG1 sourdough (Wc) on bread and sensory properties of buckwheat, quinoa, sorghum and teff bread was studied (Chapter 4). The specific loaf volume is a crucial parameter determining bread quality (Maleki et al. 1980) and influencing consumer's acceptance. Only the incorporation of sourdough into wheat bread caused an increase in specific volume (+76%) and can be linked to a better gas holding capacity of gluten in the acidified dough (Gobbetti et al. 1995; Katina et al. 2006). However, specific volumes of gluten-free sourdough breads were not increased confirming that sourdough generally does not significantly influence specific volume of gluten-free breads (Moore et al. 2007; Alvarez-Jubete et al. 2010; Galle et al. 2012).

Similarly, *in situ* produced dextran did not improve the specific volume for sorghum sourdough bread (Galle et al. 2012). Controversially, external addition of a purified dextran of higher molecular size  $(8x10^7 - 24x10^7 \text{ Da})$  produced by *Lactobacillus animalis* increased specific volume of a buckwheat/rice flour based bread (Ruehmkorf et al. 2012b). Yet, this increase might be due to the absence of organic acids in the purified EPS, which if present in sourdough could counteract the positive effect of EPS (Katina 2005).

However, in accordance with previous study, significant crumb softening upon sourdough addition was observed for all breads except for sorghum sourdough bread in our study. The favourable reduction of crumb hardness of buckwheat, quinoa and teff sourdough bread can be partially related to *in situ* produce dextran by *Weissella cibaria* MG1. The crumb softening effect of EPS was previously linked to their ability to act as hydrocolloids interfering with the starch-protein-interactions (Galle et al. 2012). The application of sourdough decreased the staling rate in buckwheat (8 vs. 3 N/ day), teff (13 vs. 9 N/ day) and wheat sourdough breads (5 vs. 1 N/ day) significantly. The reduction of staling rate upon sourdough application in buckwheat and teff bread might be enhanced by interaction of dextran with starch and confirms reduced staling rate in dextran-containing gluten-free sourdough bread (Galle et al. 2012).

The staling rate in the wheat system is mainly determined by crumb firming caused by recrystallization, water redistribution between crumb and crust, and the gluten network (Maleki et al. 1980). Associated with dough softening assessed during rheology analysis (Chapter 3), crumb porosity was significantly increased in buckwheat, sorghum and teff sourdough breads due to facilitated gas expansion. This could also be attributed to the sucrose metabolism of *Weissella cibara* MG1 which yields monosaccharides stimulating yeast metabolism and gas formation (Gobbetti et al. 1995).

Investigations on the potential of lactic acid bacteria to influence aroma profiles of breads have demonstrated that specific strains are able to generate individual aroma profiles and odorant compositions due to their metabolic properties (Czerny et al. 2005). The addition of buckwheat sourdough influenced the preference of buckwheat bread positively most probably due to the hazelnut, cooked-potato- and sourdough-like note. However, the opposite was observed for sorghum, quinoa and teff sourdough breads, which were evaluated as inferior in comparison to their control breads. This observation is most likely the result of newly generated aroma attributes or the lack to reduce negative aroma attributes during sourdough fermentation.

It can be assumed that either the level of sourdough addition was not sufficient to influence the overall aroma positively or the microorganism was not able to eliminate the odorants causing negative bread aroma. The microbial shelf life of gluten-free and wheat bread was not prolonged by application of *Weissella cibaria* MG1 sourdough. However, the rate of mould growth on buckwheat and quinoa sourdough breads was delayed possibly due to higher amounts of total titratable acids in the crumb compared to the control breads.

This study showed that dextran production and acidification did not generally lead to an improved bread quality, but that the effect depended on the flour matrix used.

# 8.3 Influence of *Lactobacillus plantarum* sourdough on gluten-free bread quality

The suitability of *Lactobacillus plantarum* FST1.7 to serve as sourdough starter for buckwheat, oat, quinoa, sorghum and teff flour and to influence dough rheology, bread texture, crumb structure, staling rate, sensory properties and microbial shelf life of gluten-free and wheat breads was investigated in Chapter 5. The facultative heterofermentative lactic acid bacterium *Lactobacillus plantarum* FST1.7 (Lp) was chosen as a strong acidifying strain and due to dominance in wheat (Gänzle et al. 2007) and gluten-free sourdoughs (Vogelmann et al. 2009). Due to the preferential use of glucose and fructose for fermentation (Gobbetti 1998; Siezen and Vlieg 2011), acidification performance of Lp was higher than for *Weissella cibaria* (Chapter 4).

Organic acid production and proteolysis during sourdough fermentation also induced dough softening of Lp sourdough samples. This is generally in accordance with dough softening found for wheat sourdough bread (Angioloni et al. 2006) as well as amaranth sourdough fermented with *L. plantarum* (Houben et al. 2010). Similar as for application of *Weissella cibaria* (Chapter 4), Lp sourdough significantly increased crumb porosity in all gluten-free and wheat sourdough breads. However, the specific volume remained unaffected in comparison to non-acidified control breads. In accordance with results on a mixed gluten-free formulation (Moore et al. 2007), crumb hardness on the day of baking of gluten-free breads was not influenced by Lp. A trend of delayed staling after five days of storage upon sourdough addition was visible for buckwheat (8 vs. 6 N/ day) and teff breads (13 vs. 10 N/ day). On the contrary, in wheat sourdough bread the staling rate was reduced significantly  $(2 \pm 1 \text{ N/ day})$  in comparison to the control bread  $(5 \pm 1 \text{ N/ day})$ . This confirms that addition of Lp sourdough delays staling also in comparison to non-acidified breads and not only compared to chemically acidified bread (Dal Bello et al. 2007; Moore et al. 2007).

Similar as previously found for *Weissella cibaria* MG1 (Chapter 4), microbial shelf life was not prolonged for gluten-free breads containing Lp sourdough. The previously discussed antifungal activity of *Lactobacillus plantarum* FST1.7 in wheat sourdough breads (Dal Bello et al. 2007) was not sufficient to increase microbial shelf life in our study. In accordance with Moore et al. (2007), organic acid production in Lp did not suffice to prolong the shelf life for gluten-free sourdough breads in comparison to non-acidified control. Consistently, amounts of glucose and fructose increased after fermentation, indicating that mould growth might be enhanced by supplementation of fermentable substrates. The aroma quality of the gluten-free sourdough breads was very low and the addition of Lp sourdough did not improve undesirable aroma notes responsible for the low odour quality in comparison to control breads.

## 8.4 *In vitro* starch digestibility of gluten-free control breads

In Chapter 6, the *in vitro* starch digestibility was analysed with a multi-enzyme dialysis system to predict glycaemic indices of gluten-free breads. High incidences of type I (insulin-dependent) diabetes mellitus in coeliac disease patients have been reported (Cronin and Shanahan 1997). Hence, the maintenance of a good glycaemic control is an important task for coeliac disease patients (Berti et al. 2004). The GI is defined as the area under the blood glucose curve upon ingestion of carbohydrate-containing food relative to a reference food (white wheat bread or glucose) (Jenkins et al. 1981). The glycaemic response has been related to the rate of digestion and absorption of carbohydrate-containing foods with help of *in vitro* methods.

These methods mimic *in vivo* digestion processes and present an indication for glycaemic response. In general, the high GI of bread is due to the fact that starch gelatinisation during baking process makes the starch granules more susceptible to  $\alpha$ -amylase attack (Fardet et al. 2006). Gluten-free breads showed significantly lower hydrolysis indices and predicted glycaemic indices (pGI) than the reference food (white wheat bread=100) but still fell into the "high" category (GI>70). This is in accordance with high GIs for gluten-free breads (Capriles and Areas 2013) (Matos Segura and Rosell 2011) (Berti et al. 2004). Predicted GIs for sorghum (72) and teff breads (74) were significantly lower than for quinoa (95), wheat (100) and buckwheat breads (80). Significantly higher gelatinisation temperatures as assessed with differential scanning calorimetry for sorghum (64-73°C) and teff (66-77°C) in comparison to buckwheat (59-72°C), oat (51-62°C) and wheat flour (55-66°C) might therefore impede starch gelatinisation and enzymatic susceptibility.

The size of starch granules in the flours was assessed with scanning electron microscopy. Smaller average granule diameters in quinoa (1.3  $\mu$ m) and buckwheat flour (5  $\mu$ m) in comparison to oat (6  $\mu$ m) and sorghum flour (10  $\mu$ m) resulted in higher specific surface area of starch granules. The rate of starch digestibility is increased and enzymatic attack is facilitated for smaller starch granules, since their specific surface area is larger (Tester et al. 2004). This explains the higher pGI of quinoa bread in comparison to oat and sorghum but not the similar GI in comparison to wheat bread. Consequently, starch granule size alone is not sufficient to explain the different GIs.

Also, presence of damaged starch increases enzymatic susceptibility (Tester et al. 2004). Wheat flour contained highest amounts of damaged starch in comparison to other gluten-free flours (Hager et al. 2012b), explaining higher pGI for gluten-free breads. Quinoa flour contained higher amounts of damaged starch than buckwheat and teff flour. This, in addition with higher starch surface area, can therefore serve to explain higher pGI of quinoa bread in comparison to teff and buckwheat bread.

Furthermore, amylopectin is more accessible to  $\alpha$ -amylase than amylose due to its branched structure (Fardet et al. 2006). Therefore, the higher amylopectin proportion in quinoa flour (Hager et al. 2012b) could be associated with higher pGI of quinoa bread. However, flour is a complex matrix and other flour components such as lipids impede starch hydrolysis due to formation of amylose-lipid complexes decreasing susceptibility (Singh et al. 2010). Higher lipid contents in oat, buckwheat, teff and sorghum flour together with higher amylose contents of these starches (Hager et al. 2012a) could therefore result in the formation of amylose-lipid complexes hindering the enzymatic access. This formation of complexed amylose might be impeded in quinoa and wheat bread, because of lower amylose content in quinoa starch on one hand and lower fat content in wheat flour (Hager et al. 2012a) on the other hand.

#### 8.5 Influence of sourdough on *in vitro* starch digestibility

Eventually, the influence of sourdough acidification on the rate of enzymatic starch hydrolysis was analysed for buckwheat, quinoa, sorghum, teff and wheat breads (Chapter 7). Sourdough can serve to improve the nutritional quality of conventional bread by decreasing starch hydrolysis linked with reduced glycaemic response (Liljeberg et al. 1995). Sourdough or lactic acid addition might enhance retrogradation of starch (Liljeberg et al. 1996) through debranching of amylopectin moieties during baking (Brighenti et al. 1998) and thereby increase formation of resistant starch (RS) (Scazzina et al. 2009).

Previously, blood glycaemia for a high-RS wheat bread was significantly reduced compared to low-RS control bread (Scazzina et al. 2009). For glutenfree bread, a reduced glycaemic response was also found upon sourdough addition which was linked to the presence of organic acids. In wheat bread, the inclusion of lactic acid prior to heat treatment reduced the rate of starch digestion by promoting gluten-starch interactions during starch gelatinisation (Östman et al. 2002).

In general, literature results are ambivalent regarding the influence of sourdoughs on hydrolysis and glycaemic indices and little research has been done on the impact of sourdough on gluten-free breads. Therefore, the influence of two lactic acid bacteria, *Weissella cibaria* (Wc) (weak acidifier) and *Lactobacillus plantarum* (Lp) (strong acidifier) on *in vitro* starch digestibility and resistant starch contents was investigated.

