Enzymatic and bacterial conversions during sourdough fermentation

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Abstract

Enzymatic and microbial conversion of flour components during bread making determines bread quality. Metabolism of sourdough microbiota and the activity of cereal enzymes are interdependent. Acidification, oxygen consumption, and thiols accumulation by microbial metabolism modulate the activity of cereal enzymes. In turn, cereal enzymes provide substrates for bacterial growth. This review highlights the role of cereal enzymes and the metabolism of lactic acid bacteria in conversion of carbohydrates, proteins, phenolic compounds and lipids.

Heterofermentative lactic acid bacteria prevailing in wheat and rye sourdoughs preferentially metabolise sucrose and maltose; the latter is released by cereal enzymes during fermentation. Sucrose supports formation of acetate by heterofermentative lactobacilli, and the formation of exopolysaccharides. The release of maltose and glucose by cereal enzymes during fermentation determines the exopolysaccharide yield in sourdough fermentations.

Proteolysis is dependent on cereal proteases. Peptidase activities of sourdough lactic acid bacteria determine the accumulation of (bioactive) peptides, amino acids, and amino acid metabolites in dough and bread.

Enzymatic conversion and microbial metabolism of phenolic compounds is relevant in sorghum and millet containing high levels of phenolic compounds. The presence of phenolic compounds with antimicrobial activity in sorghum selects for fermentation microbiota that are resistant to the phenolic compounds.

Keywords

Sourdough, *Lactobacillus sanfranciscensis*, amylase, maltose metabolism, arabinoxylan, exopolysaccharide, bioactive peptides, phenolic acids, hydroxy fatty acids, lipid oxidation.

Highlights

- Cereal enzymes are important determinants of the microbial ecology of sourdough
- Enzymatic and microbial conversion of flour components determines bread quality.
- Bacterial metabolism and cereal enzyme activity in sourdough are interdependent.

1. Introduction

Sourdough has traditionally been used as leavening agent in bread making. The use as leavening agent continues in artisanal baking and for production of specialty products; the resulting bread has an otherwise irreproducible quality. Few bakeries employ sourdough as leaving agent at an industrial scale. The industrial use of sourdough predominantly primarily aims to improve bread quality, and to replace additives. This shift of the technological aims resulted in the development of novel fermentation technologies and starter cultures with defined metabolic properties (Gobbetti and Gänzle, 2007; Brandt, 2007). The use of sourdough in bread making influences all aspects of bread quality. The technological effects of sourdough on the flavour, texture, shelf-life, and nutritional quality of bread are dependent on bioconversion of flour components at the dough stage (Table 1). Two main factors differentiate sourdough processes from straight dough processes. First, the presence of lactic acid bacteria adds the metabolic potential of this heterogeneous group of organisms to the metabolic potential of yeasts (Decock and Capelle, 2005; De Vuyst and Neysens, 2005). Second, the fermentation time of sourdough processes ranges from 8 h (sponge doughs) to over 144 h (Brandt, 2007). This long fermentation time compared to straight dough processes allows for a substantial contribution of endogenous enzymes to biochemical conversions at the dough stage.

The metabolism of sourdough microbiota and the activity of cereal enzymes are interdependent. Acidification modulates the activity of cereal enzymes and the solubility of substrates, particularly gluten proteins and phytate. Sourdough fermentations are generally dominated by obligately heterofermentative lactic acid bacteria (De Vuyst and Neysens, 2005). Carbohydrate metabolism in the phosphate pentose pathway generates an abundant supply of reduced co-factors. Heterofermentative lactobacilli use a wide array of dough constituents as electron

acceptors to regenerate these reduced co-factors (Gänzle et al., 2007). Heterolactic metabolism thus influences enzyme activities by decreasing the oxidation-reduction potential of sourdoughs, and by accumulation of low-molecular weight thiol compounds (Jänsch et al., 2007; Capuani et al., 2012). Cereal enzymes, in turn, provide substrates for bacterial growth (Hammes and Gänzle 1998). *Lactobacillus sanfranciscensis*, a key species in sourdoughs, has the smallest genome described in lactobacilli. The species has particularly abandoned the synthesis of extracellular hydrolytic enzymes and relies on substrate-derived enzymes (Vogel et al., 2011).

The use of sourdough has focused on wheat and rye baking (De Vuyst and Neysens, 2005). Wheat and rye sourdoughs do not exhibit characteristic differences in fermentation microbiota or their metabolic activity (De Vuyst and Neysens, 2005; Gänzle et al., 2007). Non-conventional substrates have recently been used for sourdough fermentations in gluten-free baking (Moroni et al., 2009). These substrates overlap with traditional fermentations in tropical climates (Nout, 2009). Studies on the microbial ecology of conventional and gluten-free sourdoughs demonstrated that the cereal substrate and substrate-derived enzymatic activities are key determinants of the microbial ecology of sourdough (Hammes and Gänzle, 1998; Vogelmann et al., 2009, Sekwati-Monang et al., 2012).

This review aims to provide an overview on microbial and enzymatic conversions in sourdough. Emphasis is placed on wheat and rye, information related to non-conventional substrates is provided where available. The carbohydrate metabolism of heterofermentative lactic acid bacteria, proteolysis in sourdough, and exopolysaccharide synthesis were subject of recent reviews (Gänzle et al., 2007; Gänzle et al., 2008; Galle and Arendt, *in press*) and are discussed only briefly. Moreover, the review emphasises metabolism of lactic acid bacteria as the

92 microbial group that differentiates sourdough from straight dough processes. For information on
93 yeast metabolism, the reader is referred to a recent review provided by Guerzoni et al. (2013).

94 **2. Starch and carbohydrate metabolism**

95 **2.1. Starch degradation and metabolism.**

96 Wheat and rye contain about 60 – 70% starch. Starch is the major determinant of the crumb
97 structure of bread and amylopectin retrogradation is the major cause for the staling of bread
98 (Table 1). Starch degradation at the dough stage is the predominant source of fermentable
99 carbohydrates and reducing sugars (Table 1). The concentration of fermentable carbohydrates in
100 wheat and rye flours is relatively low. Sucrose and raffinose are present in concentrations of 0.6
101 – 1.8% and 0.1 – 0.4%, respectively. Other mono- and disaccharides are essentially absent unless
102 the grains germinated (Brandt, 2006; Belitz et al., 2004). Resting grains of wheat and rye contain
103 α-amylase, β-amylase, and glucoamylase activities (Figure 1A, Belitz et al., 2004, Brandt,
104 2006). The amylase activity of rye flour was sufficient to attain substantial starch degradation
105 during baking (Neumann et al., 2006). Starch degradation in rye baking is further favoured by
106 the proximity of the temperature optimum of rye amylase (50 – 52°C) and the gelatinization
107 temperature of rye starch (55 – 68°C). Heating of the crumb to 100°C during baking traverses the
108 temperature range of 55 – 68°C where active amylase and gelatinized starch co-exist, leading to
109 rapid starch degradation. In flour with high amylase activity, this starch hydrolysis during baking
110 crumb results in substantial damage and rye baking thus necessitated acidification to inhibit of
111 endogenous amylases. However, the amylase activity of rye flours decreased over the last three
112 decades, corresponding to higher falling numbers. Accordingly, the use of sourdough
113 fermentation in rye baking to inhibit amylases is no longer a necessity (Neumann et al., 2006).

