Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Exopolysaccharides from Sourdough Lactic Acid Bacteria

Sandra Galle [a b] & Elke K. Arendt [a]
[a] University College Cork, Department of Food and Nutritional Science, Cork, Ireland
[b] University College Cork Biotransfer Unit, Ireland, Cork, Ireland
Accepted author version posted online: 19 Feb 2013.

To cite this article: Sandra Galle & Elke K. Arendt (2013): Exopolysaccharides from Sourdough Lactic Acid Bacteria, Critical Reviews in Food Science and Nutrition, DOI:10.1080/10408398.2011.617474

To link to this article: http://dx.doi.org/10.1080/10408398.2011.617474

Exopolysaccharides from Sourdough Lactic Acid Bacteria

Sandra Galle[1,2] and Elke K. Arendt[1]*

[1]*University College Cork, Department of Food and Nutritional Science, Cork, Ireland, e.arendt@ucc.at*

[2]*University College Cork Biotransfer Unit, Ireland, Cork, Ireland, s.galle@umail.ucc.ie*

Mailing address: School of Food and Nutritional Sciences,

University College Cork, Western Road, Cork, Ireland

Tel.:00353 (0)21 4902064

E-mail:e.arendt@ucc.ie

Fax:00353 (0)21 4270213

Abstract

The use of sourdough improves the quality and increases the shelf life of bread. The positive effects are associated with metabolites produced by lactic acid bacteria (LAB) during sourdough fermentation, including organic acids, exopolysaccharides (EPS) and enzymes. EPS formed during sourdough fermentation by glycansucrase activity from sucrose influence the viscoelastic

properties of the dough and beneficially affect the texture and shelf life (in particular starch retrogradation) of bread. Accordingly, EPS have the potential to replace hydrocolloids currently used as bread improvers and meet so the consumer demands for a reduced use of food additives. In this review the current knowledge about the functional aspects of EPS formation by sourdough LAB especially in baking applications is summarized.

1. Introduction

Lactic acid bacteria (LAB) have great industrial importance in the production of fermented food, such as dairy products, fermented sausages and sourdoughs. They contribute considerably to the microbial safety and organoleptic properties of fermented foods and posesss the generally regarded as safe (GRAS) status (Lindgren and Dobrogosz, 1990). In the last decades, the physiology and genetics of LAB were, and still remain, the subjects of major research efforts. The most important characteristic of LAB is the production of lactic acid, which lowers the pH and thus exerts an inhibitory effect on spoilage microorganisms (Hammes et al., 1996). Beside organic acid production and their the preservative effect, LAB posses numerous metabolic activities, which ranges from the formation of flavour precursors to the excretion of antimicrobial compounds including bacteriocins, which can inhibit the accompanying LAB as well as some foodborne pathogens and spoilage organisms (Gänzle, 2009a). An interesting property of some strains of various genera within the LAB group is their ability to synthesise exopolysacchrides (EPS). EPS exhibit a positive effect on the texture, mouthfeel, taste perception and stability of fermented food and, moreover, for certain EPS prebiotic effects have also been described (Korakli et al., 2002; Korakli et al., 2001; Tieking and Gänzle, 2005).

The biological role of EPS production in LAB has not been clearly established. Most of the EPS-forming bacteria cannot use their EPS as a nutrient reserve, due to the lack of EPS degrading enzymes (Cerning, 1990). In their natural environment, EPS were found to protect the microbial cell from environmental stresses as e.g. nisin, starvation, membrane stress and low pH (Kim et al., 2000; Looijesteijn et al., 2001; Schwab and Gänzle, 2006). EPS play also an important role in biofilm formation and cell-cell adherence and therefore was demonstrated to support EPS producing strains, e.g. *Lactobacillus reuteri*, to survive in intestinal passages or persist in the intestinal tract (Sim et al, 2011; Walter et al. 2008). Likewise, a protective effect of EPS against low pH was demonstrated (Kaditzky et al. 2008). It was also proposed, that EPS production may result in osmotic and energetic advantages (Korakli and Vogel, 2006).

Most of the polysaccharides used in the food industry as thickening, stabilising, texturizing and gelling agents are currently derived from plants (e.g. starch and its modified derivatives, pectin and Arabic gum) or seaweed (e.g. alginate and carrageenan). In the last decades, microbial EPS have been described as alternatives for plant polysaccharides. Examples for industrially important microbial EPS are xanthan from *Xanthomonas campestis* and gellan from *Sphingomonas paucimobilis*, which have found various food and non-food applications (Sutherland 1998) Dextran synthesised by *Leuconostoc mesenteroides* was one of the first biopolymers produced on an industrial scale and found to have several applications in medicine, separation technology and biotechnology (Soetaert et al., 1995). Due to the safe nature of most LAB, research interest in novel EPS for food and medical applications has now initiated a strong interest to these bacteria. In the last decades several reviews on EPS produced by LAB have been published (Cerning, 1990; De Vuyst et al., 2001; De Vuyst and Degeest, 1999), they focus

primarily on the classification, chemical composition and structure of EPS composition, biosynthesis and genetics of EPS (Korakli and Vogel, 2006; van Hijum et al., 2006) and on their application mainly in dairy products (De Vuyst, 2001). Although, most of the EPS- producing lactobacilli were isolated from dairy products such as fermented milk, yoghurt and kefir grains (De Vuyst and Degeest 1999), a high proportion of producer strains was isolated from cereal fermentations and the intestinal tract (Tieking and Gänzle, 2005). Different sourdough lactobacilli, *Ln. mesenteroides* and *Weissella* strains were shown to produce various EPS such as glucan and/or fructans. Suitability of EPS produced by sourdough LAB to replace or reduce plant hydrocolloids used in the bread making process has been suggested in order to improve dough rheological parameters and bread quality (Di Cagno et al., 2006; Katina et al., 2009; Lacaze et al., 2007; Schwab et al., 2008; Tieking and Gänzle, 2005; Tieking et al., 2003). Additionally, oligosaccharides and other metabolites generated during EPS formation from sucrose have also shown to effect physiological (health promoting) and technological properties in baked goods (Kaditzky et al., 2008b; Korakli, Gänzle et al., 2002). The better understanding of the fermentation performance of EPS producing LAB along with the structure-function relationship of EPS in the fermented product is crucial for further technological applications. This overview deals with the recent information of EPS forming sourdough LAB and their performance in baking applications.

2. Biosythesis of EPS and enzymes involved

Depending on their composition and biosynthesis mechanism, EPS can be classified into homo- (HoPS) and heteropolysaccharides (HePS). HoPS consists of one monosaccharide (mostly

fructose or glucose) and are usually produced in large amounts of up to 40 g l^{-1} from sucrose by the action of glycansucrases. In addition to HoPS, these enzymes also form glucooligosaccharides (GOS) and fructooligosaccharides (FOS) in the presence of acceptor sugars (Korakli et al., 2003; Monsan et al., 2001). In contrast to HoPS, HePS are composed of irregular repeating units that are synthesised from sugar nucleotides by the activity of intracellular glycosyltransferases. In comparison to the high yield obtained to from glycansucrases, EPS synthesis by glycosyltransferases of LAB typically yields less than 1g L^{-l} (De Vuyst and Degeest, 1999).

2.1. Heteropolysaccharides

The most common monosaccharides present in the HePS repeating units are galactose and glucose followed by rhamnose. Fructose and fucose and other residues such as N-actyl-glucaosamine and N-acetyl-galactosamine can also be present (De Vuyst and Degeest, 1999). Compared to extracellular biosynthesis of HoPS, the mechanism of HePS assembly appears complex. HePS are formed in the cytoplasm by polymerizing repeating units, consisting of three to eight monosaccharides. During HePS synthesis, a lipid carrier present in the membrane supports the assembly of repeating units catalysed by glycosyltransferases using sugar nucleotides as substrate. After completion the repeating units are transported across the membrane to the outer layer and polymerized to form the final HePS. The synthesis is similar to the cell wall synthesis and is energy-dependent. Several enzymes and/or proteins are involved in the biosynthesis and secretion of HePS and have been examined and reviewed in detail (De Vuyst et al., 2001; De Vuyst and Degeest, 1999; Ruas-Madiedo et al.; 2009). Despite belonging to homopolysaccharides, β-glucan biosynthesis observed in *Pediococcus damnosus*, *Pediococcus*

parvulus and lactobacilli resembles heteropolysaccharide formation (Garai-Ibabe et al.; Werning et al., 2006). Beta-glucan production among LAB is rare and to date, the formation by sourdough associated LAB has not been described. HePS are mostly found in dairy fermentation to improve texture of yoghurt and other fermented milk products, but were recently also employed in sourdough fermentation (Galle et al., 2010b).