*In vitro* starch hydrolysis and predicted GI were reduced upon sourdough addition in wheat bread (Wc 85; Lp 76). This is in accordance with previous *in vitro* results for sourdough-containing wheat bread (Liljeberg et al. 1995; De

Angelis et al. 2007; De Angelis et al. 2009). However, for gluten-free breads predicted GIs were higher for both sourdough-containing counterparts. The only exception was a significantly decreased pGI for sorghum (69) and teff (68) breads upon Lp sourdough addition. However, the content of resistant starch was not increased by the addition of any sourdough and did not correlate with *in vitro* starch hydrolysis (Wc R<sup>2</sup> = 0.583 and Lp R<sup>2</sup> = 0.239).

Only sorghum Lp bread contained significantly higher resistant starch levels (0.87% total starch) than the control (0.62%) and Wc bread (0.67%). This might explain the significantly decreased HI and reduced pGI in sorghum Lp bread. No correlations between amounts of lactic acids in sourdough breads and the HI were found within one type of sourdough (Wc  $R^2 = 0.459$  and Lp  $R^2 = 0.233$ ).

However, comparing the two sourdough strains, HI correlated negatively with lactic acid produced. Decreased *in vitro* HI for sourdough-containing wheat breads was related to pH decrease by formation of organic acids and thereby to inhibition of  $\alpha$ -amylase during the hydrolysis (Liljeberg et al. 1995; De Angelis et al. 2007; De Angelis et al. 2009).

In our study amounts of organic acids were higher in gluten-free sourdough breads than in wheat bread due to higher buffering capacity and higher acidification in gluten-free sourdoughs (Chapter 3 and 5). Therefore, the correlation between higher organic acids and lower HI does not apply for gluten-free sourdough breads in our study. The presence of lactic acid during heat treatment lowers the predicted GI of starch, but only in the presence of gluten (Östman et al. 2002) indicating that the creation of a barrier caused by heat-treatment in the presence of lactic acid leads to reduced starch availability in wheat sourdough bread (Björck et al. 2000). Hence, the presence of lactic acid in gluten-free sourdoughs might not lead to decreased HI in our study. On the contrary, higher amounts of organic acids in gluten-free sourdoughs were associated with increased HI in comparison to non-acidified controls. Possibly, the pH in gluten-free sourdoughs was still sufficient for  $\alpha$ -amylase to proceed with degradation of starch and formation of monosaccharides and thus increasing the HI.

Results suggest that the increase of HI in gluten-free breads were related to mechanism other than presence of organic acids and formation of resistant starch. For example, food structure might also have an impact on starch hydrolysis (Fardet et al. 2006; Hager et al. 2013). Sourdough fermentation results in dough softening (Wolter et al. 2014b), increased cell volume and hence higher crumb porosity in sourdoughs (Chapter 4 and 5). This renders starch which is more accessible during the digestive process than in non-acidified control breads and increases the rate of starch hydrolysis.

Concluding, the combination of microbiological investigations, analytical chemistry, and rheological, textural and microscopic measurements enlightened the factors which influence the suitability of two lactic acid bacteria strains for gluten-free fermentation and their impact on baking characteristics. The results of this PhD thesis show that the choice of flour has a considerable effect on the formation of EPS, oligosaccharides and organic acids, as well as on baking properties and crumb structure of a basic gluten-free bread formulation. In order to improve microbial shelf life and sensory characteristics, fermentation parameters might have to be adjusted and more potent lactic acid bacteria starters might have to be applied.

Furthermore, the role of starch properties, starch composition and flour components on the starch susceptibility in gluten-free breads was investigated. Sourdough acidification did not increase resistant starch levels at the chosen fermentation and baking parameters. Furthermore, the influence of sourdough addition on *in vitro* starch hydrolysis was not as distinctive in all gluten-free as in wheat sourdough breads. Our results contribute to the availability of information on predicted glycaemic indices of gluten-free breads in general and upon sourdough addition.

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# Appendix - Publications and

# presentations

#### Peer reviewed first author publications

In press

Anika Wolter; Anna-Sophie Hager; Emanuele Zannini; Sandra Galle; Michael Gänzle; Deborah M. Waters; Elke K. Arendt

# Evaluation of exopolysaccharide producing *Weissella cibaria* MG1 strain for the production of sourdough from various flours

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Anika Wolter, Anna-Sophie Hager, Emanuele Zannini, Michael Czerny, Elke K. Arendt

Influence of dextran-producing *Weissella cibaria* MG1 on baking properties and sensory profile of gluten-free and wheat breads

International Journal of Food Microbiology

# Published

Anika Wolter, Anna-Sophie Hager, Emanuele Zannini and Elke K. Arendt *In vitro* starch digestibility and predicted glycaemic indexes of buckwheat, oat, quinoa, sorghum, teff and commercial gluten-free bread *Journal of Cereal Sciences* 58, 431-436

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## Submitted

Anika Wolter, Anna-Sophie Hager, Emanuele Zannini, Michael Czerny, Elke K. Arendt

Impact of *Lactobacillus plantarum* FST1.7 as sourdough starter on baking properties of gluten-free breads

European Journal of Food Research and Technology

#### Second author and other publications

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

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#### Abstract

Bread is one of the major staple foods and is consumed daily in all parts of the world. However, a significant part of the human population cannot tolerate gluten, a protein composite found in wheat, rye and barley and therefore products made from alternative cereals such as oat, rice, maize, teff, sorghum, quinoa and buckwheat are required. In the course of this study the bread making potential of seven gluten free flours as well as wheat and wholemeal wheat flour was compared resulting in products of varying quality. Basic bread recipes were used, consisting simply of flour, water, sugar, salt and yeast. The fermentation potential of the different flours was determined with a rheofermentometer, showing that dough development height of gluten free and wholemeal wheat samples was significantly lower than for wheat and oat flour. Apart from standard bread quality parameters such as loaf specific volume and physical crumb texture, also water activity and shelf life of the final products have been determined. The results show that due to increased water activity in gluten free samples, their shelf life was reduced compared to wheat bread. With the exception of oat, gluten free breads had significantly lower volumes than wheat bread. Aroma profiles were evaluated by a trained panel, concluding that wheat, oat and wholemeal wheat breads were liked moderately, while the remaining samples had lower preference scores. Crumb grain characteristics were investigated using image analysis and microstructure was observed by means of scanning electron microscopy. Overall it can be concluded that only breads produced from oat flour were of similar quality to wheat bread and that the utilization of buckwheat, rice, maize, quinoa, sorghum and teff flours resulted in breads of inferior quality.

#### 1. Introduction

Bread is one of the major staple foods and is consumed daily in all parts of the world. Although a wide range of different types exist, the term "bread" usually refers to yeast leavened wheat products. Wheat (Triticum aestivum) is outstanding among cereals, because of its gluten protein fraction. This protein is responsible for the unique viscoelastic properties of wheat dough and hence for the exceptional bread making potential. However, this protein composite found in wheat, rye and barley triggers gluten sensitive enteropathy, i.e. coeliac disease. Consequently, there is a need for bread products made from alternative raw materials. Flours milled from oat (Avena sativa), rice (Oryza sativa), maize (Zea mays), teff (Eragrostis tef), sorghum (Sorghum bicolour) as well as the pseudocereals quinoa (Chenopodium quinoa) and buckwheat (Fagopyrum esculentum) can be included into a gluten free diet. An extensive review of the available literature showed that these flours have previously been used in baking. However, they were added to the recipe as a component in complex gluten free formulations or to replace a small proportion of wheat flour. During this study, breads were baked using 100% of the respective gluten free flour. Besides, this is the first publication directly comparing the bread making potential of such a high number of flours.

Rice flour is an economical ingredient widely used in gluten free baking. Its suitability for bread products is due to its white colour, bland taste and easy digestibility. Despite these advantages, rice proteins have poor functional properties (Rosell and Marco 2008). Therefore, many rice based gluten free formulations contain hydrocolloids: (Gujral et al. 2003; Kadan and Phillippy 2007; Nunes et al. 2009; Moore et al. 2004). Maize is a major cereal grain grown worldwide, ranking second only to wheat in total production area and second only to rice in total amount produced (Schober and Bean 2008). Limitations in

the use of maize flour for bread production are partly due to the distinctive colour and flavour. However, (Brites et al. 2010) successfully applied *broa* bread making technology (Portuguese ethnic bread) for the production of gluten free maize bread. Using different strains of lactic acid bacteria (Sanni et al. 1998) produced sour maize bread of varying quality. These authors reported a hard crumb but a shelf life of six days. Organoleptic qualities were within the acceptable limits expected for sourdough bread. (Flander et al. 2007) incorporated wholemeal oat flour into wheat breads and hence improved nutritional quality due to increased fibre and  $\beta$ -glucan content. Even though its status in a gluten free diet is controversial several publications exist on the use of oat flour for the production of gluten free bread. (Huettner et al. 2010) investigated the bread making performance of several commercial wholegrain oat flours, concluding that flours with coarse particle size, limited starch damage and low protein content are to be favoured. The same authors showed a positive effect of high pressure treatment on baking performance of oat flour (Huettner et al. 2010). Comparing the different cereals and pseudocereals, it can be stated that the main efforts of scientists were concentrated on wheat, rice, maize and oat whereas the investigation of alternative grains such as sorghum, buckwheat, quinoa and teff was less developed. Even though recently an increasing interest in the exploration of these grains has been evident, publications on their use for the production of bread are scarce. (Rosell et al. 2009) studied the effect of the addition of flours from the highly nutritious crop quinoa to wheat bread formulation. Replacement of wheat flour up to 50% still resulted in breads with acceptable sensory quality, however colour was compromised. (Alvarez-Jubete et al. 2010) investigated the potential of quinoa and buckwheat as healthy high-quality ingredients in gluten free bread. These authors found that the addition of the pseudocereals resulted in higher loaf volume and softer crumb compared to the control. Despite its name, buckwheat is not taxonomically related to wheat and hence can be considered as gluten free. In Southeast Asia it is traditionally used to make unleavened breads called chapatti. (Moore et al. 2007) produced buckwheat sourdough breads, while (Mezaize et al. 2009) used buckwheat flour in a composite formulation to produce French style gluten free breads. Teff, a small seeded tropical grain, originates from Ethiopia and is traditionally used for the production of *injera* 

(fermented flatbread) (Wrigley et al. 2004). (Mohammed 2009) supplemented wheat bread up to a level of 20% with teff flour, noticing a significant decrease of organoleptic overall acceptability. Using a simple recipe based on 100 % teff flour (Renzetti et al. 2008) produced gluten free breads of relatively low specific volume and crumb hardness. Several researchers have reported on the production of gluten free sorghum breads and much of this work is reviewed by Schober and Bean (2008). Vallons et al. (2010) attempted an improvement of sorghum bread by using high-pressure treated sorghum, while Schober et al. (2007) used sourdough fermentation. Hugo et al. (2003) showed that fermentation of sorghum flour has potential also to increase the utilisation of sorghum flour in composite wheat breads.

While a recent publication looks into the nutritional value of this wide range of flours (manuscript number JCS11-299), the aim of this study was to evaluate their potential for the production of bread and to evaluate several quality criteria of the end products. A basic bread recipe was used, consisting simply of flour, water, sugar, salt and yeast. Using a rheofermentometre, the fermentation potential of the different flours was compared. Apart from standard bread quality parameters such as loaf specific volume and physical crumb texture, also water activity and shelf life of the final products have been determined and aroma profiles were evaluated by a trained panel. As bread is an aerated product and its texture depends strongly on the size and distribution of the gas cells within, crumb grain characteristics were investigated additionally using image analysis and microstructure was observed by means of scanning electron microscopy. All flours used in this study are commercially available. Hence the research findings can be adapted to industrial bread production. This publication aims at supporting technologists in the development and improvement of gluten free breads by providing basic knowledge of the function of flours from different botanical sources.