Amylase activities in wheat and rye sourdough liberate maltodextrins, maltose, and glucose during fermentation (Röcken and Voysey, 1995; Brandt, 2006). In simulated sourdough fermentations without microbial activity, maltose accumulates at the initial stage of fermentation. After reduction of the pH of 4.5, maltogenic amylases are inhibited but glucoamylase continues to release glucose from starch and maltodextrins (Röcken and Voysey, 1995; Brandt, 2006). In keeping with the availability of maltose as main carbon source in wheat and rye sourdoughs, key sourdough lactobacilli, including *L. sanfranciscensis*, *L. fermentum*, and *L. reuteri*, are highly adapted to maltose (Figure 1A, for review, see Gobbetti et al., 2005; Gänzle et al., 2007). In *L. sanfranciscensis*, maltose phosphorylase is constitutively expressed. Maltose metabolism is preferred over metabolism of other carbon sources, or occurs simultaneously (Stolz et al., 1993; Ehrmann & Vogel, 1998, Gobbetti et al., 2005). Maltose phosphorylase is highly specific for maltose; most sourdough lactobacilli including *L. sanfranciscensis* and *L. reuteri* additionally harbour DexB, a glucosidase hydrolysing α(1→6)-linked gluco-oligosaccharides (Vogel et al., 2011; Møller et al., 2012). The contribution of DexB to carbohydrate conversion during sourdough fermentation is unknown. The widespread distribution of DexB in genomes of lactobacilli, however, implies an essential role in carbohydrate metabolism of cereal-associated lactobacilli (Gänzle and Follador, 2012).

Resting grains of C4 cereals including sorghum, pearl millet, and corn exhibit no β-amylase activity (Figure 1B, Taylor et al., 2006). The lack of β-amylase activity corresponds to low maltose concentrations in sourdough produced from these grains. In simulated sorghum sourdoughs, initial maltose levels are low and glucose but not maltose is generated by endogenous glucoamylases (Galle et al., 2010; Sekwati-Monang et al., 2012). Together with other substrate-derived factors, the absence of maltose selects against *L. sanfranciscensis*

(Sekwati-Monang et al., 2012). Only few strains of lactobacilli exhibit extracellular amylase activity (Gänzle and Follador, 2012) but amylolytic lactobacilli, e.g. *L. plantarum, L. amylolyticus,* and *L. mannihotivorans*, are frequently identified in fermentations of pearl millet, corn, or cassava (e.g. Guyot and Morlon-Guyot, 2001; Songré-Ouattara et al., 2008; Turpin et al., 2011). In fermentation of sorghum, millet, and tubers, extracellular amylases (AmyX) of lactic acid bacteria contribute to starch degradation (Figure 1B). Remarkably, the extracellular amylopullulanase AmyX is highly homologous to the intracellular glucosyl hydrolases MalN and MalL and differs mainly in its cellular location (Gänzle and Follador, 2012). In concert with the oligosaccharide transport system MalEFG and MsmK, DexB and maltose phosphorylase, many lactobacilli harbour the full complement of enzymes needed for starch hydrolysis, oligosaccharide transport, and hydrolysis (Figure 1B, Nakai et al., 2009; Turpin et al., 2011; Møller et al., 2012 Gänzle and Follador, 2012).

2.2. Solubilisation of arabinoxylans.

Wheat and rye flour contains 1.5 – 3% and 7 – 8% arabinoxylans, respectively, however, only a small fraction of arabinoxylan is water soluble (Shewry et al., 2010; Geberuers et al., 2010). Water soluble arabinoxylan contributes to dough hydration and foam stability of wheat and rye dough. In contrast, water insoluble arabinoxylans interfere with gluten formation during dough mixing, and destabilize gas cells (Table 1, for review, see Goesaert et al., 2005). The solubilisation of arabinoxylans during rye sourdough fermentation contributes to the beneficial effects of sourdough fermentation on the quality of rye bread (Neumann et al., 2006). Xylanases of wheat and rye are active in the pH range of 3.5 – 5.5 (Rasmussen et al., 2001; Gebruers et al., 2010). Accordingly, the content of water soluble arabinoxylans increased during simulated wheat and rye sourdough fermentation (Boskov-Hansen et al., 2002; Korakli et al, 2001).

Arabinoxylans solubilisation in sourdoughs and simulated sourdoughs was comparable, indicating that it is entirely attributable to cereal enzymes (Korakli et al., 2001, Loponen et al., 2009). The degradation of flour arabinoxylans in rye sourdoughs results in solubilisation of high molecular weight polysaccharides (Loponen et al., 2009). Arabinoxylan degradation to arabinose and xylose was observed in rye malt sourdoughs, and in wheat sourdough after addition of pentosanases (Gobbetti et al., 2000; Loponen et al., 2009).

2.3. Exopolysaccharide formation.

Exopolysaccharide formation by cereal-associated lactobacilli contributes to sucrose metabolism, the protection against environmental insults, and the formation of biofilms in intestinal habitats (Gänzle and Schwab, 2009). The production of exopolysaccharides by lactic acid bacteria in sourdough improves bread volume and texture and increases the dietary fibre content. However, beneficial effect of exopolysaccharides on bread texture may be mitigated by excess acidity (Table 1, for review see Poutanen et al., 2009; Galle and Arendt, *in press*). The production of homopolysaccharides from sucrose is a frequent metabolic trait of sourdough lactic acid bacteria. Remarkably, fermentation microbiota of traditional sourdoughs harbour at least one exopolysaccharide producing strain (Bunaix et al., 2009; Tieking et al., 2003). Properties of the exopolysaccharide producing glucansucrases and fructansucrases were reviewed by van Hijum et al. (2006) and Korakli & Vogel (2006). An overview on the impact of exopolysaccharide formation on bread quality is provided by Galle and Arendt (*in press*). Homopolysaccharide production by lactic acid bacteria in laboratory culture generally matches the production of homopolysaccharides during growth in sourdough (Korakli et al., 2001; Tieking et al., 2003). However, the fermentation substrate influences the polysaccharide yield (Kaditzky & Vogel, 2008; Galle et al., 2010; Rühmkorf et al., 2012). Substrates with a high buffering capacity

183 maintain the ambient pH in the optimum pH range for glucansucrases activity, pH 4.5 – 5.5, for
184 an extended period of time, and resulted in a higher yield of reuteran (Kaditzky and Vogel,
185 2008). Maltose is a strong glucosylacceptor for glucansucrases of lactic acid bacteria. High
186 concentrations of maltose thus shift glucansucrase activity from polysaccharide to
187 oligosaccharide synthesis (Figure 2, van Hijum et al., 2006; Galle et al., 2010). The presence of
188 maltose in wheat and rye favours synthesis of panose-series oligosaccharides at the expense of
189 polysaccharides (Galle et al., 2010). Fermentation of C4 cereals lacking β-amylase activity
190 allows an increased yield of exopolysaccharides (Galle et al., 2010, Rühmkorf et al., 2012). The
191 influence of substrate and acceptor carbohydrates on the polysaccharide yield, however, is strain
192 specific (Galle et al., 2010, Rühmkorf et al., 2012).