2.2. Homopolysaccharides

HoPS consist of one monosaccharide, glucose or fructose with the resulting EPS designated glucans or fructans, respectively. The biosynthesis of HoPS is cell wall bound or extracellular through the activity of glycansucrases (glycosyltransferases) and requires the specific substrate sucrose. The energy required for the reactions of the glycansucrases is obtained from the cleavage of the osidic bond in sucrose. Therefore HoPS can be produced in large amounts in comparison to energy-consuming biosynthesis of HePS. The enzyme involved in the biosynthesis of fructans or glucans are also termed glucansucrase (GS) and fructansucrase (FS), respectively. Some authors have referred to GS as glucosyltransferases and FS as fructosyltransferases. However, the glycosyltransferases which also catalyse the transfer of different sugar units, are not descriptive for the substrate and product specificity of sucrose-type enzyme (van Hijum et al., 2006). Therefore, in this review we use the terms GS and FS for enzymes using sucrose to form HoPS.

2.2.1. Fructan synthesis by fructansucrases

FS are secreted for the synthesis of high molecular fructans or low molecular fructooligosaccharides (FOS). FS can be divided into the following groups: levansucrases, which synthesize fructans composed of β (2-6)-linked fructose units (levan) and inulosucrases (van Hijum 2006), which synthesize fructans composed of β (2-1) linked fructose units (inulin). FS catalyze three different reactions depending on the nature of the acceptor molecule: (1) hydrolysis of sucrose, when water is used as acceptor, (2) transfer reaction when sucrose or kestose are use as acceptor (FOS synthesis) and (3) polymerization, when the growing fructan chain is used as an acceptor (fructan synthesis).

Carbohydrates vary in their efficiency to act as acceptors to FS. The presence of sucrose as acceptor carbohydrate results in formation of kestose and higher inulin-type fructo-oligosaccharides. The ability of *L. sanfranciscensis* levansucrase to use raffinose, maltotriose, maltose, xylose, or raffinose as fructosyl acceptors, leading to the formation of a range of heterooligosaccharides during wheat sourdough fermentation, has been reported recently (Tieking et al., 2005b). When lactose and fructose are present as acceptors, lactosucrose or lactulose are formed, respectively (Gänzle et al., 2009b).

Several FS-encoding gens from LAB have been identified in the last years (Tieking et al., 2005a; van Hijum et al., 2001). They are closely related and share a common structure, which was reviewed by Korakli and Vogel (2006). Most of the known FS enzymes are levansucrases and only few inulosucrases have been identified so far. In sourdough associated lactobacilli levansucrases have been identified and characterized, namely the levansucrase of *L. panis* (Waldherr et al., 2008), *L. reuteri* (van Hijum et al., 2004) and *L. sanfranciscensis* (Tieking et

al., 2005a). Inulinsucrase were found in cereal associated *L. reuteri* (van Hijum et al., 2003, Schwab et al., 2007) and *Ln. citreum* (Olivares-Illana et al., 2003).

2.2.2. Glucan synthesis by glucansucrases

Analog to FS, GS are extracellular enzymes cleaving their substrate sucrose transferring glucose monomers to an acceptor using the energy of cleaved glycosidic bond. Different groups of acceptor molecules are used by GS including maltose and isomaltose (OS synthesis) and glucan (resulting in polymerisation). The type of glucan produced is determined by the enzyme. According to the glucosidic linkages present in the polymer these enzymes are classified as (i) dextransucrases synthesising dextran mainly consisting of α-(1-6) backbone along with some α-(1-2), α-(1-3) and α-(1-6) branching, (ii) mutansucrases forming mutan [α-(1-3)], (iii) alternansucrase synthesising alternan composed of alternating α-(1-6) and α-(1-3) glucosidic linkages, and (iv) reuteransucrase forming reuteran containing α-(1-4) and (1-6) linkages (Korakli and Vogel, 2006, Van Hijum et al., 2006)

Acceptor carbohydrates for GS include glucose, maltose, isomaltose and lactose. Maltose and isomaltose are strong acceptors for dextransucrases leading to high yields of OS and decrease of glucan synthesis, whereas fructose and melobiose act as weak acceptor sugars and are leading to a decreased OS yield. Reuteransucrase from *L. reuteri* produces with glucose as acceptor maltose and isomaltose (Kralj et al., 2004). Panose and maltotriose are produced by the same GS from sucrose with maltose as an acceptor. Additionally, fructose was reported to be used as an acceptor to produce a disaccharide called leucrose, where the glucose from sucrose is transferred

to fructose. This reaction begins when fructose is accumulated at the end of the glucan synthesis (Korakli and Vogel, 2006).

In contrast to the widespread distribution of bacterial FS throughout different bacterial groups, GS are found only in the group of LAB. The reason for this remains unknown. Several GS have been characterized from stains of the genera *Streptococcus* and *Leuconostoc* and *Lactobacillus* (van Hijum et al., 2006). Reuteransucrase was reported to be produced by two strains of *L. reuteri* and the corresponding genes have been identified (Kralj et al., 2005). Among sourdough LAB the presence of dextransucrase genes were detected in *Ln. mesenteroides, W. cibaria, W kimchii* (Galle et al., 2010a; Schwab et al., 2008). Furthermore, the production and partial characterization of dextransucrase from several dextran-producing *W. cibaria* and *W. confusa* and alternansucrases form *Ln. citreum* strains isolated from French sourdough was recently reported (Bounaix et al., 2010a, 2010b,).

Several LAB stains were shown to possess more than one glycansucrase. The presence of several glycansucrases in a single *L. reuteri* strain is the rule rather than the exception (Kralj et al., 2002; van Hijum et al., 2001; van Hijum, van der Maarel et al., 2003; Walter et al., 2008). For example *L. reuteri* TMW 1.106, isolated from a type II sourdough, harbors two glucansucrases and one inulosucrase and produces a glucan from sucrose in MRS medium and during sourdough fermentation (Schwab et al., 2007).

2.2.3 Influence of sucrose on glycansucrases

Alternatively to sucrose turnover by extracellular glycansucrases sourdough LAB can use sucrose through intracellular sucrose phosphorylase or invertase (Schwab et al., 2007; Walter et

al., 2008). However, in some strains glycansucrases are the only enzymes able to metabolize sucrose. For example in *L. sanfranciscenis* TMW 1.392 levansucrase expression is not regulated by sucrose but deletion of levansucrase abolished the ability of the strain to grow with sucrose, indication that levansucrases is the sole active sucrose utilization system (Tieking et al., 2005a). The impact of different glycansucrases on sucrose metabolism of *L. reuteri* was also shown to be strain depended. In strain *L. reuteri* TMW 1.106, GS accounted for sucrose utilization, metabolism and growth of the organism. In contrast, FS of *L. reuteri* LTH5448 was shown to contribute to sucrose turnover but alternative routes for sucrose metabolism were functional in this strain (Schwab et al., 2007).

Glycansucrases and hydrolysis activity were reported to be dependent on sucrose concentration, pH and temperature (Korakli et al 2003, van Hijum et al. 2002). In *L. sanfranciscensis*, the yield of levan and kestose increased with increasing sucrose concentration, indicating an increase in the FS activity, while the hydrolysis activity decreased with increasing sucrose concentration (Korakli et al., 2003). Similar results were reported for GS from *L. reuteri* (Kralj et al., 2004). Furthermore, the ratio of the products (fructan or FOS) differs significantly from enzyme to enzyme. Higher sucrose levels favoured both the production of kestose and levan by *L. panis* FS (Waldherr et al., 2008) whereas only kestose formation was enhanced relative to levan formation by *L. sanfranciscensis* FS (Tieking et al. 2005).