## 2. Materials and Methods

## 2.1 Materials

The suppliers for the ingredients used were Doves Farm Foods Ltd, UK, for white rice flour and buckwheat flour; Odlums, Ireland for wholemeal wheat and bakers' flour; Trouw, The Netherlands, for teff flour; Smiths Flour Mills, UK, for maize flour; Ziegler Naturprodukte, Germany, for quinoa flour; E. Flahavan & Son Ltd, Ireland, for oat flour and Twin Valley Mills, Nebraska, USA, for sorghum flour. Dry yeast was obtained from Puratos, Belgium; sugar from Siucra, Ireland, and salt from Glacia British Salt Limited, UK. The compositional data for all flours included into this study is shown in Table 1.

#### 2.2 Compositional analysis

Crude fat, protein and moisture content of flours were determined according to the AACC methods 30-10, 46-12 and 44-15A, respectively. Protein content was calculated with a protein factor of 6.25, except for wheat flours where 5.83 was used. Dietary fibre, total starch levels and amylose/amylopectin ratio were determined using enzyme kits (K-TDFR, K-TSTA, K-AMYL) supplied by Megazyme, Ireland.

#### 2.3 Rheofermentometer analysis

Gaseous release and dough development of gluten free batters and wheat dough were measured using a Rheofermentometer (Chopin, France). Three hundred grams of dough were prepared in the same manner as described below for baking trials. The tests were performed at 30 °C over a period of 90 min. For wheat samples 1500 g weight was applied. The leavening process is described in terms of Hm (maximum height of dough development curve), T1 (time at maximum of dough development curve), (Hm-h)/Hm (dough height at the end of the test, calculated as percentage of the maximum), and Vt (total volume of carbon dioxide released by the dough).

#### 2.4 Baking tests

The water addition level for each of the gluten free breads was determined by preliminary baking trials. Breads were produced with different water levels altered in 5 % steps. Upon baking, crumb structure and bread volume were used to evaluate bread quality. The optimal water addition level for wheat as well as wholemeal wheat flour was determined using the farinograph (AACC method 54-21). Gluten free and wheat breads were prepared using 2 % salt, 2 % sugar and 3 % yeast, based on flour weight. Yeast and sugar were dissolved in the water (35 °C) and regenerated for a period of 10 min in a proofer (KOMA sunriser, Roermond, The Netherlands) set to 30 °C at a relative humidity (RH) of 85 %. This suspension was added to the premixed dry ingredients. Mixing was then carried out with a batter attachment for 1 min (gluten free batter) or with a dough hook for 30 sec (wheat dough) at low disk speed with a Kenwood chef

classic. The bowl was scraped down and a further mixing at a higher disk speed was carried out (7 min for wheat dough and 1.5 min for the gluten free formulation). Bulk fermentation for the wheat dough was carried out for 15 min at 30 °C, 85 % RH. Wheat dough and gluten free batters were scaled to 400 g into 10 baking tins of 15 x 9.5 x 7 cm and placed in a proofer for 30 min and 75 min, respectively (30 °C, 85 % RH). The breads were baked for 45 min at 190 °C top and bottom heat in a deck oven (MIWE condo, Arnstein, Germany), previously steamed with 0.3 L of water. Bread loaves were removed from the tins, cooled down at room temperature and subsequently analysed or stored in plastic bags at ambient temperature. Three batch replicas were prepared. Bake loss and loaf specific volume were analysed upon cooling using a Volscan Profiler (Stable Micro Systems, UK) and each loaf was weighed. Moisture was determined using the AACC approved air-oven method (44-15A). Crumb texture was determined at 2 and 5 days of storage. The three bread slices (25 mm thickness) taken from the centre of each loaf were used to evaluate the physical crumb texture. Texture profile analysis (TPA) was performed using a TA-XT2i texture analyser (Stable Micro Systems, Surrey, UK) equipped with a 25 kg load cell and a 35 mm aluminium cylindrical probe. The settings used were a test speed of 5 mm/s with a force of 0.98 N to compress the middle of the breadcrumb to 50 % of its original height. Three loaves per batch were analysed. Rate of staling was calculated using the equation (crumb hardness day 5 – crumb hardness day 0 / crumb hardness day 0). The structure of bread slices was characterised using a C-cell Bread Imaging system (Calibre Control International Ltd., UK). Water activity measurements of the bread crumb were determined using an AquaLab 4TE water activity meter (Decagon Devices Inc., Pullman, Washington, USA). The structure of bread slices was characterised using a C-cell Bread Imaging system (Calibre Control International Ltd., UK). The shelf life of the breads was determined using the method described by (Dal Bello et al. 2007). Each loaf was sliced transversely in a sterile manner to obtain uniform slices of 25mm thickness. Each slice was exposed to the air for 5 min on each side and then packed in a plastic bag and heat sealed, during which procedure a small slot was left open and a tip of a transfer pipette was inserted to ensure comparable aerobic conditions in each bag. Bags were incubated at room temperature and examined for mould growth during a 12-day storage

period. A series of 9 slices was inoculated. Mould growth was quantified as being the number of slice surfaces, i.e. both front and back of the slice, showing aerial mycelia. Calorie contents were calculated using specific energy factors for the food group categories published by (Schakel et al. 2009).

#### 2.5 Sensory evaluation

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

#### 2.5.1 Aroma Profile Analysis

Bread loaves were cut in slices (thickness about 2 cm) and the crusts were removed. The samples were presented to the sensory panel, which had to sniff the crumbs and describe the odour qualities they perceived during sniffing the crumbs. The panel finally agreed on characteristic odour attributes in a group discussion. Crumb samples were presented again to the panel in a second session and the intensities of the predefined odour attributes were evaluated on a scale from 0 (not detectable) over 1 (weak intensity), 2 (medium intensity), to 3 (high intensity). The results of each attribute were calculated as arithmetic mean. The assessors were trained immediately prior to analysis with aqueous odorant solutions in defined concentrations (factor 100 above the odour threshold, Czerny et al. 2008; Buttery et al. 1976, Schuh and Schieberle 2006). The odorant solutions reflected the evaluated characteristic odour attributes of the flours: buttery (butane-2,3-dione; 120 µg/L), cooked potato-like (3-(methylthio-) propanal; 140  $\mu$ g/L), malty (3-methylbutanal; 120  $\mu$ g/L), mouldy (geosmin; 2.1 µg/L), oat flakes-like ((E,E,Z)-nona-2,4,6-trienal; 2,6 µg/L), pealike (3-isobutyl-2-methoxypyrazine; 3.9 µg/L), popcorn-like, roasty (2-acetyl-1pyrroline; 12 µg/L), vinegar-like (acetic acid; 18,000,000 µg/L), vomit-like, cheesy (butanoic acid; 120,000  $\mu$ g/L). The odorant references were purchased

from Sigma-Aldrich, Taufkirchen, Germany, Acros, Geel, Belgium, and AromaLab, Freising, Germany. The attribute yeast dough-like was evaluated based on the experience of the assessors.

#### 2.5.2 Evaluation of aroma preference

Bread crumb slices were prepared as described above and presented to the panel. The assessors had to evaluate the preference of the samples on a nine-point-scale form 1 (dislike very much) over 5 (neither like nor dislike) to 9 (like very much). The results were calculated as the arithmetic mean.

#### 2.6 Scanning electron microscopy

Dough and bread samples were freeze-dried for approximately 20 h, grinded shortly with mortar and pestle and then attached onto double-sided carbon tape fixed to an aluminium specimen stub and were preliminary gold-coated in a SEM coating system (BIORAD Polaron Division) with a layer of 25 nm in thickness. Hereupon samples were examined under high vacuum in a field emission scanning electron microscope (JEOL, JSM-5510 SEM) with a working distance of 8 mm. Secondary electron images were acquired at an accelerating voltage of 5 kV.

#### 2.7 Statistical analysis

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

## 3. Results and Discussion

## 3.1 Determination of optimal water addition level

Water strongly influences dough consistency and plays an important role in starch gelatinization. Hence, it is crucial to determine the optimal water addition level before commencing baking trials. While the required water addition level for wheat flours can be determined by a standard AACC method, this procedure is not applicable for gluten free batters. Therefore, empirical trial-and-error testing was conducted and the resulting crumb structure was evaluated visually. In addition, loaf specific volume was considered. The following levels were determined as optimal: 120 % of rice flour, 95 % of oat, quinoa, sorghum and teff flour, 90 % of maize flour, 85 % of buckwheat flour, 67 % of wholemeal and 63 % of wheat flour. The gluten free flours needed higher

amounts of water to form an acceptable crumb than the wheat flours and therefore resulted in cake-like batters rather than workable dough.

#### 3.2 Rheofermentometer analysis

Rheofermentometer analysis is used to gain information on dough rise and gas formation. Wheat and oat samples reached a maximum dough development height (Hm) of 49 mm. These values are unmatched by the gluten free batters or the wholewheat dough, which reached 15 mm (maize) to 28 mm (sorghum) (Table 2). This indicates that the viscoelastic properties of oat and wheat dough are superior. Bran particles present in the wholemeal flour disrupt the gluten network and hence limit the extensibility of the dough. A statistically significant linear correlation between specific volume and maximum dough development height was observed (P<0.05). Decrease in dough volume at the end of the test, calculated as percentage of the maximum ((Hm-h)/Hm), was significantly lower for wheat dough compared to gluten free batters. The low value in wheat dough suggests that the combination of gas produced and the rheological properties of the sample were more favorable in sustaining the macrostructure of the proofed dough pieces compared to the other samples. The time for reaching maximum dough rise (T1) was significantly lower in gluten free batters when compared to wheat doughs. The total volume of gaseous release (Vt) of wheat and wholewheat dough was 1366 mL and 1570 mL, respectively. Gas production was highest in teff, buckwheat and quinoa batters (1676 mL, 1670 mL and 1583 mL, respectively). This indicates that these flours have a more favorable sugar composition for yeast fermentation.

#### 3.2 Loaf characteristics

It has been previously reported that these flours are suitable alone or as composites for the production of bread. However, the quality of the resulting end products varies widely, with gluten free breads usually being inferior to wheat bread. The quality evaluation showed that the produced breads differed in final loaf volume, crumb firmness, crumb structure, shelf life and taste attributes.

An important parameter, known to strongly influence consumer's choice is the loaf specific volume. From an economic standpoint, a high ratio of volume per weight is desired. Due to the exceptional visco-elastic properties of gluten, gas retention during proofing and baking is higher in wheat dough as compared to gluten free batters. Therefore, loaf specific volume of white wheat bread is highest (2.6 mL/g) (Table 3). However, the bran particles in wholemeal wheat flour puncture and break a high number of these gas bubbles, which results in a lower specific volume (1.7 mL/g in this study) (Seyer and Gelinas 2009). Due to the lack of a cohesive protein matrix, elasticity and extensibility of the gluten free batters is reduced and loaf volumes are low. With a specific volume of 2.4 mL/g, oat bread showed the highest value close to white wheat bread. All other gluten free loafs had significantly lower specific volumes, with maize having only half the volume of wheat bread (1.3 mL/g). Statistically significant differences were also detected in bake loss (Table 3). Again, the breads differed significantly in moisture content. Rice bread showed the highest moisture content and wheat bread the lowest. This was expected due to the different amounts of water added to the batters/doughs. A statistically significant positive correlation between water addition level and moisture content of the final loaves was detected (P<0.05). To describe the texture of the gluten free and wheat breads, crumb hardness, springiness and chewiness are shown in Table 3. Oat bread had the softest crumb (4.5 N). Wheat and rice bread had a crumb hardness of 8.5 N and 18.8 N, sorghum bread of 26.3 N. The low values found in these samples are desired, since consumers relate a firm crumb to an old product. Due to its higher fibre content, crumb hardness of wholemeal wheat bread (31.5 N) was about four times higher than that of white wheat bread. Maize bread had significantly higher crumb hardness (66.7 N). Crumb springiness, a value describing the recovery of the sample after compression, is important in separating soft, soggy bread from soft but resilient bread. Oat and wheat bread had the highest crumb springiness (1.08 and 1.00, respectively), whereas sorghum bread showed the lowest crumb springiness (0.88). Chewiness, i.e. the product of hardness, cohesiveness and springiness, gives an indication on the energy required to masticate a solid food. Wheat bread had a chewiness of 7 N, which is significantly lower than wholemeal wheat and most gluten free breads. Sorghum bread had a chewiness value of only 5N and the other gluten free breads ranged from 11 N for rice bread to 36 N for buckwheat bread. Regarding overall mechanical texture, oat bread is the most favourable, even compared to white wheat bread. Its crumb is significantly softer and springier.
#### 3.3 Crumb macrostructure