193 **3. Protein degradation and amino acid metabolism**

194 **3.1. Proteolysis.**

195 Polymeric wheat gluten proteins determine the bread making quality of wheat flours (Wieser,
196 2007). Gluten proteins contribute to dough hydration and gas retention (Table 1, Wieser, 2007).
197 The degradation and depolymerisation of proteins during sourdough fermentation is dependent
198 on bacterial metabolic activity and cereal enzymes (Figure 3; Gänzle et al., 2008). Acidification
199 and the accumulation of low molecular weight thiols increase the solubility of gluten proteins
200 and consequently their susceptibility to enzymatic degradation (Thiele et al., 2004; Jänsch et al.,
201 2007). Moreover, sourdough fermentation shifts the ambient pH to the optimum pH of aspartic
202 proteases, the major proteinase in resting grains of wheat and rye (Bleukx, et al., 1998, Brijs et
203 al., 1999). Primary proteolysis is dependent on endogenous proteinases (Thiele et al., 2004;
204 Gänzle et al., 2008). Proteolysis in wheat and rye sourdough remains limited to degradation of
205 less than 5% of the cereal proteins; extensive protein degradation requires addition of malt or

206 fungal enzymes (Gänzle et al., 2008). Lactobacilli increase the concentration of amino acids
207 relative to simulated sourdough fermentation predominantly by the activity of strain-specific
208 intracellular peptidases (Gobbetti et al., 1998; Di Cagno et al., 2002).

209 **3.2. Accumulation of bioactive peptides, amino acids, and amino acid metabolites.**

210 Peptides, amino acids, and products of microbial amino acid metabolism impact bread quality as
211 taste-active compounds, flavor precursors, or as bioactives (Table 1). The influence of strain- or
212 species specific conversion of amino acids on the sensory quality of bread was subject of several
213 recent review articles (Gobbetti et al., 2005; Gänzle et al., 2007). Recent developments focused
214 on the accumulation of peptides and amino acid metabolites with antioxidant, antihypertensive,
215 or cancer preventing activities in sourdough fermentations (Rizzello et al., 2008 and 2011; Hu et
216 al., 2011; for review, see Gobbetti, 2012). Strain-specific peptidase of sourdough lactobacilli
217 significantly influenced the accumulation of bioactive peptides in rye malt sourdough. For
218 example, the antihypertensive tripeptides LQP and LLP accumulated to higher concentrations if
219 starter cultures exhibited low PepO and high PepN activities (Hu et al., 2011). Active
220 concentrations of γ-aminobutyrate, a bioactive metabolite of glutamate, and antihypertensive
221 tripeptides were successfully incorporated in baked goods following their fermentative
222 enrichment during sourdough fermentation (Coda et al., 2010; Zhao et al., 2013).

223 **4. Metabolism of phenolic compounds, phytate, and fatty acids.**

224 **4.1. Phenolic compounds and feruloyl-esterase activity in cereals**

225 Phenolic compounds in plants were regarded as anti-nutritive factors that impart bitter taste and
226 inhibit the digestion of starch and proteins (Table 1, Taylor, 2006; Dykes and Rooney, 2006).
227 However, phenolic compounds also exert beneficial health effects as antioxidants (Ragaee et al.,

2006; Dykes and Rooney, 2006, Katina et al., 2007, Poutanen et al., 2009) and are precursor compounds for flavor formation in bread making (Czerny and Schieberle, 2002, Opperer et al., 2012). Major phenolic compounds in wheat and rye are phenolic acids and alkylresorcinols. The lipophilic alkylresorcinols are located primarily in the bran layers of the grain and have no known influence on bread quality. They were used as biomarkers for whole grain intake (Shewry et al., 2010). The major phenolic acid in rye is ferulic acid, which accounts for about 50% of total phenolic acids. Caffeic acid, dihydrobenzoic acid and sinapic acid are also present (Shewry et al., 2010). Phenolic acids in wheat and rye occur predominantly in bound form and as dimers. The concentration of free phenolic acids is low (Shewry et al., 2010; Boskov-Hansen et al., 2002). In wholemeal rye flour, the concentration of bound, dimeric and free ferulic acid was 1.1, 0.39 and 0.003 g / kg (Boskov Hansen et al., 2002). Ferulic acid is predominantly esterified with arabinoxylans. During dough making, oxidative cross links between two arabinoxylan-linked ferulate moieties, or between ferulate and tyrosine are formed (Piber and Koehler, 2005). Rye flour exhibits feruloyl esterase activity but the enzyme was inhibited at a pH of 3.5 (Boskov Hansen et al., 2002). Feruloyl esterase is activated during germination and ferulic acid is released from cell wall components during mashing of wheat or barley malt (Sancho et al., 1999; Coghe et al., 2004).

Millet and particularly sorghum have a higher content of polyphenols than wheat, barley or rye (Ragae et al., 2006; Dykes and Rooney, 2006). In sorghum, phenolic acids and glycerol esters of phenolic acids, flavonoids, condensed tannins, and deoxyanthocyanidins are the predominant compounds (Dykes and Rooney, 2006; Svensson et al., 2010). Comparable to wheat and rye, phenolic acids in sorghum occur predominantly in bound form but the concentration of free phenolic acids ranges from 50 - > 100 mg / kg (Dykes and Rooney, 2006; Svensson et al., 2010).

4.2. Metabolism of phenolic compounds by lactic acid bacteria.

The ecological role of the metabolism of phenolic compounds is unclear and may include the release of hexosides as source of metabolic energy, and the removal of noxious compounds (Table 1). Lactic acid bacteria harbour a diverse set of enzymes for conversion of phenolic compounds (Figure 4). Feruloyl esterases, which hydrolyse feruloylated sugar esters, were characterized in intestinal lactobacilli (Wang et al., 2004, Lai et al., 2009; Hole et al., 2012). Tannin acyl hydrolase, an esterase with specificity for galloyl ester bonds in gallotannins, was characterized in *L. plantarum* (Iwamoto et al., 2008). Specific glycosyl hydrolases of lactobacilli release flavonoid aglycons from the corresponding flavonoid glycosides (Avila et al., 2009, Svensson et al., 2010). Phenolic acid metabolism in lactic acid bacteria is mediated by reductases and decarboxylases. Hydroxy-benzoic and hydroxy-cinnamic acids are decarboxylated to the corresponding phenol or vinyl derivatives (van Beek & Priest, 2000; Barthelmebs et al., 2000; for review see Rodriguez et al., 2009). Hydroxy-cinnamic acids and their vinyl derivatives are converted by reductases which hydrogenate the double bond (van Beek & Priest, 2000). Bioconversion of phenolic acids is strain specific. Vinyl catechol, ethyl catechol and dihydrocaffeic acid are strain-specific alternative products of caffeic acid metabolism by *L. plantarum* (Rodriguez et al., 2009). Strains capable of ferulic acid conversion do not necessarily convert other hydroxy cinnamic acids, or produce a different pattern of metabolites (Sánchez-Maldonado et al., 2011).