Optimal glycansucrase activity lies in the temperature range of 35-50 ºC and pH optimum ranges between pH 4.5 and 5.4 (Kralj et al., 2004; Tieking, Ehrmann et al., 2005a; Waldherr et al., 2008). Accordingly, maximum dextran formation in pH static fermentations was reached at a pH

range of pH 4.7-5.7 (Kaditzky et al., 2008a). Thus, glycansucrases are active under the conditions of sourdough fermentation.

Taken together, the identification and characterization of glycansucrases will enable optimization of dough fermentations with the aim of improving bread quality through increased levels of EPS and oligosaccharides in dough.

3. Sourdough LAB

Sourdough plays an important role in baking technology by improving aroma, texture, shelf life and mineral bioavailability (Arendt et al., 2007). Sourdough hosts specific LAB and yeasts which are well adapted to the environment (De Vuyst and Vancanneyt, 2007; Gobbetti et al., 2008). The composition of the sourdough microbiota is significantly influenced by endogenous (e.g. chemical and enzyme composition of the flour) and exogenous (e.g. temperature, redox potential) factors (Hammes and Gänzle, 1998). In practice, strong impacts are caused by process parameters such as dough yield, number of propagation steps and fermentation time (De Vuyst and Neysens, 2005). Typical sourdough LAB mainly belong to the hetero- and homofermentative strains *of Lactobacillus, Leuconostoc, Pediococcus* and *Weissella* genera (Corsetti and Settanni, 2007; De Vuyst and Neysens, 2005). Traditional sourdough prepared with wheat or rye flour, are populated by a comparable micorbiota (De Vuyst and Vancanneyt, 2007). In general species of *Lactobacillus*, in particular the obligative heterofermenative *L. sanfranciscensis* or closely related organisms such as *L. rossiae*, *L. brevis*, and *L. spicheri* are invariably present in wheat and rye sourdough and frequently occur in association with *L. plantarum*, and *L. paraalimentarius*. Thermophilic lactobacilli such as *L. reuteri*,

L. fermentum or *L. points* become dominant when fermentation is carried out at higher temperature. Species belonging to the genera *Enterococcus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus* and *Weissella* are less frequent encountered. However, recently *Leuconostoc* and *Weissella* spp. were also found to dominate traditional French and Italian sourdoughs (Bounaix et al, 2009; Zotta et al., 2008). In recent years, gluten free fermentations from various gluten free cereals for the production of high quality gluten free bread has received increased interest (Moore et al., 2007; Moroni et al., 2011; Schober et al., 2007). Microbiological characterisation indicates an overlap with the microbiota of wheat and rye fermentation (Moroni et al., 2009; Vogelmann et al., 2009). Nevertheless, species such as *L. gallinarum, L. graminis, L. sakei* and *Pediococcus pentosaceus*, which are not considered endemic to traditional sourdoughs, were present in various gluten-free sourdoughs, in particular when produced by spontaneous fermentation (Huettner et al., 2009, Moroni et al 2010a, Vogelmann et al. 2009,). It can therefore be concluded that the competitiveness of LAB are not only influenced by the technological parameters but are also depended on the cereal (gluten-free) substrate, i.e. the unique sugar composition of certain flours (Galle et al., 2010a; Moroni et al., 2010a,b).

4. EPS from sourdough LAB

Screening of EPS producing sourdough strains is limited to wheat and rye fermentations. Considering the various species found in gluten free fermentations, sourdoughs made of alternative grains may be a good source for EPS producing strains. In fact, we recently isolated two dextran producing *W. cibaria* strains from buckwheat sourdough (unpublished data). In traditional fermentations homopolysaccharide production by sourdough lactobacilli belonging to

the species *L. frumenti*, *L. reuteri*, *L. pontis*, *L. sanfranciscenis*, which were shown to produce fructans (levan or inulin) as well as glucans (dextran, reuteran or mutan) as recently reviewed (Tieking and Gänzle, 2005). Furthermore strains belonging to the species *W. cibaria*, *Ln. mesenteroides*, *L. plantarum* and *L. paraplantarum* isolated from durum wheat sourdough produced EPS, unfortunately these EPS were not structural characterized (Zotta, Piraino et al., 2008). Tieking and Gaenzle (2005) postulated, that the probability of any sourdough flora containing at least one EPS producing strain is high. This is in accordance with a previous study, where each of the nine analysed traditional French sourdoughs contained at least one EPS producing strain. It was remarkable that out of thirty strains isolated twenty three produced homopolysacchairdes. Nine *Ln. citreum*, five *Ln. mesenteroides*, five *W. cibaria* and one *W. confusa* strains produced only glucan, two *L. sanfranciscenis* strains produced fructan and one strain *Ln. mesenteroides* synthesized both glucan and fructan. Interestingly, the sourdough isolated LAB exhibited diversity with regard to EPS-producing LAB species and to polymer structure. Strains that synthesised glucans showed high variation in the amount of α-(1-2), α-(1-3) and α-(1-6) linkages (Bounaix, Gabriel et al., 2009). The large structural varieties of EPS isolated from sourdough include mainly HoPS. To date, only one study reported the production of HePS from a sourdough isolate namely *L. curvatus*. The produced HePS was composed of galactosamine, galactose and glucose in a ratio of 2:3:1, respectively (Van der Meulen et al., 2007). All together this indicates, that traditional as well as gluten free sourdough offers an attractive biotope for the isolation of lactic acid bacteria producing novel polymers.

5. *In situ* EPS formation

It was demonstrated, that LAB strains producing EPS in culture media also form the same polymer during sourdough fermentation in the presence of sucrose (Katina et al., 2009; Korakli et al., 2003; Tieking and Gänzle, 2005). An overview on *in situ* formed EPS and OS of sourdough starter cultures is given in Table 1. Dough improvers such as xanthan and guar gum are effective when added at levels between 0.1-1% (1-10g kg^{-1}) (Guarda et al. 2004). EPS formation of *Weisella* spp. and *L. sanfranciscenis* reaching levels up to 16 g kg^{-1} and 5g kg^{-1} shows the potential of EPS to replace hydrocolloids. Several factors, such as dough yield, fermentation time, pH, sucrose content and fermentation substrate influences the amount of EPS formed *in situ*. Recently, Kraditzky and Vogel (2008c) reported, that glucan formation of *L. reuteri* from sucrose was higher in softer doughs, probably because of better diffusion of the substrates and extracellular glycansucrases. EPS yield was also improved when sucrose was added step wise (fed-batch) to the fermenting dough. Furthermore, with regulation of the pH to a constant value of 4.7 the EPS level increased. Interestingly, fermentation with wheat flour, rye wheat mixture or rye bran with 10% sucrose addition showed that EPS production was most efficient and fastest when in rye bran was supplied as a substrate. The higher buffering capacity of the rye bran ensured a slower decrease of pH and thus better growth conditions for the strain. In accordance, dextran forming *W. cibaria* were found to produce 5.8 g kg^{-1} in wheat sourdough and 8 g kg^{-1} in sorghum sourdough showing higher buffering capacity compared to wheat sourdough (Table 1) (Galle et al., 2010a). Also, levan producing *L. sanfranciscensis* was found to produce more EPS in substrates with higher buffering capacity, thus it can be suggested that these approaches and findings can be transferred to other EPS producing bacteria (Kaditzky et al., 2008b). It has to be considered, that the optimization of EPS formed *in situ* from sucrose

leads to alternative production of oligosaccharides, organic acids, mannitol, glucose and fructose. The choice of strain and further the flour used have a considerable effect on the oligosaccharide formation. In particular, the presence of maltose in wheat flour led to maltooligosaccharide formation by *L. reuteri* (Kaditzky et al., 2008b), and to formation of arabsucrose and erlose, 1-kestose and nystose by *L. sanfranciscenis* (Tieking et al., 2005c). *W. cibaria* and *W. kimchii* both employed as starter cultures in wheat fermentations formed two distinctive pattern of OS. *W. cibaria* rather elongated oligosaccharide during the course of wheat fermentation, whereas *W. kimchii* enriched panose and glucosylated panose up to a polymerization degree of 8 (Galle et al., 2010a). In sorghum fermentations the presence of glucose as acceptor sugar allowed the formation of isomaltose, isomaltotriose and higher isomaltooligosaccharides by *W. cibaria* (Figure 1.) (Galle et al., 2010a; Schwab et al., 2008).