Apart from physical texture, described under 3.2, also visual texture of the crumb is an important attribute of bread quality. Digital image analysis was used to quantitatively describe crumb grain and results are shown in Table 4. When comparing wheat to wholemeal wheat bread it is apparent that crumb structure and cell characteristics are very distinct. Regarding the number of cells, white wheat bread has the highest (4906), whereas wholemeal wheat bread had the lowest number between all breads of this study (2453). This difference can be explained by the high amount of bran particles present in the dough, which penetrate gas cells and cause leaks (Schober 2009). Number of cells in gluten free breads is significantly lower than in white wheat bread. Teff and quinoa bread show a relatively higher number of alveoli (3327 and 3170), whereas buckwheat counted 2985, sorghum 2788 and oat 2667. The number of cells in maize and rice is similarly low as in wholemeal wheat flour. The gas cells are incorporated through the mixing process and only their size is influenced by further bread production steps. For the production of all gluten free breads, the same mixing regime was followed. Hence, the different number of cells is due to differences in dough consistency (Rosell and Santos 2010). The area of cells as a percentage of total slice area is given in Table 4. Higher values, as found in oat or rice bread (54.55% and 53.85%, respectively) indicate a more open texture. Quinoa, buckwheat, maize and teff breads show the smallest area of cells (50.58%, 49.92%, 47.75% and 47.76%) indicating a denser structure. This is reflected in the specific volume, which was lowest for these loaves. However, not the holes themselves are the most significant contributor to mechanical strength of the baked product, but the surrounding matrix referred to as "cell walls" (Cauvain et al. 1999). Cells of rice bread crumb had the highest wall thickness (0.54 mm), followed by oat bread (0.51 mm). Thin cell walls as in wheat and maize bread (both 0.43 mm) are desirable. The mouth feel of bread is known to be strongly influenced by these cell characteristics: finer, thin-walled uniform cells yield a softer and more elastic texture, than coarse, thick-walled cell structures do (Scalon and Zghal 2001). Cell elongation is a measure of how far the pore shape differs from a circle, with values close to 1 indicating rounded cells and higher values indicating greater elongation. White wheat bread showed the most elongated cells, whereas voids of wholemeal wheat bread

were less elongated. The latter contains a high proportion of dietary fibre which disrupts the starch-gluten matrix and hence restricts gas cell expansion, forcing the alveoli to expand in a certain way. The most rounded cells were observed in rice bread.

## 3.4 Shelf life

The shelf life of bread is determined by the staling behaviour of the product and its microbial deterioration. Bread staling involves crumb firming, which has been attributed mainly to recrystallization of amylopectin and water redistribution between crumb and crust. Sciarini et al. (2010) proposed previously that in wheat breads the gluten network slows down the movement of water, thus gluten free breads are more prone to stale. This assumption cannot be confirmed by the data of this study, as the rate of staling of most gluten free breads is lower than that of wheat bread (Table 3). Oat bread had the highest rate of staling (4.10), followed by white wheat bread (3.55) and maize bread (2.41). Staling rate of teff was significantly lower (1.29). This was expected since teff starch has a lower tendency to retrograde compared to maize and wheat starches (Bultosa et al. 2002). Wholewheat and sorghum had a comparable staling rate of 1.58 and 1.59, respectively. In bread made with buckwheat and rice flour staling was far less pronounced (0.82 and 0.83, respectively). Quinoa flour resulted in breads with the lowest rate of staling (0.18). These values show that other factors than the presence or absence of gluten is influencing the staling rate. The changes in compressibility and crumbliness of bread crumb is in large parts attributable to the retrogradation behaviour of cereal starch and hence the ratio of amylose to amylopectin (Singh et al. 2003). The significantly lower amylose content in quinoa flour (Table 1) is reflected in a much slower staling of quinoa bread compared to others.

The quality of bread is lost rapidly not only due to staling but also due to microbial spoilage. Under ambient conditions mold grows on well-packaged wheat bread within four to six days (Sluimer 2005). White wheat bread of this study had a microbial shelf life of four days. As expected, shelf life of gluten free breads was lower. With the exception of rice bread, the first mold growth was observed on day four, giving the breads a shelf life of three days. Also wholemeal wheat bread was spoiled on day four. Rice bread had a shelf life of only two days. The microbial stability of gluten free breads is mainly

compromised because of the high water activity (aw). Rice bread has the highest water activity (0.987) (Table 3). Wheat bread, having the lowest water activity (0.969), had the longest microbial shelf life. The gluten free flours of this study generally showed significantly higher values for water activity than the two wheat flours (Table 3).

## 3.5 Energy content

Studies showed that the exclusion of gluten from the diet very often results in a significant increase of body fat stores and weight gain in coeliac patients (Capristo et al. 2000; Dickey and Kearney 2006; Smecuol et al. 1997). As bread is a major source of energy in our daily diet, the calorie content of gluten free products is of importance. For the breads produced in this study, values have been calculated according to (Schakel et al. 2009) using the compositional data of the flours shown in Table 1. As expected, calorie content was highest in white wheat bread (224 kcal/100 g). Wholemeal wheat bread had less calories (195 kcal/100g), since it contains a higher amount of fibre, which does not contribute significantly to the energy usable by humans. Due to the fact that the gluten free breads of this study contained significantly higher amounts of water, their calorie content was lower compared to white wheat bread: oat 199 kcal/100g, buckwheat 196 kcal/100g, maize 195 kcal/100g, sorghum 191 kcal/100g, teff 180 kcal/100g and rice 177 kcal/100g.

# 3.6 Sensory evaluation

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

Aroma profile analyses were performed in order to characterize and describe the crumb aroma in detail. Therefore, the characteristic odor attributes were identified by the sensory panel, which then also determined the intensities of

these odor qualities. Using this approach and by comparing the profiles, specific odor characteristics of each bread crumb were evaluated. The aroma profiles of wholemeal and wheat crumb as well as the seven gluten free flours are shown in Figure 1. Wheat bread crumb was dominated by a medium intense yeast-like note and the profile was completed by weak malty and buttery notes. It can be concluded that these attributes were responsible for the positive evaluation of preference. In contrast to wheat, the yeast-like, malty and buttery intensities were in part much lower in the buckwheat crumb where pea-like, moldy and vinegar-like notes with weak intensities were detectable. These attributes can be correlated with the low acceptance of buckwheat crumb evaluated in the preference test. Again, the maize crumb exhibited a reduced veast-like and malty aroma in comparison to wheat and an undesirable vomit-like note was perceivable, which was responsible for the low preference of maize bread. The aroma profile of oat bread was very similar to the wheat profile. Although the yeast-like note was decreased, the malty intensity in oat was comparable to wheat and the buttery note was even more intense. Undesirable odor notes like moldy, pea-like and vomit-like, which have been detected in maize and buckwheat, were not perceivable in oat crumb. The high resemblance of the aroma profiles is therefore the reason why oat and wheat crumb had a similar preference. The pea-like odor attribute was the outstanding note of quinoa crumb and its intensity ranged from medium to high. The low acceptance of quinoa aroma is therefore explainable with the dominant presence of this odor note. Additional notes, which were reminiscent of cooked potato and mold, were also perceived with weak to medium intensity. Rice and wheat bread crumb aroma agreed in nearly all the intensities of the attributes yeast, doughlike and malty. However, the low preference of rice crumb was obviously caused by a vomit-like odor with a weak to medium intensity. Low intensities were determined for the evaluated odor characteristics in the sorghum crumb. Although a weak pea-like note was detectable in the crumb, but only a little influence on aroma preference was observed. Teff bread crumb showed reduced intensities of yeast, dough-like, malty and buttery, which have been found as the positive attributes in wheat crumb. The absences of these attributes, in combination with the detected vinegar-like quality, were responsible for the negative evaluation. The comparable preference scores of the crumbs made from wholemeal and baker's flour was correlated with the aroma profiles. The attributes "yeast dough-like", "malty" and "buttery" were almost identical. Only the vinegar- and oat flakes-like attributes were somewhat higher in wheat and wholemeal wheat crumb.

## 3.7 Crumb microstructure

Batters and breads were investigated by means of scanning electron microscopy. Representative micrographs are shown in Figure 2 and Figure 3, respectively. The batters/ doughs preserved some characteristics of the flours (JCS11-299): starch granules of various sizes and shapes as well as protein aggregates (Figure 2). Bread dough represents a limited-water-system and therefore starch cannot fully gelatinize. Nevertheless, during mixing the granules swell and get deformed. This is most obvious for maize samples. While granule size in the flour was below 10  $\mu$ m, they are up to 20  $\mu$ m in diameter in the dough. The foam structure of dough consists of a continuous starch-protein matrix containing discrete gas cells, starch granules and in case of wholemeal, bran particles. The transformation of dough to bread is a complex process during which several structural changes take place. These changes include the gelatinization of starch, which can be observed by means of scanning electron microscopy. During baking, the combination of gas production and evaporation turns the foam into a sponge structure with interconnected cells (Rojas et al. 2000). Figure 3 shows the resulting breads, where only a reduced number of starch granules are present. Due to partial gelatinisation they appear distorted. For the examination of bread microstructure, scanning electron microscopy seems unfavourable as gelatinised starch and proteins cannot be distinguished. Therefore the gluten network cannot be visualised and no real structural differences between gluten free and wheat breads can be observed. Compared to all other breads, oat and rice bread seem more aerated. This finding is in accordance with macrostructure observations by image analysis, where these samples showed the highest percentage area of cells.