4.3. Antimicrobial activity of phenolic acids: selective pressure in cereal fermentations?

Phenolic acid metabolism was predominantly characterized in lactobacilli from wine and vegetable fermentations (Rodriguez et al., 2009). Phenolic acid metabolism was also demonstrated for lactobacilli from malt whisky fermentation, sorghum fermentations, and the

wheat sourdough isolate *L. hammesii* DSM 16381 (van Beek & Priest, 2000; Valcheva et al., 2005; Svensson et al., 2010; Sánchez-Maldonado et al., 2011). Phenolic acids inhibit the growth of lactobacilli at concentrations ranging from 0.5 – 4 g / L (Figure 5, Sánchez-Maldonado et al., 2011); the sensitivity of lactobacilli to phenolic acids is strain-specific. Metabolites of phenolic acid conversion by lactobacilli have a reduced antimicrobial activity when compared to the substrates (Figure 5). Remarkably, those lactobacilli that are capable of phenolic acid conversion also exhibit higher resistance to their antimicrobial activity (Figure 5, Sánchez-Maldonado et al., 2011). These findings indicate that phenolic acid metabolism is a means of detoxification.

In wheat or rye sourdoughs, the concentration of phenolic acids remains several orders of magnitude below their inhibitory concentration (Boskov-Hansen et al., 2002). In sorghum sourdoughs, however, their concentration is higher than the inhibitory concentration for sensitive lactobacilli (Svensson et al., 2010; Sánchez-Maldonado et al., 2011; Sekwati-Monang et al., 2012). *L. sanfranciscensis* is inhibited by phenolic compounds in sorghum and thus fails to grow in sorghum sourdough. In contrast, *L. casei* and *L. parabuchneri* are resistant to sorghum phenolics and persist in sorghum sourdoughs. These strains, however, are out-competed by *L. sanfranciscensis* in wheat sourdough propagated at comparable conditions (Sekwati Monang et al., 2012). Taken together, these findings indicate that the antimicrobial activity of phenolic compounds in sorghum selects for fermentation microbiota that resist the antimicrobial activity of phenolic compounds, particularly phenolic acids (Sekwati-Monang et al. 2012).

4.4. Conversion of phenolic compounds in sourdough fermentations.

Few studies have identified the specific contribution of cereal enzymes and defined starter culture on the conversion of phenolic during sourdough fermentation. Free phenolic compounds and free ferulic acid increased in rye bran fermentations started with baker's yeast (Katina et al.,

297 2007 and 2012). During imitated sourdough fermentation, the amount of free ferulic acid in
298 wholemeal rye increased more than twofold but it still accounted for less than 0.5% of the total
299 (bound) phenolic compounds (Boskov Hansen et al., 2002). Imitated sourdough fermentation of
300 whole grain oats and barley also increased the concentration of phenolic acids more than 5 fold
301 (Hole et al., 2012). The use of starter cultures with feruloyl esterase activity increased the content
302 of free phenolic acids up to 20 fold (Hole et al., 2012). However, the effect of cultures with
303 feruloyl esterase activity on the content of phenolic acids in was strain specific. This was
304 attributed to metabolism of phenolic acids by individual strains, or to difference in the expression
305 and specificity of feruloyl esterases (Hole et al., 2012).

306 A detailed characterization of the conversion of phenolic compounds during sourdough
307 fermentation was carried out with sourdoughs prepared from the red sorghum variety PAN3860
308 (Svensson et al., 2010). Simulated sourdoughs without microbial activity were characterized by
309 an increase of phenolic acids, indicating release of bound phenolic compounds, partial hydrolysis
310 of glycerol esters of phenolic compounds, and the partial conversion of flavonoid hexosides to
311 the corresponding flavonoids. During sourdough fermentation, two binary strain combinations
312 metabolized hydroxy-cimmacic acids but only one of the two strain combinations was capable of
313 metabolism of hydroxy-benzoic acids. Lactic fermentation strongly enhanced the release of
314 flavonoids from flavonoid glucosides (Svensson et al., 2010).

315 **4.5. Enzymatic conversion of phytate.**

316 Wheat and rye flours contain about 1% phytate (Belitz et al., 2004). Complexes formed by
317 phytate and divalent cations reduce the bioavailability of calcium, magnesium, and iron (Reddy
318 et al., 1989). Phytate is degraded in wheat and rye sourdoughs. Phytase activity of sourdough
319 lactobacilli has been described, however, evidence for their contribution to phytate hydrolysis

during sourdough fermentation is lacking (De Angelis, 2003). Phytate hydrolysis in dough is primarily dependent on cereal phytases. Enzymatic hydrolysis of phytate occurs in the pH range of 3.5 – 5 (Tangkongchitr et al., 1982; Fretzdorff and Brümmer, 1992; Leenhardt et al., 2005). Phytate complexes with divalent cations are insoluble above pH 5.0 and thus not accessible to enzymatic hydrolysis. Below pH 3.5, phytases of wheat and rye are inhibited (Tangkongchitr et al., 1982; Fretzdorff and Brümmer, 1992; Leenhardt et al., 2005).

4.6. Enzymatic and microbial conversion of fatty acids.

Lipid oxidation during dough mixing generates flavour volatiles, and influences dough rheology through oxidation of flour components (Table 1, Belitz et al., 2004). During mixing of wheat and rye doughs, oxygen is consumed by endogenous lipoxygenase activity (Graveland, 1973; Mann & Morrison, 1975; Belitz et al., 2004). Lipoxygenases oxidize linoleic acid to hydroxyperoxy acids. Wheat lipoxygenase preferably forms 9 hydroperoxy lineolic acid, rye lipoxygenase preferably forms the 13 hydroperoxy isomer (Figure 6, Belitz et al., 2004). Enzymatic or non-enzymatic reactions degrade hydroperoxydes to flavour active aldehydes (Czerny and Schieberle, 2002; Belitz et al., 2004). Hydroperoxy linoleic acid is alternatively reduced to hydroxy-linoleic acid with concomitant oxidation of other flour constituents. In presence of cysteine, peroxides are converted to the corresponding hydroxy-fatty acids (Figure 6, Shahzadi, 2011). Several of the resulting hydroxy fatty acids have potent biological activities. Coriolic acid (13-(*S*)-hydroxy-9Z,11E-octadecadienoic acid) has antifungal activity with an MIC of 0.1 – 0.7 g / L and 0.15% addition of coriolic acid to bread increased the mould-free shelf life of read more than twofold (Kobayashi et al., 1987, Black et al., 2013). Unsaturated di- and trihydroxy fatty acids impart a bitter taste with a taste threshold of 2 – 4 g / L (Baur et al., 1977; Biermann et al.,

342 1980). The enzymatic formation of hydroxy fatty acids during oat processing contributes to the
343 bitter taste of oat products (Biermann et al., 1980).

