Food Fermentations for improved digestibility of plant foods – an essential *ex situ* digestion step in agricultural societies?

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Abstract

The fermentation of plant foods detoxifies and eliminates compounds that are inherently present in grains and legumes and have antinutritive properties, including cyanogenic glycosides, vicine and convicine, phytate, phenolic compounds, immune-reactive proteins and fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs). Contemporary food production has partially replaced fermentation of plant foods by alternative processes for production of nutritious plant foods. This communication explores the question whether the conversion of noxious components in plants by food fermentations remains relevant in contemporary food production, or, more pointedly worded, whether food fermentations are an essential *ex situ* digestion step for agricultural societies. Noxious compounds in grains and legume seeds contribute to irritable bowel syndrome, non-celiac wheat intolerance, and food allergies. Food fermentations provide an effective unit operation to improve tolerance of plant foods to sensitive individuals. In addition, they are a source of viable and active microorganisms that may provide additional health benefits.

Keywords. Food fermentations, *Lactobacillus*, sourdough, probiotics, fructans, FODMAP, amylase-trypsin inhibitor, phytate.

Introduction

The transition of groups of hunterer-gatherers to agricultural societies in the Neolithic Revolution about 14,000 years ago paved the way to modern civilization [1] and substantially impacted the human diet. Hunterer-gatherer diets included a high proportion of animal protein and diverse plants including fruits, vegetables and tubers. In contrast, the diet of agricultural societies relied on only few plant species, particularly cereals, legumes and oilseeds. Nutritional consequences of this transition include a reduced intake of protein and dietary fibre, a reduced supply of minerals, and increased exposure to anti-nutritive factors in seeds [2, 3]. The origin of food fermentations, which are defined as the preparation of foods or beverages by controlled microbial growth and enzymatic conversions [4], predates the origin of agriculture [5, 6]. The case was made that cereals were domesticated to enable fermentation of alcoholic beverages for rituals or festivities about 14,000 years ago [5]. While this hypothesis is not undisputed, the knowledge on how to ferment cereals and legumes to improve the digestibility of grains and legumes, and to remove anti-nutritive compounds likely was a prerequisite for the Neolithic Revolution. Because fermented foods have unique sensory properties and are often deeply rooted in local culture, they have remained a staple in the human diet after the shift from artisanal to industrial food production in the last 150 years. However, alternative preservation methods including thermal processing or refrigeration, alternative separation and processing methods including extrusion or isolation of protein fractions, and simplified and accelerated fermentation methods e.g. in production of bread or soy sauce, have reduced the intake of fermented foods as well as the intake of live and active microbes. This communication explores whether the removal of anti-nutritive, pro-inflammatory or toxic compounds in cereals grains and legumes by traditional fermentation processes remains relevant in contemporary human nutrition by improving the tolerance of sensitive individuals to legumes or cereal products.

Are seeds meant to be eaten?

Toxic, conditionally toxic or anti-nutritive components in grains and legumes. Plants are sessile organisms that cannot escape pathogens or predators and therefore employ chemical defenses against microbial pathogens and herbivores. These chemical defenses are termed phytoalexins or phytoanticipins depending on whether they are pre-formed in the plant (phytoanticipins) or produced after tissue damage by pathogen or herbivores attack (phytoalexins) [7, 8]. Phytoalexins and phytoanticipins include phenolic compounds and antifungal agents that receive attention as antioxidative food components, food preservatives and biological fungicides [9] but also include compounds that have antinutritive properties, or are outright toxic to humans. Compounds that protect plants against abiotic stress, e.g. raffinose-family oligosaccharides in legumes and fructans in cereals [10, 11], also result in adverse digestive symptoms. Toxic, conditionally toxic or antinutritive food components in plants (Table 1) were identified in the last century and are described only briefly [12–14].