## 4. Conclusion

Due to differences in the composition of gluten free flours (manuscript number JCS11-299), although nutritionally superior, the loaf quality of gluten free and wholemeal wheat breads is inferior to that of white wheat bread. Oat represents an exception, as the breads produced from this flour were comparable to wheat

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

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	Wheat	Wholewheat	Rice	Oat	Quinoa
Moisture [g/100g]	12.69 ± 0.01 °	$13.10 \pm 0.00$ <sup>b</sup>	12.83 ± 0.15 °	10.36 ± 0.20 <sup>f</sup>	12.26±0.03 <sup>d</sup>
Fat [g/100g]	1.81 ± 0.05 <sup>d</sup>	3.63 ± 0.104 °	₀ 90.0 ∓ 0.06 e	$6.74 \pm 0.80$ b	8.59 ± 0.25 <sup>a</sup>
Protein [g/100g]	11.54 ± 1.07 c	9.89 ± 0.17 <sup>d</sup>	7.33 ± 0.03 e	6.91 ± 0.08 e	13.48 ± 0.04 ª
Total starch [g/100g]	$68.06 \pm 2.34^{b}$	57.24 ± 0.26°	77.52 ± 0.42ª	69.38 ± 1.66°	48.88 ± 2.07 <sup>d</sup>
Amylose [% of total starch]	21.10 ± 1.29ª	$21.10 \pm 2.08^{ac}$	$21.38 \pm 0.90^{ab}$	20.42 ± 2.43 <sup>bc</sup>	$4.62 \pm 0.83^{e}$
Total dietary fibre [g/100g]	3.44 ± 0.01 <sup>cd</sup>	$11.42 \pm 1.27^{a}$	$0.43 \pm 0.15^{f}$	$4.05 \pm 0.40^{\circ}$	$7.14 \pm 0.23^{b}$
	Buckwheat	Sorghum	Maize	Teff	
Moisture [g/100g]	12.63 ± 0.06 °	11.08 ± 0.18 <sup>e</sup>	13.97 ± 0.12 ª	9.53 ± 0.06 g	
Fat [g/100g]	4.21 ± 0.74 °	3.50 ± 0.31 °	2.48 ± 0.46 <sup>d</sup>	4.39 ± 0.26 <sup>c</sup>	
Protein [g/100g]	$12.19 \pm 0.38$	$4.68 \pm 0.04$ f	5.50 ± 0.19 <sup>f</sup>	12.84 ± 0.51 <sup>ab</sup>	
Total starch [g/100g]	61.35 ± 2.15°	73.20 ± 1.52 <sup>a</sup>	71.52 ± 0.42 <sup>a</sup>	57.77 ± 5.94°	

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Table 1

Amylose [% of total starch]  $15.95 \pm 0.61^{d}$   $18.18 \pm 0.55^{cd}$   $22.91 \pm 0.82^{a}$   $19.72 \pm 0.99^{bc}$ 

**Total dietary fibre [g/100g]**  $2.18 \pm 0.11^{\circ}$   $4.51 \pm 0.01^{\circ}$   $2.62 \pm 0.45^{de}$   $4.54 \pm 0.57^{\circ}$ 

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

Table 2 Dough development and gaseous release of wheat doughs and gluten free batters determined with the rheofermentometre at  $30^{\circ}$ C for 1.5h

Table 4 Crumb cell characteristics

	Number of cells	Cell elongation	pore/area	wall thickness
			[%]	[mm]
Wheat	4906 ± 38 ª	$1.517 \pm 0.021^{a}$	51.29 ± 0.05 °	0.427 ± 0.006 <sup>e</sup>
Wholewheat	2453 ± 2 g	$1.393 \pm 0.015^{\text{ef}}$	55.16 ± 0.54 ª	0.478 ± 0.005 °
Rice	$2507 \pm 170$ g	$1.380 \pm 0.000^{\text{f}}$	53.85 ± 0.11 <sup>b</sup>	$0.539 \pm 0.008$ a
Sorghum	2788 ± 100 <sup>e</sup>	$1.410 \pm 0.000^{\text{ef}}$	53.88 ± 1.24 <sup>b</sup>	$0.479 \pm 0.004$ <sup>c</sup>
Oat	2667 ± 39 ef	$1.440 \pm 0.030^{cd}$	$54.55 \pm 0.35$ <sup>ab</sup>	$0.505 \pm 0.018$ b
Quinoa	3170 ± 82 °	$1.417 \pm 0.006^{de}$	$50.58 \pm 0.71$ <sup>cd</sup>	$0.455 \pm 0.002$ d
Teff	3327 ± 21 <sup>b</sup>	$1.443 \pm 0.015$ <sup>cd</sup>	47.75 ± 0.41 <sup>e</sup>	$0.452 \pm 0.005$ d
Buckwheat	2985 ± 15 <sup>d</sup>	$1.477 \pm 0.006^{b}$	49.92 ± 0.09 <sup>d</sup>	$0.446 \pm 0.002$ d
Maize	$2576 \pm 16^{\text{ fg}}$	$1.453 \pm 0.006^{bc}$	47.76 ± 0.22 <sup>e</sup>	0.430 ± 0.003 <sup>e</sup>

Table 3 Loaf and slice characteristics of gluten free and wheat breads

	Specific Volume	Bake loss [g]	Moisture [%]	Hardness [N]	Springiness	Chewiness [N]	Rate of staling	Water activity
	[ml/g]		1	1	)	1	)	
Wheat	2.62 ± 0.18 <sup>a</sup>	42.89 ± 2.83 <sup>f</sup>	$40.96 \pm 1.51^{f}$	8.48 ± 1.33 <sup>f</sup>	$1.004 \pm 0.018$ <sup>b</sup>	$7.74 \pm 1.41$ f	3.55 ± 0.29 <sup>ab</sup>	0.967 ± 0.002 <sup>d</sup>
Wholewheat	1.70 ± 0.06 <sup>d</sup>	50.18 ± 2.52 <sup>f</sup>	46.28 ± 1.98 <sup>d</sup>	$31.52 \pm 3.56$ °	0.913 ± 0.025 <sup>cd</sup>	<b>18.03</b> ± 4.95 <sup>d</sup>	1.58±0.09°	0.969 ± 0.004 d
Rice	1.80 ± 0.05 <sup>cd</sup>	63.89 ± 2.73 c	55.20 ± 2.16 <sup>a</sup>	18.79 ± 1.90 €	0.953 ± 0.015 c	11.04 ± 2.29 e	0.83 ± 0.12 <sup>df</sup>	0.987 ± 0.003 ª
Sorghum	1.85 ± 0.14 °	72.46 ± 3.39 <sup>a</sup>	44.29 ± 4.49 e	26.28±5.05 <sup>d</sup>	0.878 ± 0.039 <sup>d</sup>	$5.26 \pm 2.24$ g	1.59 ± 0.24 <sup>cd</sup>	0.980 ± 0.002 <sup>b</sup>
Oat	$2.40 \pm 0.12$ <sup>b</sup>	66.42 ± 4.04 <sup>b</sup>	49.42 ± 1.80 bc	4.47 ± 0.90 g	1.083 ± 0.215 <sup>a</sup>	20.37 ± 2.08 <sup>cd</sup>	$4.10 \pm 1.77$ a	0.985 ± 0.002 ª
Quinoa	$1.51 \pm 0.07$ f	58.91 ± 3.24 <sup>d</sup>	48.23 ± 2.31 <sup>cd</sup>	31.98 ± 2.50 °	0.930 ± 0.030 c	$32.93 \pm 2.47$ b	$0.18 \pm 0.07$ gef	0.974 ± 0.002 c
Teff	1.60 ± 0.03 €	52.66 ± 5.01 <sup>e</sup>	$50.25 \pm 1.59$ b	$43.13 \pm 5.20$ b	0.942 ± 0.017 c	$31.91 \pm 7.48$ <sup>b</sup>	1.29 ± 0.19 <sup>cd</sup>	0.978 ± 0.002 <sup>b</sup>
Buckwheat	1.69 ± 0.06 <sup>d</sup>	53.89 ± 1.49 <sup>e</sup>	50.64 ± 2.40 <sup>b</sup>	$42.92 \pm 4.03$ b	0.952 ± 0.014 °	36.25 ± 3.38 ª	0.82 ± 0.05 gd	0.971 ± 0.003 <sup>d</sup>
Maize	1.33 ± 0.10 g	60.82 ± 6.41 <sup>d</sup>	46.09 ± 1.78 <sup>d</sup>	66.66±3.56ª	0.902 ± 0.018 <sup>cd</sup>	22.70 ± 2.59 c	2.41 ± 0.62 <sup>bc</sup>	0.979 ± 0.003 <sup>b</sup>



Figure 1 Aroma profile analysis of bread crumbs made from different flours



Figure 2 Micrographs of gluten free and wheat doughs: (a) oat, (b) buckwheat, (c) teff, (d) maize, (e) quinoa, (f) rice, (g) wheat, (h) wholemeal wheat (i) sorghum



Figure 3 Micrographs of gluten free and wheat breads: (a) oat, (b) buckwheat, (c) teff, (d) maize, (e) quinoa, (f) rice, (g) wheat, (h) wholemeal wheat (i) sorghum

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

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#### Abstract

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

iron. Therefore these flours represent nutrient dense raw materials for the production of gluten free foods.

#### **1** Introduction

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

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

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

# 2 Experimental

#### 2.1 Materials

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

(10.4 % moisture) and Twin Valley Mills, Nebraska for sorghum flour (11.1 % moisture).

## 2.2 Compositional analysis

Crude fat, protein and moisture content of flours were determined according to the AACC methods 30-10, 46-12 and 44-15A, respectively. Protein content was calculated with a protein factor of 6.25, except for wheat flours where 5.83 was used. Ash content was determined according to Matissek (2006). Dietary fibre, phytate, total and damaged starch levels as well as amylose/amylopectin ratio were determined using enzyme kits (K-TDFR, K-PHYT, K-TSTA, K-SDAM, K-AMYL) supplied by Megazyme, Ireland. Polyphenol content was determined according to Alvarez-Jubete, Wijngaard et al. (2010). The fatty acid profile was determined chromatography using gas following the trimethylsulfoniumhydroxide (TMSH) derivatisation method described by the DGF (Deutsche Gesellschaft für Fettwissenschaften) (method number: DGF C-VI 11e). Minerals were analyzed by ICP-AES following the method EN ISO 11885 E22. The chloride concentration of flours was determined according to Analysenkommision (1996). Calorie contents were calculated using the specific energy factors for the food group categories published by Schakel, Jasthi et al. (2009). Folate levels were determined according to AOAC 944.12 / 45.2.03 (1990). Folate was extracted from the sample in an autoclave using a buffer solution, followed by an enzymatic digestion with human plasma and pancreas V and finally by a second autoclave treatment. After dilution with basal medium containing all required growth nutrients except folic acid the growth response of Lactobacillus rhamnosus (ATCC 8043) to extracted folate was measured turbidimetrically and was compared to calibration solutions with known concentrations.

#### 2.3 Capillary electrophoreses of extracted proteins

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

## 2.4 Scanning electron microscopy

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

## 2.5 Statistical analysis

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

## **3 Results**

## 3.1 Carbohydrates

Cereals usually comprise of about 50-80 % carbohydrate on a dry weight basis. Starch is the main cereal polysaccharide and a major food reserve providing a bulk nutrient and energy source in the human diet (Dewettinck, Vanbockstaele et al. 2008). It is stored in granular form of variable size and shapes characteristic of the species (Figure 1). Granules consist of starch molecules which are arranged radially forming a series of concentric layers that alternate as amorphous and semi-crystalline regions. Wheat starch differs to that of other botanical sources in that it contains two, possible three, distinct populations of granules differing in shape, dimension, composition, and properties (Maningat and Seib 2010). Apart from large lenticular starch granules (A-granules) also smaller spherical granules (B-granules) can be observed. On the contrary to wheat starch, granules found in the other cereals have a simple size distribution, being of similar shape and diameter. Maize shows only spherical granules with a diameter of approximately 10 µm (Figure 1e). The starch granules in rice flour are polyhedral and very small ( $<5 \mu m$ ). The individual granules are organised together forming compound granules. Figure 1f shows indentations where granules have been broken off during of milling. Also oat starch is a compound

Appendix

starch, comprised by bigger granules than in rice (up to 10  $\mu$ m). The micrographs of sorghum flour show polygonal starch granules of approximately 10  $\mu$ m. These are surrounded by smaller spherical bodies of only a few micrometers, likely to be protein bodies (Delcour and Hoseney 2009). Teff granules are polygonal in shape and between 2 and 7  $\mu$ m in diameter. They are packed together and protein seems to attach outside of the compound starch granule. Also buckwheat starch has granular shape (up to about 5  $\mu$ m in diameter) and is organised in bigger compounds. Quinoa has significantly smaller starch granules than all other flours (<2  $\mu$ m). They are polygonal and present both singly and in aggregates. The scanning electron micrographs show that although in several cereal species starch is organised compound-like, on milling individual starch granules are released.

Table 1 shows the total starch contents of the studied flours, which ranged from 49 g/100g (quinoa) to 78 g/100g (rice). Wholemeal wheat flour contains 57 g starch per 100 g flour. Wheat flour, due to a greater proportion of endosperm material, shows a higher starch level (68 g/100g). From a chemical point of view, starch is comprised of two polymers of D-glucose: amylose and amylopectin. Amylose is linear (only lightly branched) and completely amorphous. Amylopectin is a highly branched polymer and provides partial crystallinity to the starch granule. The ratio of amylose to amylopectin is of technological relevance especially in connection to bread staling. The percentage amylose of total starch is approximately 22% for most of the flours studied (Table 1). Sorghum and buckwheat flour had a lower percentage of amylose (19 and 16 %, respectively). Quinoa starch showed a significantly lower amylose content of only 4 % of total starch.