The formation of oligo- and polysaccharides from added sucrose results in the release of either glucose or fructose and thus affects organic acid formation. In obligate heterofermentative LAB, glucose released from sucrose is metabolized to lactate, ethanol and CO2. In *L. sanfranciscenis* and *L. reuteri*, fructose is preferably used as electron acceptor by the activity mannitol dehydrogenase, which results in reduced ethanol formation and formation of mannitol and acetate in a molar ration of 2:1. Interestingly, sourdoughs fermented *W. confusa* were shown to be less acidic than sourdoughs produced with other LAB (Katina et al., 2009). Schwab and Galle reported that the closely related species *W. cibaria* and *W. kimchii* lack the activity of mannitol dehydrogenase as no mannitol and only small amounts of acidic acid are formed during sorghum and wheat fermentations and, thus leading to less acidification. In addition to the preservative effect, acetate affects the sensorial quality of the resulting bread. Syntheses of organic acids are

also enhanced through prolongation of the fermentation time. Kaditzky et al (2008) found, that EPS formation did not increase proportional compared to organic acid synthesis. Since intensive acidification also negatively influences bread quality an optimal fermentation time for *in situ* EPS production of 24h was proposed. The accumulation of glucose and fructose not only affects organic acid formation it also may affect yeast metabolism in co-cultures of yeast and lactobacilli. High glucose concentrations support the gas production by yeast and thus contribute to dough leavening but repress maltose utilization in baker's yeast (Gobbetti et al., 1995; Korakli et al., 2001).

Overall, these factors have to be taken into consideration when applying EPS sourdough in baking applications. However more research is required to identify the suitable starter and fermentation condition for achieving optimal EPS production *in situ*. Improved knowledge will allow in future, depending on the flour used, to adjust EPS, oligosaccharides and organic through strain selection and fermentation condition.

6. Effect of *in situ* formed EPS on the bread quality

Bacterial HoPS act in sourdough systems as hydrocolloids. Hydrocolloids modify starch gelatinisation and are applied as fat replacers and gluten substitutes in the production of gluten free bread and as fibre source (Rosell et al., 2001). In the baking industry their potential as bread improvers is of growing importance. EPS formed from sucrose can be used to replace or reduce expensive hydrocolloids currently used as bread improvers. The beneficial influence of EPS from LAB on one or more of the following technological properties has been suggested: (i) water absorption of the dough, (ii) dough rheology and machinability, (iii) dough stability during

frozen storage, (iv) loaf volume, (v) bread staling (Tieking and Gänzle, 2005). In bread two different ways of applications of EPS are possible: Either the *ex situ* production and addition of the EPS as pure substance or *in situ* production by the use of starter cultures during fermentation. *In situ* EPS production has the advantage to enhance dough and bread properties without having them mentioned on the ingredients list, which meets the increasingly consumer demands for fewer additives in food products. Additionally, EPS are not obtained separately and thus decreases production costs. Furthermore, when added *in situ*, EPS was more effective compared to externally added levan (Brandt et al., 2003).

In conventional wheat baking the application of EPS forming starter cultures has shown to lead to improved bread quality (Table 2.). Compared to sourdough prepared with an EPS-negative strain, the sourdough started with EPS forming *W. cibaria* and *L. plantarum* increased the viscosity of the sourdough and when added at 20% the resulting bread had higher specific volume and lower firmness (Di Cagno et al., 2006). Recent studies have shown a significant impact of sourdough rich in dextran on wheat, rye and gluten free bread quality (Figure 2). Dextran can bind high amounts of water and, thus leads to an improved freshness of the end product. Furthermore, dextran improves dough stability and gas retention through a structure build up of the dextrans and interaction with the gluten network. Dextran with a high molecular weight and a linear chain structure are more efficient in their effect on increasing bread volume than dextran with a high molecular weight and more branching. (Decock and Cappelle, 2005; Lacaze et al., 2007; Ross et al., 1992). In panettone, a traditional Italian sweet bread, dextran from *Ln. mesenteroides* is responsible for the long storage stability (Decock and Cappelle, 2005). In this product, dextran production has been optimized by refreshing the doughs seven times

with increasing amounts of sugar. Due to this process, the microflora is able to adapt to high sucrose levels and can reach 25% of dextran based on dry matter. Also Lacaze (2007) obtained improved freshness, crumb structure, mouthfeel and softness of wheat and rye breads using sourdoughs prepared with *Ln. mesenteroides* producing a dextran. When used in milk bread, a sweet wheat bread, significant reduction in bread firmness during two weeks of storage was obtained, while in rye bread the dextran enriched sourdough accounted for significant decrease in firming and a major increase of the specific volume. Remarkably, strains belonging to the genera *Weissella spp.* form also significant amounts of dextran *in situ* (Galle et al., 2010a; Katina et al., 2009; Schwab et al., 2008) and due to its low acetate formation differ from heterofermenative lactobacilli and *Leuconostoc spp*. Previously, Katina et al. (2009) employed dextran forming *W. confusa* at a level of 43% sourdough addition to improve bread quality. This approach enables a higher concentration of dextran in the final bread dough without negative impacts on the flavour and texture of the bread. In wheat baking normally the addition levels of sourdough are between 10-20% (Brandt and Gänzle, 2005), since intensive acidification is known to negatively influence bread volume, crumb hardness and firming kinetics (Barber et al., 1992; Kaditzky et al., 2008b). It has to be noted, that the concomitant production of organic acid during *in situ* EPS formation can counterbalance the positive effects generated by the presence of EPS. Kaditzky et al. (2008b) described *in situ* production of levan from *L. sanfranciscensis*, which was counteracted by the increased acidification by the strain and therefore, did not achieve the same positive effect on bread quality compared to bread with externally added levan. Beside EPS yield and the metabolites formed by the microorganisms parallel to EPS formation, the impact of the polymere properties have to be also considered. In comparative studies, fructan and reuteran was

compared to dextran and, it was found when added at 5g kg^{-1} dextran had a greater effect on viscoelastic properties of wheat doughs and on the volume of breads compared to the same level of fructan or reuteran (Tieking and Gänzle, 2005).

To date, most of the studies describe the effect of *in situ* formed EPS on wheat and rye bread quality but only little information is available on EPS formation and their role in gluten free bread. Schwab *et al.* (2008) recently analyzed the applicability of the EPS-producers *L. reuteri* and *W. cibaria* in gluten-free sorghum sourdoughs. In sorghum sourdoughs, *L. reuteri* and *W. cibaria* produced significant amounts of levan and dextran respectively, which were comparable to those measured in wheat and rye sourdough. Improvements in the sorghum bread quality was only achieved when dextran forming, low acidifying *W. cibaria* was added to the formulation, indicating that the polymer properties as well as metabolic traits play an important role in gluten free baking (Schwab et al., 2008). Recently, HePS produced by *L. buchneri* were shown to have a significant impact on the dough rheology of sorghum sourdough (Galle et al., 2010b). Interestingly, HePS influenced the rheological properties only in sorghum sourdoughs but not in wheat sourdoughs. Remarkably, it was the first report of HePS production and application in cereal fermentations. The use of LAB producing HePS could be advantageous in baking applications, since in comparison to HoPS formation, no sucrose is needed for HePS production and consequently does not affect acetate levels. Moreover, the use of LAB producing HePS expands the variety of cultures as well as the diversity of polysaccharides produced by sourdough starter cultures for the use in baking.

The results collected so far suggests, when applied in baking, it is necessary to select an EPS producing starter culture not solely along its polymer properties and polymer yield but also based on its by-products and fermentation performance.