344 The metabolism of lactobacilli in sourdough can favor lipid oxidation during fermentation, or
345 exert strong antioxidative effects. Homofermentative lactobacilli enhance lipid oxidation and the
346 formation of nonenal and decadienal during sourdough fermentation (Vermeulen et al., 2007).
347 This effect was attributed to the formation of hydrogen peroxide during homofermentative
348 glucose metabolism. In contrast, obligate heterofermentative lactobacilli decrease the oxidation-
349 reduction potential of sourdoughs, and specifically accumulate glutathione or related low-
350 molecular weight thiol compounds (Jänsch et al., 2007; Capuani et al., 2012). Thiol
351 accumulation through heterofermentative metabolism is linked to the generation of reducing
352 equivalents in the pentose phosphate pathway (Jänsch et al., 2007), providing abundant reducing
353 power to convert lipid peroxides to hydroxydes (Figure 6). Moreover, alcohol dehydrogenases of
354 heterofermentative lactobacilli reduce the flavour–active (E)-2-nonenal and (E,E)-2,4-decadienal
355 to the corresponding alcohols during growth in sourdough (Vermeulen et al., 2007). A
356 comparable reduction of the flavour active heptenal, nonenal, nonedienal, and decadienal was
357 also observed in sourdough fermentations with a commercial starter culture containing *L.*
358 *sanfranciscensis* as dominant species (Czerny and Schieberle, 2002).

359 Lactobacilli hydrate oleic, linoleic, and linoleic acids to hydroxyl fatty acids. Linoleic acid is
360 converted to 13-hydroxy-9-octadecenoic acid or 10-hydroxy-12-octadecenoic acid (Shahzadi,
361 2011; Ogawa et al., 2001). The reaction is catalysed by a fatty acid hydratase (Volkov et al.,
362 2010; Yang et al., 2013). The physiological role of fatty acid conversion by lactic acid bacteria
363 likely relates to membrane homeostasis (Fernández-Murga et al., 1999). Formation of hydroxy

364 fatty acids during growth of *L. hammesii* in sourdough significantly extended the mould-free
365 shelf life of bread (Black et al., 2013).

366 **5. Conclusions**

367 The effect of sourdough fermentation on bread quality is dependent on enzymatic and microbial
368 conversions at the dough stage, and the activity of cereal enzymes is an important determinant of
369 the microbial ecology of sourdough. Examples for metabolic activities that are present in
370 fermentation microbiota in specific substrates include maltose metabolic enzymes and amylases
371 that are present in strains from wheat and rye sourdoughs or fermentations of C4 cereals and
372 tubers in tropical climates, respectively (Figure 1); levansucrase activity of strains in traditional
373 wheat and rye sourdoughs in response to the sucrose content of the flour (Bunaix et al., 2009;
374 Tieking et al., 2003); and the metabolism of phenolic acids by isolates from sorghum sourdoughs
375 (Figures 4 and 5). It is noteworthy that these metabolic activities are not species specific but
376 present in strains of many cereal-adapted species (e.g. maltose phosphorylase) or found as strain
377 specific metabolic traits in many different species (levansucrase, metabolism of phenolic acids).
378 Cereal substrates thus appear not to select for specific species but for "metabolic consortia" that
379 are best adapted to the substrate supply.

380 The persistence of cereal-adapted lactobacilli in wheat and rye sourdoughs was attributed to the
381 activity of cereal amylases, and the presence of maltose as main carbon source (Hammes and
382 Gänzle, 1998; Gobbetti, 2005). The contribution of endogenous enzyme activities to the
383 selection of fermentation microbiota is substantiated by recent investigations on the fermentation
384 microbiota of sourdoughs prepared from C4 cereals. These differ in their enzymatic activities
385 and support different fermentation microbiota when compared to wheat and rye sourdoughs
386 (Vogelmann et al., 2009; Sekwati-Monang et al., 2012). Likewise, proteolysis by endogenous

enzymes provides substrates for microbial metabolism, and determines the microbial ecology of type II sourdoughs (Su et al., 2011). Proteolysis in gluten-free sourdoughs, however, is currently poorly understood.

Phenolic compounds and lipids are minor constituents of cereal flours. These compounds and their derivatives are potent bioactive compounds and influence bread quality already in micro- or nanomolar concentrations (Opperer et al., 2012, Jänsch et al., 2007). The emerging knowledge of their conversion by cereal enzymes and microbial metabolism during sourdough fermentation will provide innovative fermentation processes and starter cultures for improved bread quality.

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681

Figure legends

Figure 1. Starch metabolism and conversion of maltodextrins in sourdough. Panel A represents enzymes and metabolic pathways that relevant in wheat and rye sourdoughs. Panel B represents enzymes and metabolic pathways that are relevant in substrates with low amylase activity, e.g. corn, sorghum, and tubers. Conversions by cereal enzymes are indicated in blue colour; conversions by microbial enzymes are indicated in red colour.

Panel A. Wheat and rye flours exhibit α-amylase, β-amylase and glucoamylase activity liberating maltodextrins, maltose, and glucose, respectively, from starch during sourdough fermentation. Amylases but not glucoamylases are inhibited by acidification to pH < 4.5 (Brandt, 2006; Belitz et al., 2004). Key organisms in sourdoughs, including *L. sanfranciscensis*, *L. reuteri* and *L. fermentum*, harbour maltose phosphorylase (MalP) and an 1,6-α-glucosidase (DexB) as sole glucan-hydrolysing enzymes (Gänzle and Follador, 2012). MalP phosphorylyses maltose to D-glucose and β-D-glucose 1-phosphate and is highly specific for maltose (Ehrmann and Vogel, 1998); DexB hydrolyses α(1→6) linkages but not α(1→4) linkages in gluco-oligosaccharides.

Panel B. Starch degradation in substrates with low amylase activity, including sorghum, pearl millet, corn, and tubers (cassava, potatoes). Resting grains of C4-cereals generally have no β-amylase activity (Taylor et al., 2006). In these grains as well as in fermentations of tubers or porridges of cooked cereals, starch degradation depends on extracellular amylases (AmyX) of lactic acid bacteria and amylolytic strains of *L. fermentum* or *L. plantarum* are frequently isolates in these fermentations (Songré-Ouattara et al., 2008; Turpin et al., 2011). Many lactobacilli, including *L. plantarum*, *L. acidophilus*, and *L. gasseri*, harbour a full complement of enzymes need for maltodextrin transport and hydrolysis (Gänzle and Follador, 2012). Maltodextrins are transported by the ATP-binding cassette transport system , (Nakai et al., 2009); the intracellular

705 glucosyl hydrolases **MalN** and **MalL** amylopullulanases, hydrolyse α(1→6)- and α(1→4)-
706 glucosidic linkages in maltodextrins and isomaltodextrin (Nakai et al., 2009). Phosphorolysis of
707 maltose and hydrolysis of isomaltodextrins are catalysed by MalP and DexB, respectively (see
708 PanelA).

709 **Figure 2.** Schematic representation of glucansucrase activity in wheat and rye flours (Panel A)
710 and sorghum flours (Panel B). Sucrose conversion by glucansucrases proceeds through a
711 covalent linkage of glucose to the catalytic site of the enzyme as catalytic intermediate (van
712 Hijum et al., 2006). Glucose is subsequently transferred to a glucosyl-acceptor; suitable
713 acceptors for reuteransucrase or dextransucrase include water, maltose, panose-series
714 oligosaccharides (POS) and reuteran or dextran. In wheat and rye sourdoughs, maltose is present
715 in high concentrations throughout the fermentation and results in formation of high levels of POS
716 or maltodextrins (MD) at the expense of reuteran or dextran formation (Panel A). In sorghum,
717 buckwheat or quinoa fermentations, initial maltose concentrations are low and maltose is rapidly
718 depleted during fermentation. Correspondingly, POS formation is low or absent and the yield of
719 reuteran or dextran is increased (Kaditzky et al., 2008; Galle et al., 2010; Rühmkorf et al., 2012).