The milling of grains causes physical damage to a proportion of the starch granules (Table 1). Their altered properties are of technological significance, as damaged starch granules increase water absorption and are also more susceptible to enzyme hydrolysis, thereby promoting yeast fermentation. In this study, the highest amount of damaged starch was found in rice flour (15.24 g/100g), followed by white wheat flour (7.85 g/100g). Buckwheat and teff flour contained the lowest amounts (2.63 g/100g and 2.08 g/100g respectively).

Apart from starch, cereals also contain significant amounts of carbohydrates, which are resistant to digestion in the human small intestine and are completely or partly fermented in the large intestine (i.e. non-starch polysaccharides or dietary fibre). However, compared to their grain counterparts, flours are significantly lower in fibre because the milling process removes the bran and germ to a certain extent. Due to differences in chemical composition of the grains and applied milling procedures, the fibre content of the flours in this study varies widely (Table 1). Endosperm-derived white wheat flour contains only 3.4 g/100g dietary fibre, while wholemeal wheat flour, where the bran fraction is reintroduced into the milled white flour, contains 11.4 g/100g. Quinoa flour contained the highest amount of dietary fibre among the gluten free flours screened (7.1 g/100g). This high level is due to the fact that quinoa flour is made by milling the whole seed. The milling process of oat includes a dehulling step and results in flour containing 4.1 g/100g fibre. Sorghum and teff flours have a similar fibre content (both 4.5 g/100g). The fibre content of the maize kernel is naturally lower than that of other cereals and therefore the fibre content of the resulting flour is as low as 2.6 g/100g. Buckwheat is dehulled and milled into flour, containing mainly starchy endosperm and therefore fibre content in buckwheat flour is lower than in most other flours (2.2 g/100 g). During production of white rice flour, hull and bran are removed from paddy rice. Therefore the resulting product contains only negligible amounts of fibre (0.4 g/100 g) in this study). Dietary fibre is commonly fractionated into insoluble and soluble dietary fibre, the first being associated with intestinal regulation (increased stool weight and frequency and reduced intestinal transit time) and the latter being linked to reduction of serum cholesterol levels and an attenuation of postprandial glycaemic response. The soluble fibre contents of the gluten free and wheat flours screened are shown in Table 1. White wheat flour, quinoa and maize as well as buckwheat are characterized by a high proportion of soluble fibre (39%, 24%, 24% and 22% of total dietary fibre).

# **3.2 Proteins**

Amino acids, peptides and proteins are important constituents of food. Besides their nutritional significance, they contribute to flavour and texture of food. Wheat flour commonly used for bread making has a protein content of approximately 11 % (11.5 % in this study). In comparison teff and buckwheat flour showed higher protein contents (12.8 % and 12.2 %). With 13.48 %, quinoa flour showed the highest protein content. This pseudocereal is higher in

protein and fat and lower in carbohydrates due to the proportional size of the embryo within the grain (up to 30 % of the grains cross weight, compared with 1% for most cereals) (Wrigley, Corke et al. 2004). Sorghum and maize flour had the lowest protein contents of 4.7 % and 5.5 %. This value was also relatively low for rice and oat flour (7.3 % and 6.9 %). The wide variation in protein content is not only due to genetic factors, but also environmental effects. Protein is synthesized during the fruiting period, whereas starch synthesis starts later. If growing conditions in the late fruiting period are good, starch yield will be high but protein content will be relatively low (Lasztity 1996). Capillary electrophoresis was used to further investigate the proteins present in the flours of this study. Electropherotograms were obtained for each extract and a representative gel is shown in Figure 2. When comparing results of the current study to literature it has to be kept in mind, that the protein banding pattern is characteristic of species but also variety. Extracts of wheat and wholemeal wheat flour produce several bands between 14 and 223 kDa. The results of these two flours compare well, indicating that the same wheat variety was used for their production. Protein peaks were found at 14, 16, 40, 45, 59, 97, 147 and 170 kDa. Wholemeal wheat flour extracts also showed bands at 53 and 139 kDa. These additional bands could be due to the fact that aleurone layer and embryo of wheat grains are rich in protein and these fractions are removed during milling of white wheat flour. The peptides triggering coeliac disease are contained in the prolamin fraction of wheat protein. Molecular weights of prolamins vary greatly from approximately 10 kDa to 100 kDa (Shewry and Halford 2002). Even though also panicoideae such as maize, sorghum and teff, contain significant amounts of prolamins, this group of storage proteins has separate evolutionary origins of those in triticeae (Shewry and Halford 2002). The 33-mer gluten peptide, responsible for the immune reaction in genetically susceptible persons, is absent in these grains and therefore they are not coeliactoxic. The major group in maize prolamins are  $\alpha$ -zeins, which result in bands at 19 kDa and 22 kDa on the electropherogram. As also shown by Moroni, Iametti et al. (2010), for maize flour only three protein components in the 9-25 kDA range can be detected. The low molecular weight storage protein of sorghum, named kafirin, produced major bands at 22 kDa (corresponding to  $\alpha$ -kafirin) and at 19 kDa (corresponding to  $\beta$ -kafirin) (Lasztity 1996). Additional bands

were observed at 14, 39, 43 and 53 kDa. In teff extracts major bands were present at 25, 40 and 61 kDa, possibly corresponding to the prolamin fraction (Tatham, Fido et al. 1996). Bands could also be observed at 15, 32, 37, 53 and 77 kDa. As previously observed, the protein fractions of teff are less complex than those of wheat, in terms of their apparent molecular size differences, and resemble more the pattern found in maize (Shewry and Tatham 1990). The major storage proteins in wheat, maize, sorghum and teff are prolamins. This is not the case for other plants such as oats and pseudocereals, where globulins are the major storage proteins; or rice, where glutelins are most abundant (Gorinstein, Pawelzik et al. 2002). Oat prolamins (avenins) are similar to wheat gluten. However, due to different composition and amino acid sequence, oat might not belong to the grains harmful to coeliac disease patients (Vader, de Ru et al. 2002). This was supported by clinical observations (Janatuinen, Kemppainen et al. 2002). The electropherogram of the extracted oat flour proteins showed bands at 14 and 17 kDa and several bands between 23 and 28 kDa as well as between 44 and 54 kDa and at 70 kDa. These findings compare well with those of Hüttner, Bello et al. (2010). Capillary electrophoreses of quinoa proteins resulted in major bands at 23 kDa and at 30 and 38 kDa. These bands are likely to represent the principal protein of quinoa, the chenopodina. Electropherotograms of buckwheat protein shows bands at 14, 15, 22 and 53 kDa as well as between 32-44 kDa, corresponding to the albumin and globulin fractions. This banding pattern was also observed by Vallons, Ryan et al. (2011). Additionally, buckwheat flour extract showed a major band at 53 kDa. The extracted rice proteins result in a protein peak at 16 kDa and a minor one at 22 kDa, corresponding to the low molecular weight storage proteins (prolamins). The electropherogram shows proteins of molecular weights between 35 and 40 kDa, representing the  $\alpha$ -glutelin subunits and between 19 and 25 kDa, representing  $\beta$ -glutelin subunits (Van der Borght, Vandeputte et al. 2006).

# 3.3 Lipids

Although lipids comprise only about 1.5-7.0 % of cereal grains, they are of nutritive and physiological importance due to their role as energy supply and source of essential fatty acids. Furthermore, they play a role in food quality as they may cause off-flavours in stored flours. Fat content was significantly different in the analysed samples, ranging from 0.9 % for rice flour up to 8.6 %

for quinoa flour (Table 2). Oat, teff, buckwheat, wholewheat and sorghum flour had relatively higher fat contents (6.7 %, 4.4 %, 4.2 %, 3.6 % and 3.5 % respectively), as compared to wheat flour (1.8 %). The amount of fat in the pseudocereal quinoa is higher than in any other grain. However, the fat is characterised by a high content of nutritionally valuable unsaturated fatty acids, with linoleic acid accounting for 52 % of total fatty acids. The results were in agreement with literature (Schoenlechner 2008). In the flours of this study, palmitic acid (C16:0) was the most abundant saturated fatty acid, being especially high in wheat, oat and rice (19.7%, 20.6% and 22.4%, respectively). Cereal lipids include a range of essential fatty acids such as linoleic and linolenic acid. In teff, sorghum and maize, guinoa and wholemeal wheat flour, linoleic acid is the most abundant fatty acid (Table 2). Oats contain a considerable amount of oleic acid (42.1 % w/w). Also in wheat, buckwheat and rice flour, oleic acid makes the highest proportion of the fatty acids (31.1 %, 36.5 % and 40.0 %, respectively). These findings are in accordance with Dewettinck, Vanbockstaele et al. (2008). Also in buckwheat flour unsaturated fatty acids (Oleic and Linoleic) prevail (36.4 % and 33.0 %, respectively). The major fatty acids in sorghum flour are palmitic (13.5 %), oleic (30.4 %) and linoleic acid (49.3 %), making up over 90 % of the total fatty acids. These values compare well with literature (Wrigley, Corke et al. 2004). Sorghum oil is very similar to maize oil in quality and fatty acid content (Table 2). Wholewheat, buckwheat and guinoa flour show a high amount of linolenic acid (5.1 % w/w, 4.6 % w/w and 3.8 % w/w respectively). Buckwheat was found to be high in eicosenoic acid (3.3%). Comparing omega-6/omega-3 it can be seen that the pseudocereals quinoa and buckwheat have the most favourable ratio (11/1 and 9/1). Also wheat and rice flours have similar ratios (12/1 for wholemeal wheat, 14/1 for wheat and 15/1 for rice flour). The other flours have much higher ratios up to 37/1 for oat flour.

# 3.4 Folate and minerals

Folate, an essential component in the human diet, is involved as a cofactor in metabolic reactions (e.g. the biosynthesis of nucleotides, the building blocks of DNA and RNA) and plays a critical role in the prevention of neural tube defects. Determination of folate content of the different flours showed big variations between the samples. Wheat and wholemeal wheat flour as well as rice and oat flour contained low levels: 18, 34, 33, and 30  $\mu$ g/100g, respectively. Sorghum (77  $\mu$ g/100g), maize (37  $\mu$ g/100g) and teff (96  $\mu$ g/100g) contain notably higher levels. The pseudocereals quinoa and buckwheat contain the highest amounts of folate among the flours screened: 180  $\mu$ g/100g and 132  $\mu$ g/100g, respectively. Results for wheat and quinoa flour compare well with those of (Schoenlechner, Wendner et al. 2010), but folate content of buckwheat flour was lower in the study of these authors (24  $\mu$ g/100g).