7. Health beneficial of EPS

Sourdough-originated EPS also provide opportunity to improve gut health on individuals consuming the product. Prebiotic oligosaccharides are defined as substances, which are not digested in the small intestine, stimulate colonic carbohydrate fermentation to short-chain fatty acids, and increase cell counts of the intestinal microbiota, particularly bifidobacteria. (Cummings et al., 2001). Dextran is metabolised by gut microbes to acetate, butyrate and propionate. Propionate has been postulated to have several beneficial effects, such as reducing cholesterol and triglyceride levels and increased insulin sensitivity (Jann et al. 2006). Among the EPS from sourdough LAB, levan produced by *L. sanfranciscensis* and isomaltooligosaccharides (IMO) from *Ln. mesenteroides* has shown to posses prebiotic properties *in vitro* (Dal Bello et al., 2001; Korakli et al., 2002). Fructooligosaccharides are not suitable for baking applications as they are hydrolyzed by yeast invertase at the dough stage, in contrast, gluco-oligosaccharide (i.e. IMO) are not fermented by *S. cervisiae* and are still available in the final bread product (Schwab et al., 2008). The formation of IMO was achieved during fermentation in kvass (Dlusskaya et al., 2008), in wheat and gluten free bread (Galle et al., 2010a; Katina et al., 2009, Schwab et al., 2008). In particular, in sorghum fermentation the amount of IMO in 300 g sorghum bread accounts for 20% of the daily intake known to have prebiotic activity (Schwab et al., 2008).

Furthermore, oligosaccharides can act as soluble receptor analogs of epithelial cell surface carbohydrates, and therefore inhibit pathogens or bacterial toxin adhesion to epithelial surfaces, an initial stage of an infective process. The protective effect of breast milk against many infectious agents was attributed to the large number of oligosaccharides present in the breast milk (Kunz et al. 2000). The inhibitory effect of oligosaccharides towards epithelial binding of *Heliobacter pylori* and *E. coli* was recently reviewed (Korakli and Vogel, 2006). The large variety of oligosaccharide produced by enzymes also involved in HoPS production makes LAB harbouring those enzymes potential candidates in prevention and therapy of infection or inflammatory bowl diseases.

In conclusion, EPS and oligosaccharide formation during sourdough fermentations have good potential to promote gut health in future applications, but research in this area is still in its infancy.

8. Conclusion

Overall, EPS formation from sucrose is a metabolic activity that is widespread among sourdough lactic acid bacteria. Sourdough is therefore an attractive biotope for the isolation of LAB producing polymers and the corresponding synthesising-enzymes. *In situ* production of EPS positively influences all aspects of bread quality: texture, aroma, nutritional properties and shelf life. Recently, *in situ* formed EPS have also been successfully applied for the improvement of the qualities of gluten-free bread. The use of well-characterized EPS-producing starter cultures in sourdough fermentation enables the introduction of natural bread improvers and health-promoting compounds into cereal products. Thus, application of *in situ* formed EPS meets the

consumer demands for clean labels and for a reduced use of additives. However, it has to be considered that the metabolism of the fermentation flora can negatively influence EPS yields and counteract beneficial EPS effects. Nevertheless these metabolic traits can also have other functional properties in baking, which may even be required e.g. to achieve bakeability in rye breads. In future, with the improved knowledge on polymer properties, polymer yield and the concomitant formation of oligosaccharides and organic acids of EPS forming starter-cultures and their dependence on production condition, strains can be selected to achieve the desired optimal bread quality.

9. Acknowledgement

This study was financially supported by the Food Institutional Research Measure, administered by the Department of Agriculture, Fisheries and Food Ireland, Ireland.

10. References

Arendt, E.K., Ryan, L.A.M., Dal Bello, F. (2007). Impact of sourdough on the texture of bread. *Food Microbiology* **24**, 165-174.

Barber, B., Ortolá, C., Barber, S., Fernández, F. (1992). Storage of packaged white bread. *Zeitschrift für Lebensmitteluntersuchung und-Forschung* **A 194**, 442-449.

Bounaix, M.-S., Gabriel, V., Morel, S., Robert, H., Rabier, P., Remaud-Simeon, M., Gabriel, B., Fontagne-Faucher, C. (2009). Biodiversity of exopolysaccharides produced from sucrose by sourdough lactic acid bacteria. *Journal of Agricultural and Food Chemistry* **57**, 10889-10897.

Bounaix, M.-S., Gabriel, V., Robert, H., Morel, S., Remaud-Siméon, M., Gabriel, B., Fontagné-Faucher, C. (2010a). Characterization of glucan-producing Leuconostoc strains isolated from sourdough. *International Journal of Food Microbiology* **144**, 1-9.

Bounaix, M.S., Robert, H., Gabriel, V., Morel, S., Remaud-Simeon, M., Gabriel, B., Fontagne-Faucher, C. (2010b). Characterization of dextran-producing Weissella strains isolated from sourdoughs and evidence of constitutive dextransucrase expression. *Fems Microbiology Letters* **311**, 18-26.

Gänzle, M.G. (2005). Physiologie und Biochemie der Mikroflora des Sauerteiges. In: *Handbuch Sauerteig*, pp. 109-183, Brandt, M.J., Gänzle, M.G. Eds, B. Behr's Verlag.

Gänzle, M.G. (2009a). From gene to function: Metabolic traits of starter cultures for improved quality of cereal foods. *International Journal of Food Microbiology 134*, 29-36.

Gänzle, M.G., Zhang, C., Monang, B.-S., Lee, V., Schwab, C. (2009b). Novel metabolites from cereal-associated lactobacilli - Novel functionalities for cereal products? *Food Microbiology* **26**, 712-719.

Brandt, M.J., Roth, K., Hammes, W.P. (2003). Effect of an exopolysaccharide produced by Lactoabacillus sanfranciscensis LTH1729 on dough and bread quality. **In** *Sourdough from fundamentals to application*, p. 80, Vyust L, Vrije Eds, de Universiteit Brussels (VUB).

Cerning, J., 1990. Exocellular polysaccharides produced by lactic acid bacteria. *FEMS Microbiology Letters* **87**, 113-130.

Corsetti, A., Settanni, L. (2007). Lactobacilli in sourdough fermentation. *Food Research International* **40**, 539-558.

Cummings, J.H., Macfarlane, G.T., Englyst, H.N. (2001). Prebiotic digestion and fermentation. *American Journal of Clinical Nutrition* **73**, 415S-420S.

Dal Bello, F., Walter, J., Hertel, C., Hammes, W.P. (2001). In vitro study of Prebiotic Properties of Levan-type Exopolysaccharides from Lactobacilli and Non-digestible Carbohydrates Using Denaturing Gradient Gel Electrophoresis. *Systematic and Applied Microbiology* **24**, 232-237.

De Vuyst, L., De Vin, F., Vaningelgem, F., Degeest, B. (2001). Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. *International Dairy Journal* **11**, 687-707.

De Vuyst, L., Degeest, B. (1999). Heteropolysaccharides from lactic acid bacteria. *Fems Microbiology Reviews* **23**, 153-177.

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

De Vuyst, L., Vancanneyt, M. (2007). Biodiversity and identification of sourdough lactic acid bacteria. *Food Microbiology* **24**, 120-127.

Decock, P., Cappelle, S. (2005). Bread technology and sourdough technology. *Trends in Food Science & Technology* **16**, 113-120.

Di Cagno, R., De Angelis, M., Limitone, A., Minervini, F., Carnevali, P., Corsetti, A., Gaenzle, M., Ciati, R., Gobbetti, M. (2006). Glucan and fructan production by sourdough Weissella cibaria and Lactobacillus plantarum. *Journal of Agricultural and Food Chemistry* **54**, 9873-9881.

Dlusskaya, E., Jansch, A., Schwab, C., Gänzle, M.G. (2008). Microbial and chemical analysis of a kvass fermentation. *European Food Research and Technology* **227**, 261-266.

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

Galle, S., Schwab, C., Arendt, E.K., Gänzle, M.G. (2010b). Structural and rheological characterisation of heteropolysaccharides produced by lactic acid bacteria in wheat and sorghum sourdough. *Food Microbiology* **28**, 547-553.

Gänzle, M.G. (2009a). From gene to function: Metabolic traits of starter cultures for improved quality of cereal foods. *International Journal of Food Microbiology* **134**, 29-36.

Gänzle, M.G., Zhang, C., Monang, B.-S., Lee, V., Schwab, C. (2009b). Novel metabolites from cereal-associated lactobacilli - Novel functionalities for cereal products? *Food Microbiology* **26**, 712-719.