Cyanogenic glucosides. Cyanogenic glucosides release cyanide after hydrolysis of the β-glucosidic bond [14]. Among edible parts of plants, the content of cyanogenic glucosides is high (0.5 – 4 g HCN /kg) in bitter tubers of cassava, lima beans, bitter almonds, and flaxseed. Chronic cyanide intoxication, leading to a disease termed konzo, is a consequence of improperly processed cassava and has repeatedly been observed in Africa in times of famine [15]. Comparable diseases are not reported from South American countries, where cassava originates; this may relate to the long tradition of safe preparation of cassava roots in South America, which reduces the likelihood of improper preparation even at times when food is scarce.

Vicine, convicine and favism. Faba beans contain the pyrimidine glucosides vicine and convicine, which constitute up to 2% of the dry weight of the beans. The aglycones are redox-reactive and have antifungal properties. During digestion, the aglycones are released through β-glucosidases from faba beans or intestinal microorganisms. The aglycones oxidize glutathione *in vivo* and cause hemolytic anemia termed favism in individuals with glucose-6-phosphate dehydrogenase deficiency [16]. Favism is more prevalent in countries where malaria occurs because the resistance of individuals with low glucose-6-phosphate dehydrogenase activity to malaria provides selective pressure for favism [16].

Phytic acid. Phytic acid is the main storage compound for phosphorous and minerals in cereal and legume seeds; their phytate content ranges from 5 to 20 g / kg [13, 17]. Phytates are not hydrolysed in the monogastric digestive tract until digesta reach the large intestine. The complexes formed by phytic acid and divalent minerals including Ca^{2+}, Zn^{2+} and iron are insoluble; hence, phytates reduce the bioavailability of minerals. In affluent countries, an adequate supply of Ca^{2+} and Zn^{2+} is warranted by animal products in the diet [18]. Ca^{2+} and Zn^{2+} deficiencies are more commonly observed in developing countries and complexation of dietary minerals by phytates in plant foods contributes to the mineral deficiency [18]. Iron-deficiency anemia is observed in developed as well as developing countries, particularly in young women [19]. Iron uptake from plant foods is not only impeded by complexation with phytate but also by complexation with tannins [19, 20].

Phenolic compounds. Proanthocyanidins, gallotannins and ellagitannins, commonly referred to as tannins, are phenolic compounds that occur in a wide variety of plant foods. Their presence in cereals and legumes is dependent on the plant species and the cultivar [21]. For example, the content of proanthocyanidins and 3-deoxyanthocyanins in pea and sorghum varieties, respectively, is highly variable [22, 23]. Tannins impart bitter taste, reduce protein and starch digestibility by inhibition of pancreatic enzymes, and reduce iron uptake [21, 23]. In affluent countries, the presence of tannins reduces the caloric content and the glycemic index of foods [24] while in countries with low food security, their presence reduces the supply of macro- and micronutrients.

Enzyme inhibitors. Specific inhibitors of digestive enzymes further reduce the digestibility of starch and proteins in legumes and cereals. Wheat and other cereals contain amylase-trypsin inhibitors, which account for up to 4% of the total protein in the grain endosperm [25]. Protease and amylase inhibitors are also present in seeds of legumes and oilseeds [12, 21].

Fermentable oligosaccharides, disaccharides, monosaccharides, and polyols. The content of raffinose-family oligosaccharides pulses ranges from 1% to 6% with stachyose as most abundant compound. In cereals, the content ranges from 0.5 – 1.5% and raffinose is the sole or the most abundant compound [26, 27]. Cereals, in turn, contain 1 – 5% of fructans with a DP of 3 – 6 [27]. Ingestion of non-digestible oligosaccharides results in adverse digestive symptoms when a threshold of about 15 g / person and day is exceeded [28], a threshold that is readily exceeded in cereal- or legume based diets unless the content of these oligosaccharides is reduced by fermentation or germination.