720 **Figure 3.** Overview on proteolysis and amino acid metabolism in wheat and rye sourdoughs
721 (modified from Stromeck et al., 2011). Conversions by cereal enzymes are indicated in blue
722 colour; conversions by microbial enzymes are indicated in red colour.

723 Insoluble or polymeric prolamins of wheat and rye are solubilized by microbial acidification and
724 the disruption of intermolecular disulfide bonds, which is dependent on glutathione
725 dehydrogenase and related activity of sourdough lactobacilli. Primary proteolysis (conversion of
726 proteins to peptides) is dependent on substrate-derived enzymes or enzymes from added malt or
727 fungal enzyme preparations. Lactobacilli convert peptides to amino acids by strain-specific

728 intracellular peptidases, and convert amino acids to specific metabolites. For review, see Gänzle
729 et al. (2008).

730 **Figure 4.** Overview on conversion of phenolic compounds during sourdough fermentation.
731 Conversions by cereal enzymes or chemical reactions are indicated in blue colour; conversion by
732 microbial enzymes are indicated in red colour.

733 Lactobacilli harbour enzymes catalyzing the release of bound phenolic acids by feryloyl esterase
734 hydrolysing esters of ferulic acid (Wang et al., 2004); tannase (tannin acylhydrolase),
735 hydrolysing galloyl ester bonds of gallotannins (Iwamoto et al., 2008), and glycosyl hydrolases
736 releasing the flavonoid aclycons from flavonoid hexosides (Avila et al., 2009). These
737 conversions are also observed in acid aseptic cereal fermentations (Svenson et al., 2010; Hole et
738 al., 2012) but corresponding cereal enzymes are not characterized. Phenolic acids are converted
739 in cereal fermentations by strain-specific phenolic acid decarboxylases and cinnamic acid
740 reductases of cereal-associated lactobacilli (Svensson et al., 2010). See Rodriguez et al., 2009 for
741 review.

742 **Figure 5**. Antimicrobial activity of phenolic acids and phenolic acid metabolites against
743 *Lactobacillus hammesii* and *Lactobacillus plantarum*. Shown is the minimum inhibitory
744 concentration of caffeic acid and dihydrocaffeic acid, the product of microbial hydration of
745 caffeic acid as well as the activity of protocatechuic acid and catechol, the product of microbial
746 decarboxylation. Data from Sánchez-Maldonado et al. (2011).

747 **Figure 6.** Conversion of fatty acids. Conversions by cereal enzymes are indicated in blue colour;
748 conversion by microbial enzymes are indicated in red colour.

749 Fatty acid hydratases of lactobacilli convert oleic acid, linoleic acid, and linolenic acid to
750 hydroxy-fatty acid. 10-hydroxy-12-octadecenoic acid is the predominant product of linoleic acid
751 conversion by sourdough lactobacilli (Shahzadi, 2011; Volkov, 2010; Ogawa et al., 2001).

752 Cereal lipoxygenase activity oxidizes linoleic acid to linoleic peroxide (Belitz et al., 2004). In
753 presence of cysteine, the peroxide is chemically converted to the corresponding hydroxy-fatty
754 acid coriolic acid (Shahzadi, 2011), a compound with antifungal activity (Kobayashi et al.,
755 1987). Thiol levels in wheat sourdough are increased by the metabolism of heterofermentative
756 lactobacilli (Jänsch et al., 2007). Chemical degradation of linoleic acid peroxide results in
757 formation of flavour-active aldehydes, including hexanal, nonenal, and decadienal (Belitz et al.,
758 2004). During sourdough fermentations, these flavour-active aldehydes are converted to the
759 corresponding alcohols by alcohol dehydrogenase activity of heterofermentative lactobacilli
760 (Vermeulen et al., 2007; Czerny and Schieberle, 2002).

761

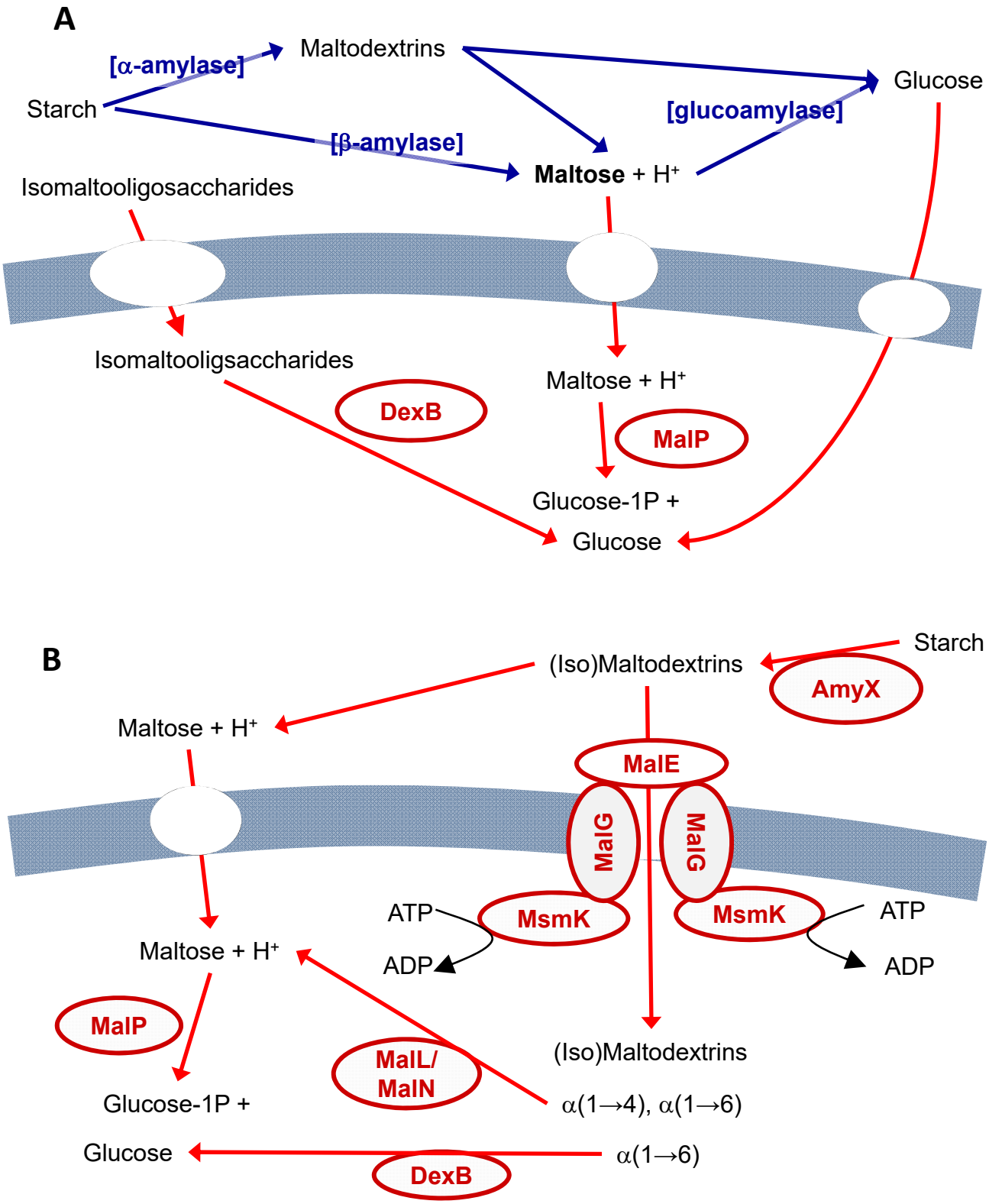
762 **Table 1.** Overview on the role of microbial and enzymatic conversions during sourdough

763 fermentation in microbial physiology, and their contribution to bread quality.