Garai-Ibabe, G., Dueñas, M.T., Irastorza, A., Sierra-Filardi, E., Werning, M.L., López, P., Corbí, A.L., Fernández de Palencia, P. (2010). Naturally occurring 2-substituted (1,3)-[beta]-d-glucan producing Lactobacillus suebicus and Pediococcus parvulus strains with potential utility in the production of functional foods. *Bioresource Technology* **101**, 9254-9263.

Gobbetti, M., Corsetti, A., Rossi, J. (1995). Interaction between lactic acid bacteria and yeasts in sour-dough using a rheofermentometer. *World Journal of Microbiology and Biotechnology* **11**, 625-630.

Gobbetti, M., De Angelis, M., Di Cagno, R., Rizzello, C.G. (2008). Sourdough lactic acid bacteria. **In:** *Gluten-Free Cereal Products and Beverages*, pp. 267-288, Arendt, E. K., Dal Bello, F Eds., Academic Press, San Diego.

Guarda, A., Rosell, C.M., Benedito, C., Galotto, M.J. (2004). Different hydrocolloids as bread improvers and antistaling agents. *Food Hydrocolloids* **18**, 241-247.

Hammes, P. W., Stolz, P., Gänzle, M. (1996). Metabolism of lactobacilli in traditional sourdoughs. *Advances in Food Science* **18**, 176-184.

Hammes, W.P., Gänzle, M. (1998). Sourdough breads and related products. **In:** *Microbiology of Fermented Foods*, pp. 199-216, Woods, B.J.B. Eds, Blackie Academic/Professional, London.

Huttner, E.K., Dal Bello, F., Arendt, E.K. (2010). Identification of lactic acid bacteria isolated from oat sourdoughs and investigation into their potential for the improvement of oat bread quality. *European Food Research and Technology* **230**, 849-857.

Jann A., Arrigoni, E., Rochat, F.,Schmid, D., Bauche, A. (2006). Methode for increasing the production of propionate in the gastrointestinal tract. *United Sates Patent*. US7091194B1

Kaditzky, S., Behr, J., Stocker, A., Kaden, P., Gänzle, M., Vogel, R.F. (2008a). Influence of pH on the formation of glucan by Lactobacillus reuteri TMW 1.106 exerting a protective function against extreme pH values. *Food Biotechnology* **22**, 398-418.

Kaditzky, S., Seitter, M., Hertel, C., Vogel, R.F. (2008b). Performance of Lactobacillus sanfranciscensis TMW 1.392 and its levansucrase deletion mutant in wheat dough and comparison of their impact on bread quality. *European Food Research and Technology* **227**, 433-442.

Kaditzky, S., Vogel, R. (2008c). Optimization of exopolysaccharide yields in sourdoughs fermented by lactobacilli. *European Food Research and Technology* **228**, 291-299.

Kang, I.-C., Kim, S.-M., Lee, H.-C., Oh, J.-S. (2006). Effect of Leuconostoc spp. on the formation of Streptococcus mutans biofilm. *Journal of Microbiology* **45**, 291-296.

Katina, K., Maina, N.H., Juvonen, R., Flander, L., Johansson, L., Virkki, L., Tenkanen, M., Laitila, A. (2009). In situ production and analysis of Weissella confusa dextran in wheat sourdough. *Food Microbiology* **26**, 734-743.

Kunz, C., Rudloff, S., Baier, W., Klein, N., Strobel, S. (2000). Oligosaccharides in human milk: structural, functional, and metabolic aspects. *Annual review of Nutrition* **20**, 699-722.

Kim, D.-S., Thomas, S., Fogler, H.S. (2000). Effects of pH and Trace Minerals on Long-Term Starvation of Leuconostoc mesenteroides. *Applied and Environmental Microbiology* **66**, 976-981.

Korakli, M., Gänzle, M.G., Vogel, R.F. (2002). Metabolism by bifidobacteria and lactic acid bacteria of polysaccharides from wheat and rye, and exopolysaccharides produced by Lactobacillus sanfranciscensis. *Journal of Applied Microbiology* **92**, 958-965.

Korakli, M., Pavlovic, M., Gänzle, M.G., Vogel, R.F. (2003). Exopolysaccharide and Kestose Production by Lactobacillus sanfranciscensis LTH2590. *Applied and Environmental Microbiology* **69,** 2073-2079.

Korakli, M., Rossmann, A., Gänzle, M.G., Vogel, R.F. (2001). Sucrose metabolism and exopolysaccharide production in wheat and rye sourdoughs by Lactobacillus sanfranciscensis. *Journal of Agricultural and Food Chemistry* **49**, 5194-5200.

Korakli, M., Vogel, R. (2006). Structure/function relationship of homopolysaccharide producing glycansucrases and therapeutic potential of their synthesised glycans. *Applied Microbiology and Biotechnology* **71**, 790-803.

Kralj, S., Stripling, E., Sanders, P., van Geel-Schutten, G., Dijkhuizen, L. (2005). Highly hydrolytic reuteransucrase from probiotic Lactobacillus reuteri strain ATCC 55730. *Applied and environmental microbiology* **71,** 3942.

Kralj, S., van Geel-Schutten, G., Rahaoui, H., Leer, R., Faber, E., van der Maarel, M., Dijkhuizen, L. (2002). Molecular characterization of a novel glucosyltransferase from Lactobacillus reuteri strain 121 synthesizing a unique, highly branched glucan with alpha-(134) and alpha-(136) glucosidic bonds. *Applied and environmental microbiology* **68**, 4283-4291.

Kralj, S., Van Geel-Schutten, G.H., van der Maarel, M.J.E.C., Dijkhuizen, L. (2004). Biochemical and molecular characterization of Lactobacillus reuteri 121 reuteransucrase. *Society for General Microbiology* **150**, 2099-2112.

Lacaze, G., Wick, M., Cappelle, S. (2007). Emerging fermentation technologies: Development of novel sourdoughs. *Food Microbiology* **24**, 155-160.

Lindgren, S.E., Dobrogosz, W.J. (1990). Antagonistic activities of lactic acid bacteria in food and feed fermentations. *FEMS Microbiology Letters* **87**, 149-163.

Looijesteijn, P.J., Trapet, L., de Vries, E., Abee, T., Hugenholtz, J. (2001). Physiological function of exopolysaccharides produced by Lactococcus lactis. *International Journal of Food Microbiology* **64**, 71-80.

Monsan, P., Bozonnet, S., Albenne, C., Joucla, G., Willemot, R.-M., Remaud-Siméon, M., (2001). Homopolysaccharides from lactic acid bacteria. *International Dairy Journal* **11**, 675-685.

Moore, M.M., Juga, B., Schober, T.J., Arendt, E.K. (2007). Effect of Lactic Acid Bacteria on Properties of Gluten-Free Sourdoughs, Batters, and Quality and Ultrastructure of Gluten-Free Bread. *Cereal Chemistry* **84**, 357-364.

Moroni, A.V., Arendt, E.K., Dal Bello, F. (2010a). Biodiversity of lactic acid bacteria and yeasts in spontaneously-fermented buckwheat and teff sourdoughs. *Food Microbiology* **28**, 497-502.

Moroni, A.V., Arendt, E.K., Morrissey, J., Brandt, M.J., Bello, F.D. (2010b). Competitiveness of commercial starters for the production of gluten-free buckwheat and teff sourdoughs. *International Journal of Food Microbiology* **142**, 142-148.

Moroni, A.V., Bello, F.d., Arendt, E.K. (2009). Sourdough in gluten-free bread-making: an ancient technology to solve a novel issue? *Food Microbiology 7*, 676-684.

Moroni, A.V., Dal Bello, F., Zannini, E., Arendt, E.K. (2011). Impact of sourdough on buckwheat flour and batter and bread: biochemical, rheological and textural insights. *Journal of Cereal Science,* in press.

Olivares-Illana, V., Lopez-Munguia, A., Olvera, C. (2003). Molecular Characterization of Inulosucrase from Leuconostoc citreum: a Fructosyltransferase within a Glucosyltransferase. *Journal of Bacteriology* **185**, 3606-3612.

Poutanen, K., Flander, L., Katina, K. (2009). Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiology* **26**, 693-699.