Lactose is not present in plant foods, however, adverse effects of lactose ingestion relate to those caused by other indigestible oligosaccharides and are thus also briefly discussed in this communication. Infants digest lactose through the activity of brush-border β-galactosidase (lactase); generally, the expression of brush border lactase is reduced after weaning and most human adults do not digest lactose [29]. About 25 – 30% of human adults are lactase persistent; the current high prevalence in European populations evolved in less than 5000 years [29]. The ability of humans to digest lactose is thus substantially predated by the consumption of fermented dairy products, which dates back to about 5,000 BCE [30].

Are noxious components of plants and animal foods degraded in food fermentations?

Cyanogenic glycosides. Cyanogenic glycosides are degraded by substrate-derived β-glucosidase after injury of plant tissue by milling (Fig 1). β-Glucosidase activity of *Lactiplantibacillus plantarum* (previously *Lactobacillus plantarum* [31]) and other fermentation organisms contributes to the degradation of cyanogenic glycosides to glucose and the volatile cyanide during fermentation of cassava [32].

Vicine and convicine in beans. Comparable to cyanogenic glycosides, the β-glucosides of vicine and convicine are degraded during fermentation of faba beans by substrate-derived or microbial β-glucosidases (Fig 1) [33]. The aglycones divicine and isouramil are reactive and unstable, and are rapidly removed during fermentation [33].

Degradation of phytate. The phytase activity of cereals [13] is sufficient to degrade phytates if the insoluble salts of phytate and divalent cations are solubilized by acidification [34, 35]. Phytate degradation in sourdough thus occurs independent of microbial phytases. Cereal phytases are optimally active pH 5.5 but remain active at the lower pH-values that are achieved in sourdough and legume fermentations [35]. The use of sourdough in bread production, and lactic fermentations for production of cereal porridges or beverages thus increase the bioavailability of minerals [36].

The phytase activity in legumes is lower when compared to cereals [13, 37], however, processing of legumes by soaking, milling and lactic fermentation prior to cooking substantially reduces phytate levels. One example for such a product is idli, which is produced by fermentation of rice and legumes in South Asia [38]. Fermented soy products in South East Asia are produced by steaming or cooking of raw materials prior to fermentation [39]. In these products, substrate-derived phytases are inactivated and phytate degradation is achieved by fermentation with bacilli or fungal cultures, e.g. *Rhizopus stolonifer* or *Aspergillus oryzae,* which hydrolyse phytate with extracellular enzymes [40, 41]. Examples of these products include tempe (or tempeh) produced in Indonesia, stinky tofu produced in China and natto produced in Japan.

Phenolic compounds. The content of tannins is reduced in fermentations of plant foods [42]. In particular, red and black sorghum varieties, which are cultivated in sub-Saharan Africa because of their superior drought and pest resistance, are considered essentially inedible unless they are processed by germination and / or fermentation. Only few studies, however, document the biochemical conversions of phenolic compounds including tannins at the molecular level [43–45]. Lactic acid bacteria metabolise tannins by tannases and related phenolic acid esterases (Fig 1) [46]. Tannase releases gallic acid from tannins, thus reducing their interaction with proteins as well as the affinity to iron. In cereal fermentations, phenolic acid esterases of lactobacilli release phenolic acids that are esterified with plant cell wall polysaccharides [46, 47]. Glycosides of phytochemicals including flavonoids are metabolised by diverse glycosyl hydrolases, releasing the corresponding aglycons [44]. Phenolic acids are metabolized by phenolic acid reductases, phenolic acid decarboxylases, and vinyl phenol reductases [48, 49]. Phenolic acid metabolism by lactic acid bacteria is strain specific and frequently observed in organisms of the genera *Lactiplantibacillus*, *Levilactobacillus* and *Furfurilactobacillus* (previously *L. plantarum*, *Lactobacillus brevis* and *Lactobacillus rossiae* groups, respectively [31]) as well as *Limosilactobacillus fermentum* (previously *Lactobacillus fermentum* [31, 48], which are dominant fermentation organisms in spontaneous cereal, legume and vegetable fermentations [39]. The products of phenolic acid metabolism include dihydro-derivatives of hydroxycinnamic acids, and decarboxylated volatiles which contribute to the food flavor [46]. Lactic fermentation also generates pyranoanthocyanidins or pyrano-3-deoxyanthocyanidins, which are formed by condensation of vinylphenols, products of decarboxylation of hydroxycinnamic acids by lactobacilli, and anthocyanidins or 3-

deoxyanthocyanidins [45]. The biological activities of the metabolites that are formed from phenolic compounds including their nutritional properties, however, remain to be explored [43].