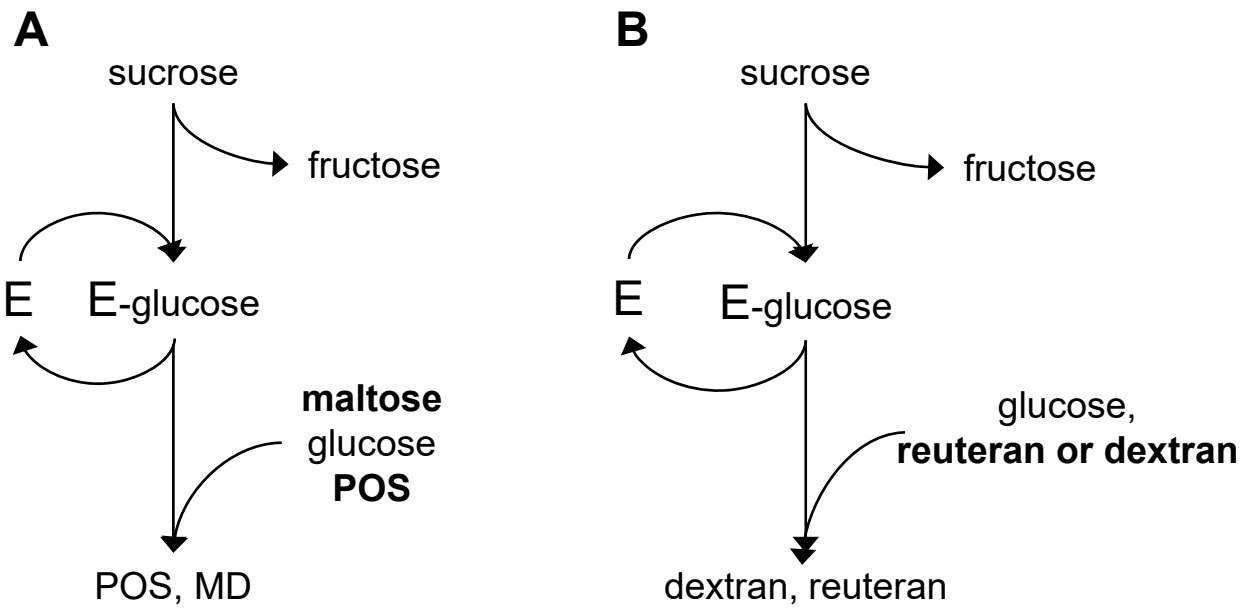
Role in microbial physiology	Contribution to bread quality
Carbohydrate conversion and metabolism	
Metabolic energy (maltose, sucrose) Cofactor regeneration (fructose) Protection against environmental insults (oligosaccharides, exopolysaccharides) Biofilm formation (exopolysaccharides)	Texture (starch) Water binding, staling (starch, pentosans, EPS) Taste and shelf life (organic acids) Generation of reducing sugars for flavour generation during baking Dietary fibre and prebiotic oligosaccharides
Protein conversion and metabolism	
Nitrogen source Metabolic energy (alanine) Acid resistance (Gln, Glu, Arg) Cofactor regeneration (Glu, glutathione); and protection against oxidative stress (Cys)	Volume (gluten) Taste and flavour (glutamate, ornithine, other amino acids) Bioactive compounds (γ-aminobutyrate) bioactive peptides (taste-active, ACE-inhibitory)
Conversion of phenolic compounds	
Metabolic energy (hydrolysis of flavonoid hexosides) Removal of noxious compounds	Elimination of antinutritive factors (enzyme inhibitors) Elimination of bitter taste (tannins) Increased bioavailability of phenolics as antioxidants Flavour volatiles
Lipid metabolism	
Metabolic energy (cofactor regeneration) Membrane homeostasis (synthesis of unsaturated and hydroxy fatty acids)	Control of lipid oxidation (taste, flavour) Formation of antifungal compounds

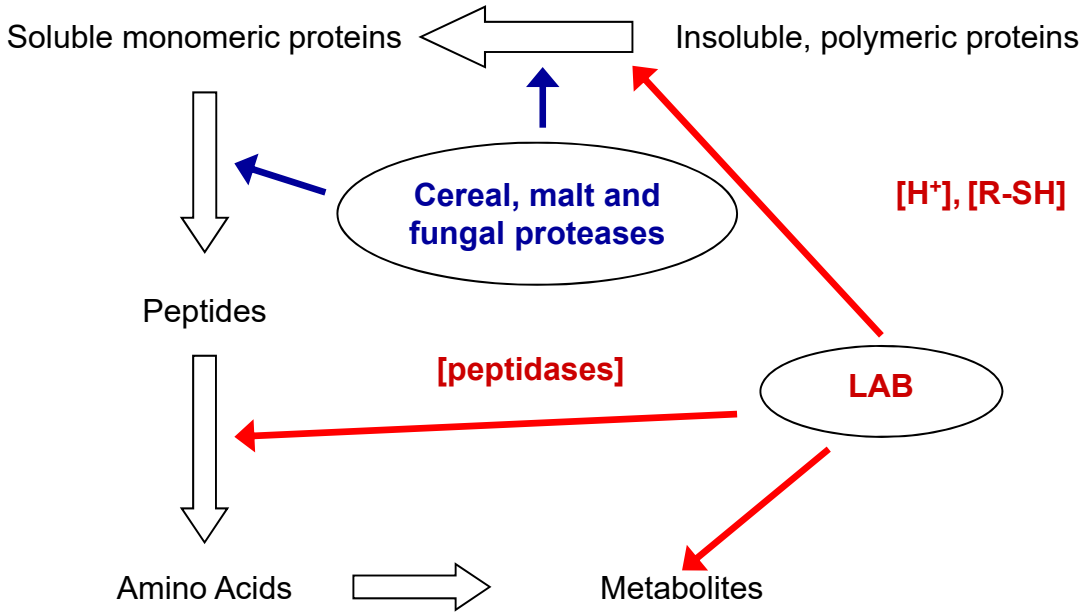
764

Gänzle, Figure 1.