Minerals are important for various physiological functions in the human body. Per day, more than 100 mg of the major minerals (Na, Mg, K, Ca, P, and Cl) and less than 100 mg of trace elements (Fe, Cu, Zn) are required (Insel 2004). Table 3 shows the ash content of the flours as well as the mineral composition. In this study ash content ranged from 0.4 mg/100g (maize) to 2.4 mg/100g (quinoa). The element in highest concentration was phosphorus with up to 441.6 mg/100g (quinoa). Only wheat flour had lower phosphorus content (10% of total ash). The majority of the phosphorus in cereals occurs as phytic acid, an inositol hexaphosphoric acid. Potassium and Sodium, two elements of concern with regard to health care, were also detected in the flours screened. Potassium contents were high, ranging from 97.4 mg/100g (rice) to 553.8 mg/100g (quinoa). However, cereal flours are not considered a high or even moderate source of sodium. Contents in this study were between 0.5 mg/100g for sorghum and maize and about 3.7 mg/100g for wheat and quinoa flour and contribute to less than 1% of the dietary reference amount (Table 4). Mineral content in quinoa grain is superior to most cereals. Content of minerals in quinoa is more than twice as high as in the other cereals, with potassium, phosphorous, magnesium and calcium prevailing (553.8, 441.6, 229.9 and 49.8 mg/100g). Additionally quinoa is high in iron and zinc (5.4 and 3.7 mg/100g). Teff can be considered a good source of calcium (154.3 mg/100g). The high amount of calcium in white wheat flour of this study (179.8 mg/100g) compared to literature (17 mg/100g, Wrigley, Corke et al. 2004) is due to the fact that calcium carbonate is added to this product (personal communication with producer). Magnesium levels are relatively high in buckwheat and teff flour (173.6 mg/100g and 169.0 mg/100g). Wheat is known to be a source of iron with contents ranging from 1 mg/100g to 5 mg/100g (1.3 mg/100g in this study) (Dewettinck, Vanbockstaele et al. 2008). This study however showed

that flours made from buckwheat, quinoa or teff are even higher in their iron content (2.9 mg/100g, 5.4 mg/100g and 8.5 mg/100g, respectively). It is well known from literature that wheat is a good source of zinc (1-5 mg/100g) and copper (0.1-1 mg/100g) (Dewettinck, Vanbockstaele et al. 2008). However, also the other cereal flours analyzed, apart from maize flour, contained comparable or even higher levels of zinc and copper (Table 3). Buckwheat is a richer mineral source (except for calcium) than many cereals such as rice, sorghum and maize, with high levels of magnesium (173.6 mg/100g), zinc (1.88 mg/100g), potassium (402.3 mg/100g) and copper (0.51 mg/100g).

# **3.5 Phytate and polyphenols**

Cereal grains being an important source of minerals also contain phytic acid. Phytate is considered to be an anti-nutritional factor as it has a high chelating activity, which may decrease the bioavailability of certain elements. Phytate also adversely affects the absorption of other nutrients such as amino acids, proteins and starch. In this study, teff and quinoa flour contained high amounts of phytate (1.52 g/100g and 1.34 g/100g), followed by wholewheat, buckwheat and sorghum (0.77 g/100g, 0.64 g/100g and 0.49 g/100g). White wheat flour, rice, oat and maize flour showed low phytate concentrations (Table 1). These results compare well with literature (Garcia-Estepa, Guerra-Hernandez et al. 1999), apart from maize where a much lower level of phytate was detected in this study.

Polyphenols are a heterogeneous group of molecules (benzene rings with one or more hydroxyl groups) produced as secondary plant metabolites which affect nutritional and sensory properties. The total phenol content is shown in Table 1. Among the different flours this value was significantly higher in buckwheat (465.47 mg/100g) and teff (175.65 mg/100g) and decreased in the following order buckwheat > teff > sorghum > maize > wholewheat > quinoa. Wheat, rice and oat flour showed significantly lower values.

# 3.6 Energy content

Looking at the calculated calorie content of the different flours, oat and rice had the highest values of 402 kcal/100g, followed by sorghum, maize and wheat flour (386, 384 and 381 kcal/100g). Buckwheat, teff and quinoa had calorie contents of 377 kcal/100g, 365 kcal/100g and 359 kcal/100g, respectively. Wholemeal wheat flour shows the lowest calorie content of only 340 kcal/100g.

#### 4 Discussion

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

Table 5 summarises the Dietary Reference Intakes of selected nutrients set by the United States Department of Agriculture (2010) and shows the percentage contribution of 100g of each of the flours. One of the most noticeable differences in contribution between the different breads was the protein content of gluten free flours. By consuming 100g of wheat flour, 21% (for men) and 25% (for women) of the daily required amount of protein is reached. Most gluten free flours contribute less to the protein content. Intakes would be significantly higher if quinoa, buckwheat or teff were used for the production of gluten free breads instead of rice, maize or sorghum.

Low bone mineral density (osteopenia, osteomalacia and osteoporosis) in children and adults with coeliac disease has been described. Osteopenia is reversible in time with a gluten free diet (Kennedy and Feighery 2000). However, the study of Pazianas, Butcher et al. (2005) provided evidence, that even after prolonged gluten withdrawal, calcium absorption remains impaired. Therefore a higher calcium intake by coeliac patients is necessary. From this point of view teff flour is interesting for the production of gluten free products. It contains over 30 times more calcium than for example maize or rice flour and 100g contribute to 15% of the daily recommended intake. The calcium content of quinoa is also higher than in other gluten free raw materials. The mechanism of bone loss in coeliac disease is considered multifactorial and has also been attributed to trace element and magnesium deficiencies (Sategna-Guidetti, Grosso et al. 2000). Ohlund, Olsson et al. (2010) showed a too low magnesium intake in children on a gluten free diet. Incorporation of quinoa, buckwheat and teff flour would have a positive influence on magnesium levels. Already 100g of these flours supply the human body with over 40% of the daily required amount of magnesium (Table 4). Adoption of a gluten free diet may also reduce the intake of iron (Mariani, Viti et al. 1998). (Thompson 2000) could show that gluten free products often contain lower amounts of iron than their glutencontaining counterparts. Sorghum, rice and maize flour are deficient in iron. On the contrary, teff, quinoa and buckwheat flour show a high iron level. Their incorporation in gluten free products would therefore be beneficial. The consumption of 100g quinoa flour contributes 67% of the male and 30 % of the female daily recommended allowance. Teff flour is outstanding, with 100g providing 107% and 47% (male and female, respectively) of the daily required iron (Table 4). However, it has to be kept in mind that despite quinoa and teff show a favourable mineral composition, their high phytic acid levels are considered an antinutritional factor, since this compound binds minerals such as calcium, iron, magnesium, manganese and zinc. This is disadvantageous especially for coeliac patients who often suffer from micronutrient deficiencies. Dietary fibre is another highly important nutrient, which was repeatedly shown

Dietary fibre is another highly important nutrient, which was repeatedly shown not to be consumed in sufficient amounts among coeliac disease patients as well as the general population (Hager, Axel et al. 2011) to wheat flour, oat, quinoa, sorghum and teff flour show higher fibre contents. However, none of the flours screened contain fibre amounts comparable to wholemeal wheat flour. Therefore many gluten free products on the market are fibre enriched (Hager, Axel et al. 2011). Rice and maize flour, the most commonly used raw materials for gluten free products, show significantly lower fibre levels than wheat flours. The consumption of 100g rice flour provides only 1-2% of the dietary reference intake (Table 4).

Fatty acids of specific chain length and saturation are required by humans for structural and metabolic needs. Linoleic and linolenic acid are two essential fatty acids, which cannot be synthesized by the body but have to be taken up as part of the daily diet. All studied cereals are a good source of linoleic acid. Quinoa is relatively higher in linolenic acid, contributing to 26% and 37% (male

and female) of the dietary reference intake. Excessive amounts of omega-6 fatty acids and a very high omega-6/omega-3 ratio, as is found in today's Western diets, promotes diseases such as cardiovascular disease or cancer, whereas increased levels of omega-3 fatty acids, exert a suppressive effect (Simopoulos 2006). Ohlund, Olsson et al. (2010) could show that children on a gluten free diet frequently have a too high intake of saturated fatty acids and a too low intake of polyunsaturated fatty acids. This trend can also be observed in the general population. The characterization of the fatty acid profiles show that in all cereal flours the majority of the lipids are unsaturated.

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

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

Several studies could show, that overweight and obesity are problems often connected with a strict gluten free diet (Mariani, Viti et al. 1998, Castelluzzo, Massoud et al. 2011). Moreover, once coeliac disease has been diagnosed, regeneration of the intestinal mucosa and normalisation of absorptive processes often result in weight gain (Castelluzzo, Massoud et al. 2011). This can be partly related to the fact that gluten free bread products often contain higher amounts of calories than wheat breads of the same category (Hager, Axel et al. 2011). Therefore the use of raw materials with low energy content such as buckwheat, teff and quinoa flour are recommended. Oat and rice flour provide the most calories. This is due to the high starch level of rice and the high fat content of oat flour.

## **5** Conclusion

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

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	Wheat	Wholewheat	Rice	Oat	Quinoa
Protein [g/100g]	$11.54 \pm 1.07c$	9.89 ± 0.17d	7.33±0.03e	6.91±0.08e	13.48 ± 0.04a
Total starch [g/100g]	68.06±2.34b	57.24 ± 0.26c	77.52 ± 0.42a	69.38±1.66c	48.88 ± 2.07d
Amylose [% of total starch]	21.10 ± 1.29ab	21.10 ± 2.08abc	21.38±0.90ab	20.42 ± 2.43bc	4.62 ± 0.83e
Damaged Starch [g/100g]	$7.85 \pm 0.41b$	$4.06 \pm 0.68c$	15.24 ± 1.53a	$4.91 \pm 0.06c$	4.71 ± 0.70c
Total dietary fibre [g/100g]	$3.44 \pm 0.01$ cd	11.42 ± 1.27a	$0.43 \pm 0.15f$	$4.05 \pm 0.40c$	$7.14 \pm 0.23b$
Soluble dietary fibre [g/100g]	$1.34 \pm 0.11a$	1.60 ± 0.40a	$0.14 \pm 0.06d$	$0.36 \pm 0.02$ cd	1.77 ± 0.14a
Phytate [g/100g]	0.16±0.03e	$0.77 \pm 0.01b$	$0.21 \pm 0.01e$	0.27 ± 0.01de	1.34 ± 0.00a
Polyphenols [mg/100g]	$13.04 \pm 0.23d$	$82.20 \pm 0.42c$	$14.16 \pm 2.45d$	22.16±0.16d	78.24 ± 0.46c

	Ruckwheat	Sorahiim	Maiza	Taff
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Protein [g/100g]	$12.19 \pm 0.38^{bc}$	$4.68 \pm 0.04^{f}$	$5.50 \pm 0.19^{f}$	$12.84 \pm 0.51^{ab}$
Total starch [g/100g]	$61.35 \pm 2.15^{\circ}$	$73.20 \pm 1.52^{a}$	$71.52 \pm 0.42^{a}$	57.77 ± 5.94°
Amylose [% of total starch]	$15.95 \pm 0.61^{d}$	$18.18 \pm 0.55^{cd}$	$22.91 \pm 0.82^{a}$	$19.72 \pm 099^{bc}$
Damaged Starch [g/100g]	$2.63 \pm 0.25^{d}$	$4.66 \pm 1.03^{\circ}$	$4.52 \pm 0.30^{\circ}$	$2.08 \pm 0.22^{d}$
Total dietary fibre [g/100g]	$2.18 \pm 0.11^{e}$	$4.51 \pm 0.01^{\circ}$	$2.62 \pm 0.45^{de}$	4.54 ± 0.57°
Soluble dietary fibre [g/100g]	$0.48 \pm 0.17$ cd	$0.72 \pm 0.04$ bc	$0.64 \pm 0.14^{bd}$	$0.85 \pm 0.17^{b}$
Phytate [g/100g]	$0.64 \pm 0.06^{bc}$	$0.49 \pm 0.02$ cd	0.09 ± 0.03€	$1.52 \pm 0.21^{a}$
Polyphenols [mg/100g]	$465.47 \pm 22.41^{a}$	$103.30 \pm 6.06^{\circ}$	97.85 ± 0.64°	$175.65 \pm 1.48^{b}$