Rosell, C., Rojas, J., Benedito de Barber, C. (2001). Influence of hydrocolloids on dough rheology and bread quality. *Food Hydrocolloids* **15**, 75-81.

Ross, A.S., McMaster, G.J., David Tomlinson, J., Cheetham, N.W.H. (1992). Effect of dextrans of differing molecular weights on the rheology of wheat flour doughs and the quality

characteristics of pan and arabic breads. *Journal of the Science of Food and Agriculture* **60**, 91-98.

Ruas-Madiedo, P., Salazar, N., de los Ryes-Gavilán, C. G. (2009) Biosynthesis and chemical composition of exopolysaccharides produced by lactic acid bacteria. **In:** *Bacterial Polysaccharides: Current Innovations and Future Trends*, pp 279-312, Ullich, M. Eds, Caister Academic Press, UK.

Schober, T.J., Bean, S.R., Boyle, D.L. (2007). Gluten-free sorghum bread improved by sourdough fermentation: Biochemical, rheological, and microstructural background. *Journal of Agricultural and Food Chemistry* **55**, 5137-5146.

Schwab, C., Gänzle, M.G. (2006). Effect of membrane lateral pressure on the expression of fructosyltransferases in Lactobacillus reuteri. *Systematic and Applied Microbiology* **29**, 89-99.

Schwab, C., Mastrangelo, M., Corsetti, A., Gänzle, M. (2008). Formation of oligosaccharides and polysaccharides by Lactobacillus reuteri LTH5448 and Weissella cibaria 10M in sorghum sourdoughs. *Cereal Chemistry* **85**, 679-684.

Schwab, C., Walter, J., Tannock, G.W., Vogel, R.F., Gänzle, M.G. (2007). Sucrose utilization and impact of sucrose on glycosyltransferase expression in Lactobacillus reuteri. *Systematic and Applied Microbiology* **30**, 433-443.

Sims, I.M., Frese, S.A., Walter, J., Loach, D., Wilson, M., Appleyard, K., Eason, J., Livingston, M., Baird, M., Cook, G., Tannock, G.W. (2011). Structure and functions of exopolysaccharide produced by gut commensal Lactobacillus reuteri 100-23. *ISME Journal* **5**, 1115-1124.

Soetaert, W., Schwengers, D., Buchholz, K., Vandamme, E.J., Steffen B. Petersen, B.S., Sven, P. (1995). A wide range of carbohydrate modifications by a single micro-organism: Leuconostoc mesenteroides. *Progress in Biotechnology*, 351-358.

Sutherland, I.W. (1998). Novel and established applications of microbial polysaccharides. *Trends in Biotechnology* **16,** 41-46.

Tieking, M., Ehrmann, M.A., Vogel, R.F., Gänzle, M.G. (2005a). Molecular and functional characterization of a levansucrase from the sourdough isolate Lactobacillus sanfranciscensis TMW 1.392. *Applied Microbiology and Biotechnology 66*, 655-663.

Tieking, M., Gänzle, M.G. (2005). Exopolysaccharides from cereal-associated lactobacilli. Trends in Food Science & Technology **16**, 79-84.

Tieking, M., Kaditzky, S., Valcheva, R., Korakli, M., Vogel, R.F., Gänzle, M.G. (2005b). Extracellular homopolysaccharides and oligosaccharides from intestinal lactobacilli. *Journal of Applied Microbiology* **99**, 692-702.

Tieking, M., Korakli, M., Ehrmann, M.A., Gänzle, M.G., Vogel, R.F. (2003). In situ production of exopolysaccharides during sourdough fermentation by cereal and intestinal isolates of lactic acid bacteria. *Applied and Environmental Microbiology* **69**, 945-952.

Tieking, M., Kuhnl, W., Gänzle, M.G. (2005c). Evidence for formation of heterooligosaccharides by Lactobacillus sanfranciscensis during growth in wheat sourdough. *Journal of Agricultural and Food Chemistry* **53**, 2456-2461.

Van der Meulen, R., Grosu-Tudor, S., Mozzi, F., Vaningelgem, F., Zamfir, M., Font de Valdez, G., De Vuyst, L. (2007). Screening of lactic acid bacteria isolates from dairy and cereal products

for exopolysaccharide production and genes involved. *International Journal of Food Microbiology* **118**, 250-258.

van Hijum, S., Kralj, S., Ozimek, L.K., Dijkhuizen, L., van Geel-Schutten, I.G.H. (2006). Structure-function relationships of glucansucrase and fructansucrase enzymes from lactic acid bacteria. *Microbiology and Molecular Biology Reviews 70*, 157.

van Hijum, S.A.F.T., Bonting, K., van der Maarel, M.J.E.C., Dijkhuizen, L. (2001). Purification of a novel fructosyltransferase from Lactobacillus reuteri strain 121 and characterization of the levan produced. *FEMS Microbiology Letters* **205**, 323-328.

van Hijum, S.A.F.T., Szalowska, E., Van der Maarel, M.J.E.C., Dijkhuizen, L. (2004). Biochemical and molecular characterization of a levansucrase from Lactobacillus reuteri *Society for General Microbiology* **150**, 2099-2112.

van Hijum, S.A.F.T., van der Maarel, M.J.E.C., Dijkhuizen, L. (2003). Kinetic properties of an inulosucrase from Lactobacillus reuteri 121. *FEBS Letters 534*, 207-210.

Vogelmann, S.A., Seitter, M., Singer, U., Brandt, M.J., Hertel, C. (2009). Adaptability of lactic acid bacteria and yeasts to sourdoughs prepared from cereals, pseudocereals and cassava and use of competitive strains as starters. *International Journal of Food Microbiology* **130**, 205-212.

Waldherr, F., Meissner, D., Vogel, R. (2008). Genetic and functional characterization of Lactobacillus panis levansucrase. *Archives of Microbiology* **190**, 497-505.

Walter, J., Schwab, C., Loach, D.M., Gänzle, M.G., Tannock, G.W. (2008). Glucosyltransferase A (GtfA) and inulosucrase (Inu) of Lactobacillus reuteri TMW1.106 contribute to cell aggregation, in vitro biofilm formation, and colonization of the mouse gastrointestinal tract. *Microbiology.* **154**, 72-80.

Werning, L. M., Ibarburu, I., Duneas, T. M., Irastorza, A., Navas, J., Lopez, P. (2006). *Pediococcus parvulus* gtf gene encoding the GTF glycosyltransferase and its application for specific PCR detection of (1-3)-β-D-glucan-producing bacteria in foods and beverages *International Association for Food Protection* **69**, 161-169.

Zotta, T., Piraino, P., Parente, E., Salzano, G., Ricciardi, A. (2008). Characterization of lactic acid bacteria isolated from sourdoughs for Cornetto, a traditional bread produced in Basilicata (Southern Italy). *World Journal of Microbiology& Biotechnology* **24**, 1785-1795.

Figure caption

Figure 1. Formation of oligosaccharides in wheat (A) and sorghum (B) sourdough by *W. kimchi* F28 (1) and *W. cibaria* MG1 (2). Oligosaccharides were analyzed using HPAEC-PAD. Abbreviations: suc sucose, mal maltose, pan panose, pan-$(glu)_n$ glucosylated panose, n degree of polymerization, IM isomaltose, IM3 isomaltotriose, IMO isomaltooligosaccharides (Reprinted with permission from {Galle, S., Schwab, C., Arendt, E., Gänzle, M. (2010a). Exopolysaccharide-Forming Weissella Strains as Starter Cultures for Sorghum and Wheat Sourdoughs. *Journal of Agricultural and Food Chemistry* **58**, 5834-5841.} © 2010 American Chemical Society)

Figure 2. Comparison of EPS sourdough bread with a non acidified control wheat bread (A) and sourdough bread fermented with *W. cibaria* without sucrose (B) (EPS negative control). Dextran containing bread were prepared with 20% *W. cibaria* sourdough fermented with 15% sucrose addition (C) (Galle et al. unpublished results).

.Table1. Amount of EPS and oligosaccharide formed during sourdough fermentation.