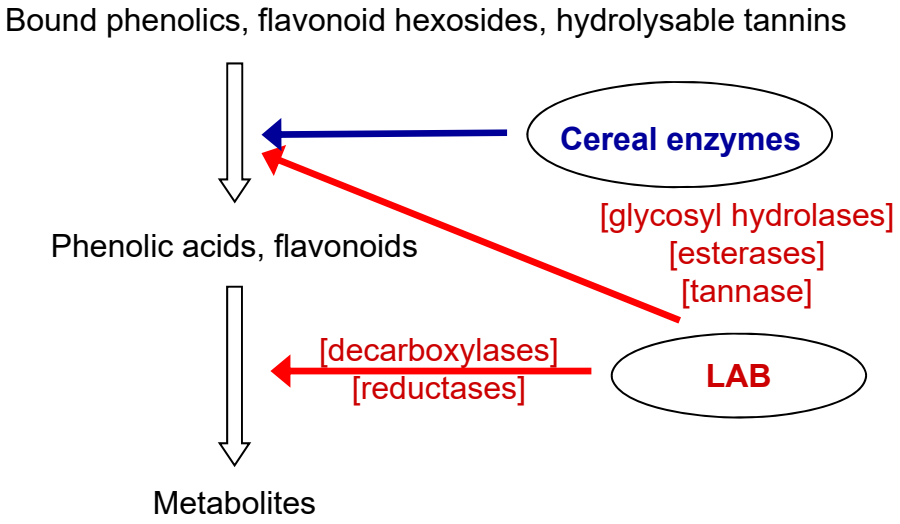


Gänzle, Figure 2





Gänzle, Figure 3



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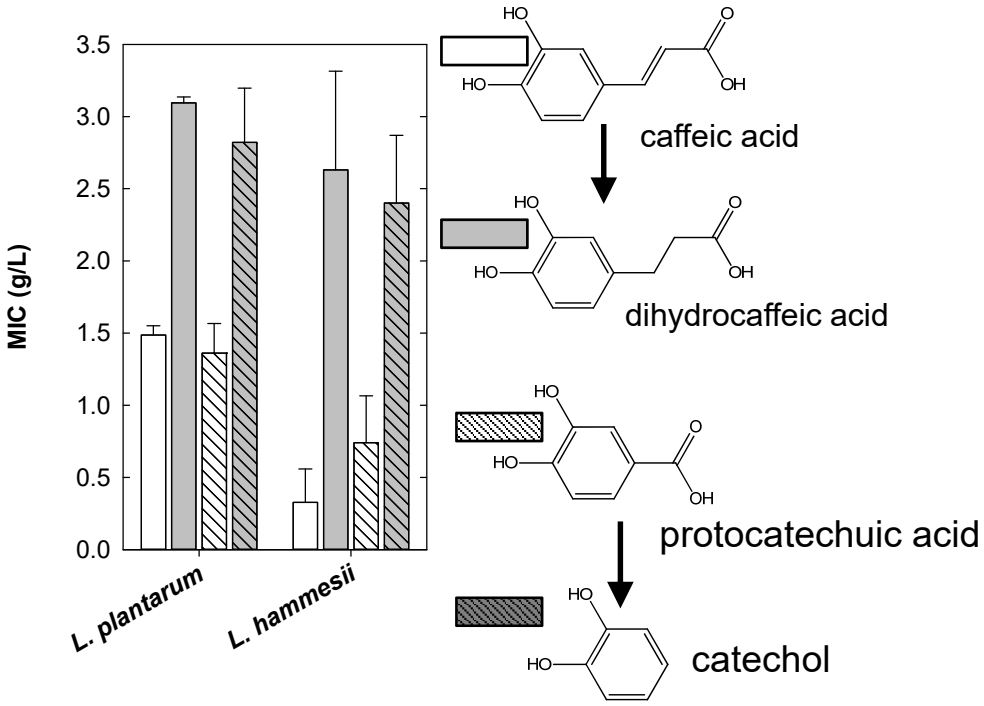

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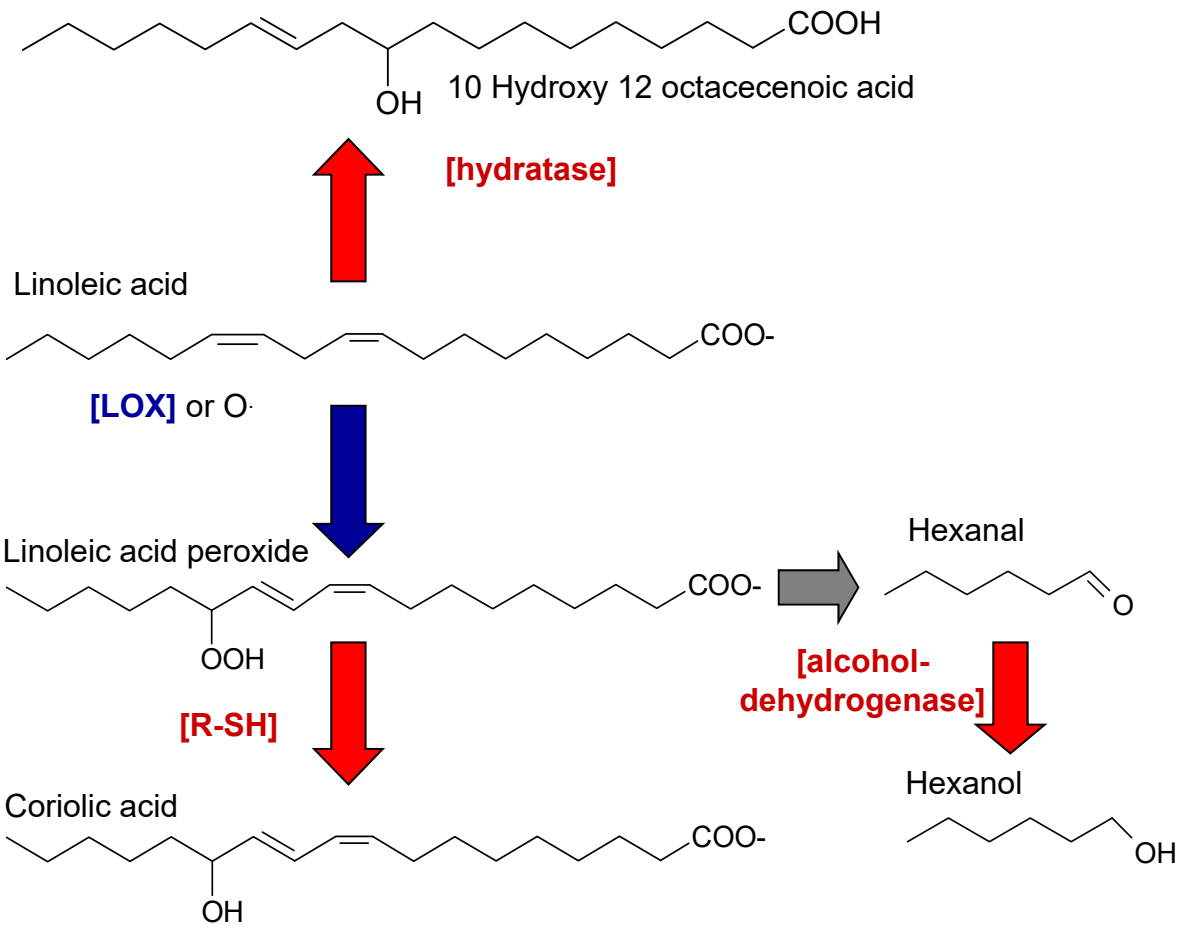

Gänzle, Figure 6