8 Tables

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	Wheat	Wholewheat	Rice	Uat	Quinoa	Buckwheat	Sorghum	Maize	Tett
Fat	<b>1.81 ± 0.05</b> <sup>d</sup>	3.63 ± 0.104 °	0.90 ± 0.06 e	6.74 ± 0.80 <sup>b</sup>	8.59 ± 0.25 <sup>a</sup>	4.21 ± 0.74 c	3.50 ± 0.31 °	2.48 ± 0.46 <sup>d</sup>	4.39 ± 0.26 °
Myristic 14:0	$1.48 \pm 0.014$	$0.10 \pm 0.000$	$0.44 \pm 0.002$	$0.24 \pm 0.001$	$0.12 \pm 0.000$	$0.11 \pm 0.000$	0	0	$0.22 \pm 0.003$
Palmitic 16:0	$19.74 \pm 0.076$	$516.97 \pm 0.011$	$22.43 \pm 0.014$	$20.62 \pm 0.001$	$9.77 \pm 0.004$	$15.78 \pm 0.03$	$13.52 \pm 0.21$	$12.62 \pm 0.01$	$10.86 \pm 0.042$
Stearic 18:0	$10.41 \pm 0.094$	ł 0.75 ± 0.000	$2.45 \pm 0.012$	$1.71 \pm 0.007$	$0.63 \pm 0.004$	$2.08 \pm 0.001$	$1.28 \pm 0.00$	$2.07 \pm 0.00$	$4.14 \pm 0.031$
Oleic 18:1, 9c	$31.14 \pm 0.006$	$512.73 \pm 0.007$	$40.01 \pm 0.019$	$41.85 \pm 0.004$	$23.93 \pm 0.004$	$36.53 \pm 0.012$	$30.40 \pm 0.05$	$26.08 \pm 0.01$	29.47 ± 0.320
Linoleic 18:2 9, 12	$23.74 \pm 0.034$	↓ 60.79 ± 0.020	29.38 ± 0.003	$26.56 \pm 0.011$	$52.68 \pm 0.012$	$33.01 \pm 0.010$	$49.31 \pm 0.13$	$54.73 \pm 0.01$	$49.99 \pm 0.183$
α-Linolenic 18:3 9, 12 15	$1.74 \pm 0.004$	$5.04 \pm 0.002$	$1.91 \pm 0.009$	$0.71 \pm 0.014$	$4.60 \pm 0.001$	$3.78 \pm 0.005$	$2.22 \pm 0.01$	$2.08 \pm 0.00$	$2.29 \pm 0.072$
Eicosenoic 20:1 11	$1.61 \pm 0.016$	$0.72 \pm 0.001$	$0.53 \pm 0.007$	$1.06 \pm 0.001$	$1.56 \pm 0.001$	$3.27 \pm 0.007$	$0.32 \pm 0.01$	$0.26 \pm 0.00$	$0.78 \pm 0.017$
Saturated fatty acids	$38.94 \pm 0.038$	$318.97\pm0.004$	26.35 ± 0.035	23.42 ± 0.001	$11.56 \pm 0.005$	$21.43 \pm 0.021$	$15.19 \pm 0.21$	$15.21 \pm 0.00$	$16.14 \pm 0.075$
Unsaturated fatty acids	$60.06 \pm 0.044$	↓80.72 ± 0.001	73.25 ± 0.038	71.78 ± 0.005	85.44 ± 0.003	60.06 ± 0.044	83.81 ± 0.21	84.29 ± 0.00	83.66 ± 0.075
w6/w3	14/1	12/1	15/1	37/1	11/1	9/1	22/1	26/1	21/1
Table 3 Ash content [% (w/w)] and mineral composition of flours [mg/kg]

	Wheat	Wholewheat	Rice	0at 0	Quinoa	Buckwheat	Sorghum	Maize '	ſeff
Ash*	0.92 ± 0.02 f	1.32 ± 0.01 <sup>d</sup>	$0.51 \pm 0.01$ h	0.82 ± 0.01 g	2.43 ± 0.03 <sup>a</sup>	1.65 ± 0.01 ℃	0.97 ± 0.01 <sup>e</sup>	<b>0.37 ± 0.03</b> <sup>i</sup>	2.15 ± 0.05 <sup>b</sup>
Calcium***	$1797.7 \pm 10.5$	$307.7 \pm 4.5$	$50.7 \pm 0.5$	$224.3 \pm 2.1$	$497.3 \pm 1.2$	$148.2 \pm 1.7$	$97.6 \pm 1.4$	$33.2 \pm 1.1$	$1543.0 \pm 2.0$
Magnesium***	$244.0 \pm 1.0$	782.7 ± 2.5	$338.0 \pm 4.0$	392.7 ± 4.5	$2299.0 \pm 17.0$	$1736.0 \pm 13.0$	$849.7 \pm 8.5$	$315.7 \pm 1.5$	$1689.7 \pm 14.5$
Sodium***	$38.1 \pm 3.7$	$19.9 \pm 0.2$	$15.1 \pm 0.3$	$16.7 \pm 3.5$	$37.0 \pm 0.2$	$10.8 \pm 2.6$	$4.9 \pm 0.1$	$4.8 \pm 0.3$	59.8 ± 2.1
Potassium***	$1520.3 \pm 8.5$	3997.7 ± 29.5	973.7 ± 4.5	$1743.7 \pm 16.5$	5537.7 ± 23.5	$4022.7 \pm 24.5$	$2581.0 \pm 5.0$	$1487.0 \pm 7.0$	3827.7 ± 4.5
Iron***	$13.4 \pm 0.1$	$26.9 \pm 0.2$	$6.0 \pm 0.3$	$16.4 \pm 0.1$	$53.5 \pm 0.4$	$28.5 \pm 0.0$	$26.7 \pm 0.2$	$9.1 \pm 0.1$	85.3 ± 2.0
Copper***	$1.51 \pm 0.0$	$4.0 \pm 0.0$	$2.2 \pm 0.0$	$2.7 \pm 0.1$	$7.71 \pm 0.1$	$5.1 \pm 0.0$	$1.8 \pm 0.0$	$0.9 \pm 0.0$	$9.3 \pm 0.1$
Manganese	$8.25 \pm 0.1$	$23.4 \pm 0.1$	$7.3 \pm 0.02$	$27.7 \pm 0.4$	$13.5 \pm 0.0$	$11.8 \pm 0.0$	$11.3 \pm 0.1$	$1.5 \pm 0.0$	$34.5 \pm 0.4$
Zinc***	$7.59 \pm 0.00$	$17.5 \pm 0.4$	$17.8 \pm 0.2$	$11.3 \pm 0.1$	$32.77 \pm 0.1$	$18.8 \pm 0.0$	$10.0 \pm 0.0$	$6.6 \pm 0.1$	$41.5 \pm 0.1$
Chloride <sup>***</sup>	825.6 ± 42.6	998.0 ± 23.7	$350.6 \pm 31.5$	$670.0 \pm 48.5$	$433.8 \pm 0.3$	$144.0 \pm 31.0$	$402.8 \pm 25.36$	$484.2 \pm 23.1$	$481.0 \pm 39.1$
Phosphorus***	908.7±3.5	$2040.7 \pm 3.5$	954.7±16.5	$1476 \pm 25$	$4415.7 \pm 57.5$	$2787.0 \pm 14.0$	$1631.0 \pm 13.0$	$813.7 \pm 8.5$	$3617.0 \pm 11.0$

Table 4 Dietary Reference Intakes (DRIs) for the female and male adult general population and the contribution of 100g flour to the DRIs. Recommended Dietary Allowances (RDAs) are presented in ordinary type, Adequate Intakes (AIs) are followed by an asterix (\*).ª Source: USDA Dietary Reference Intakes

Nutrient	Gender	DRIS <sup>a</sup>						Contr	ibutions of 100	g flour to DRI	s (%)	
				Wheat	Wholewheat	Rice	0at	Quinoa	Buckwheat	Sorghum	Maize	Teí
Protein	Μ	56	g/d	21	18	13	12	24	22	8	10	23
	Ц	46	g/d	25	22	16	15	29	27	10	12	28
Linoleic Acid	Μ	$17^{*}$	g/d	3	13	2	11	26	8	10	8	13
	Ч	$12^*$	g/d	4	18	2	15	37	12	14	11	18
α-Linolenic Acid	Μ	$1.6^{*}$	g/d	2	11	1	3	24	10	ß	3	9
	Ч	$1.1^{*}$	g/d	3	17	2	4	36	15	7	ß	6
Dietary Fibre	Μ	38*	g/d	6	30	7	11	19	9	12	7	12
	Ч	25*	g/d	14	46	2	16	29	6	18	10	18
Folate	M/F	400	hg/d	S	6	8	7	45	33	19	6	24
Calcium	M/F	1000	mg/d	18	£	1	2	ß	1	1	0	15
Phosphorus	M/F	700	mg/d	13	29	14	21	63	40	23	12	52
Sodium	M/F	$1.5^{*}$	g/d	41	<1	4	41	~ 1	<1	4	41	$^{\prime}$
Potassium	M/F	4.7*	g/d	3	6	2	4	12	6	ß	3	8
Chloride	M/F	2.3*	g/d	4	4	2	3	2	1	2	2	2
Copper	M/F	006	hg/d	17	44	24	30	86	57	20	10	103
Magnesium	Μ	420	mg/d	9	19	8	6	55	41	20	8	40
	Ч	320	mg/d	8	24	11	12	72	54	27	10	53
Zinc	Μ	11	mg/d	7	16	16	10	30	17	6	9	38
	Ч	8	mg/d	10	22	22	14	41	24	13	8	52
Iron	Μ	8	mg/d	17	34	8	21	67	36	3	11	107
	ц	18	mg/d	7	15	ŝ	6	30	16	2	S	47

## 9 Figures



Figure 1 Scanning electron micrographs of the gluten free and wheat flours (magnification x2000): (a) buckwheat; (b) quinoa; (c) teff; (d) sorghum; (e) maize; (f) rice; (g) oat; (h) wholemeal wheat; (i) wheat;



Figure 2 Gel view representative of banding patterns: (L) Ladder; (1) teff; (2) sorghum; (3) rice; (4) buckwheat; (5) maize; (6) wholewheat; (7) wheat; (8) oat; (9) quinoa

## Oral and poster presentations

Wolter, A., Galle, S., Hager, A.-S., Zannini, E., Arendt, E.K. (2011) Importance of Sourdough to Improve quality of gluten-free bakery products, Oral presentation in German: GDL-Forum "Sourdough IV", Minden, Germany (Society of German Food Technologists)

Wolter, A., Galle, S., Hager, A.-S., Zannini, E., Arendt, E.K. (2011)
Screening of various gluten-free flours related to nutritional value, ultrastructure and suitability for bread production,
Oral presentation in German: 62<sup>nd</sup> Conference Cereal Chemistry,
Arbeitsgemeinschaft Getreideforschung e.V., Detmold, Germany

Wolter, A., Hager, A.-S., Zannini, E., Arendt, E.K. (2011) Structural comparison of gluten-free flours, dough and breads, 40<sup>th</sup> Annual UCC Food Research Conference, 31<sup>st</sup> March, 2011

Wolter, A., Hager, A.-S., Zannini, E., Arendt, E.K.

*In vitro* starch digestibility and estimated glycaemic index of various gluten-free breads upon sourdough addition,

42<sup>nd</sup> Annual UCC Food Research Conference, Teagasch, Ashtown, 26<sup>th</sup> June 2013

## **Poster presentation**

Wolter, A, Hager, A.-S., Zannini, E, Galle, S, Gänzle, MG, Arendt, EK (2013) Exopolysaccharide producer as sourdough starter in gluten-free flours? 3<sup>rd</sup> Gluten-free Symposium, Vienna, Austria, June 2013

Wolter, A, Hager, A.-S., Zannini, E, Arendt, EK (2013) Can sourdough influence starch digestibility and *in vitro* glycaemic indices of gluten-free breads?

Best Poster Award – International Association for Cereal Science and Technology