Strain	EPS	EPS *in situ*	OS *in situ*	Reference
L. sanfranciscensis	levan	wheat (1-5 g kg^{-1})	HeOS, FOS	Korakli et al., 2005; Tieking et al. 2003, Kaditzky et al., 2008
L. pontis, *L. frumenti*	fructan	wheat (0.3-1 g kg^{-1})	n.d.	Tieking et al., 2003
L. reuteri	reuteran, levan	wheat, sorghum (0.8-1 g kg^{-1})	FOS, GOS	Kadtizky et al., 2008; Schwab et al., 2008
W. cibaria *W. kimchii*	dextran	wheat (0.8-5.8 g kg^{-1}) sorghum (4.3 – 8 g kg^{-1})	IMO, GOS	Di Cagno et al., 2006, Schwab et al., 2008; Galle et al., 2010
W. confusa	dextran	wheat (11-16 g kg^{-1})	IMO	Katina et al., 2009
L. buchneri	HePS	wheat, sorghum[a]	-	Galle et al., 2010

*n.d. not determined

[a] HePS amount not detectable, HePS formation in sourdough was confirmed by gene expression.

Table 2. *In situ* formed EPS and their effect on bread quality

Bread type	Strain	Sourdough addition	EPS properties/amount [g kg^{-1} sd[a]]	Sucrose addition and EPS Co-products [mMol kg^{-1} sd]	Bread quality	Reference
Wheat bread	*W. cibaria* / *L. plantarum*	20%	2.5 g/ kg^{-1} (glucan)	10% sucrose n.d[b]	increased dough viscosity, increased volume, decreased firmness	Di Cagno et al., 2006

Wheat bread	*L. sanfranci scensis*	10%	5.2 g kg^{-1} (levan)	10% sucrose FOS[c] lactate 140 acetate 55 glucose 187 fructose 18	no improvement	Kadtitzky et al., 2008
Wheat bread	*W. confusa*	43%	11-16 g kg^{-1} (dextran)	10% sucrose IMO[d]	increased volume, decreased firmness, improved freshness	Katina et al., 2009
Sorghum bread	*W. cibaria*	14%	0.6 g kg^{-1} (dextran)	15% sucrose IMO lactate 140 acetate 30	decreased firmness, improved freshness	Schwab et al., 2006
Sorghum bread	*L. reuteri*	14%	1.5 g kg^{-1} (levan)	15% sucrose FOS lactate 214 acetate 121	no improvement	Schwab et al., 2006
Mixed rye bread	*Ln. mesente roides*	-	n.d. (dextran)	n.d.	increased volume, decreased firmness, improved freshness	Lacaze et al., 2007
Wheat milk bread	*Ln. mesente roides*	5%	dextran	n.d.	decreased firmness, improved freshness and mouthfeel	Lacaze et al., 2006

[a]sd…sourdough,

[b]n.d. not determined

[c]FOS…fructooligosaccharides.

[d]IMO…isomaltooligosaccharides

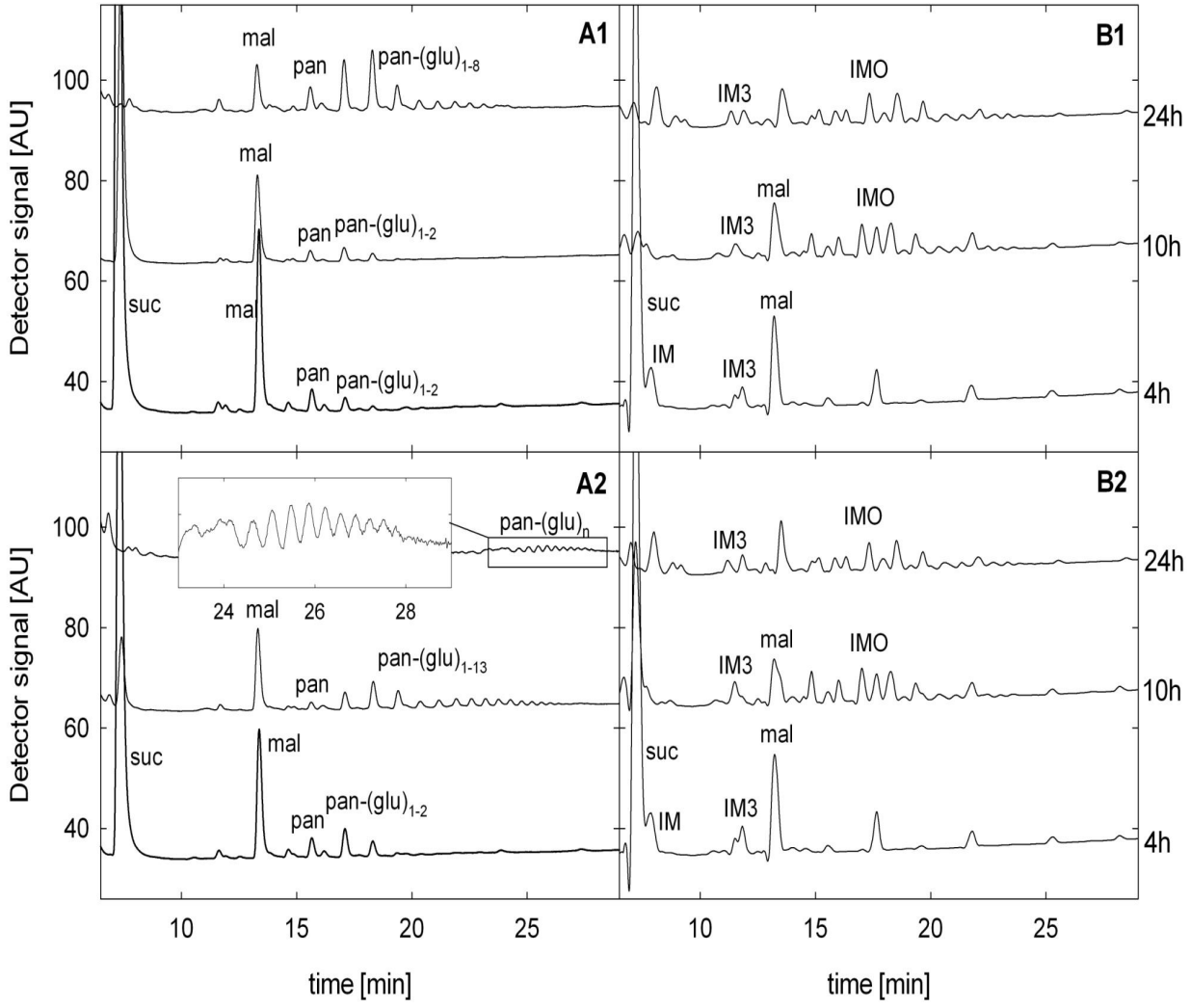

Figure 1.

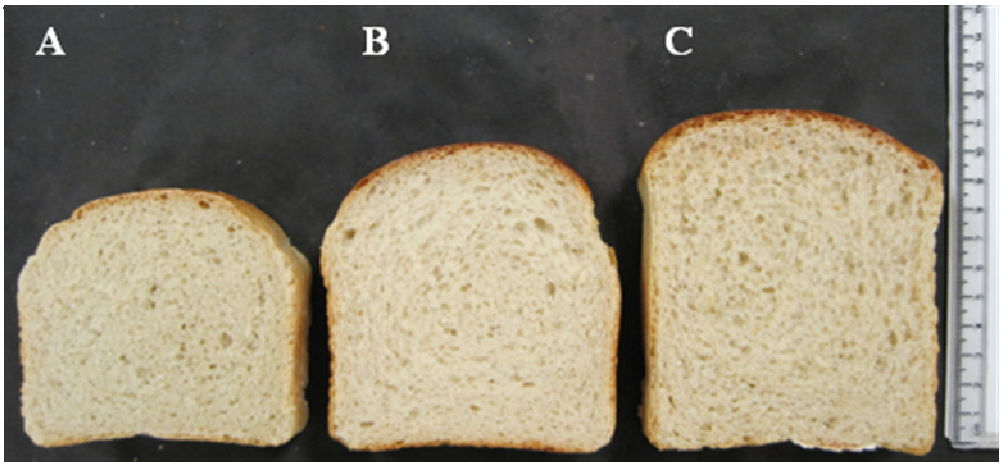

Figure 2. Galle et al.