Degradation of fermentable oligosaccharides, disaccharides, monosaccharides, and polyols. FODMAPs including lactose, raffinose-family oligosaccharides and fructans are partially or completely degraded by fungal or bacterial enzymes during food fermentations (Fig 1). Fermentation of yoghurt and related fermented dairy products removes only 10 – 20% of the lactose in milk [50], however, β-galactosidases of fermentation organisms remain active throughout gastrointestinal transit, hydrolyse lactose, and thus alleviate lactose intolerance [51].

Raffinose family oligosaccharides are hydrolysed through the activity of α-galactosidases, levansucrase and sucrose-phosphorylase activities of lactic acid bacteria [52, 53] or corresponding enzymes of fungal cultures; their removal in legume fermentations has been amply documented (Fig 1) [54].

The removal of fructans in cereal flours has been explored only recently. In fermentations for bread production, extracellular yeast invertase and intracellular fructanases of lactic acid bacteria hydrolyse sucrose and low molecular weight oligosaccharides but fructans with a higher degree of polymerization are not degraded [27]. The use of lactobacilli or yeasts expressing extracellular fructanases, which is currently employed only in few commercial applications, achieves hydrolysis of all fructans for production of low-FODMAP bread [27, 55]. Because the adverse effects of FODMAPs are dose dependent, even their partial reduction in food fermentations alleviates or even eliminates adverse symptoms.

Degradation of patulin and other mycotoxins. Patulin is a mycotoxin that is produced by *Penicillium expansum* and other fungi growing on fruits [56]. Its toxicity relates to the reactivity with thiols [57], which depletes cellular glutathione levels; accordingly, generation of thiols by yeasts or heterofermentative lactobacilli during alcoholic or lactic fermentation of fruit juices inactivates patulin. *L. plantarum* also converts patulin by uncharacterized esterase and reductase activities [58].

Aflatoxin levels were reduced in cereal and legume fermentations [59], however, pure cultures of lactobacilli do not convert aflatoxin [59] and enzyme activities that are known to degrade aflatoxin are not expressed by food-fermenting lactobacilli [60]. It thus remains unknown whether the apparent reduction of aflatoxin levels in food fermentation [59] is attributable to absorption of the toxin to bacterial biomass [61], or to the co-operative activity of bacterial and substrate-derived enzymes. It is thus uncertain whether the reduction of mycotoxins in food fermentations is achieved only by specific combinations of raw materials and fermentation cultures, or relate to a principle that is more generally applicable to fermented foods.

Is the fermentative degradation of noxious compounds in plant foods relevant today?

In developed countries with high food security, offending plant foods can be replaced by other plant or animal foods, as is the case e.g. in gluten-free or low-FODMAP diets, and food processing provides alternative technologies for detoxification of plant materials. Moreover, antinutritive compounds may be health beneficial in affluent societies, where diets are often characterized by an excess of rapidly digestible carbohydrates and a low intake of dietary fibre. For example, inhibition of starch digestion by phenolic compounds decreases the glycemic index and the risk of diabetes and the metabolic syndrome [24, 28]. Likewise, raffinose-family oligosaccharides cause digestive discomfort when consumed in large amounts but have beneficial prebiotic properties when consumed at an adequate dose [28]. Nutritional benefits of food fermentations, however, remain relevant even in affluent societies. As outlined further in this section, these nutritional benefits relate to the intake of viable and non-pathogenic microbes, the reduction of the content of FODMAPs, and modification of immune-reactive proteins in food.

Live and active dietary microbes. Although not all fermented foods contain viable fermentation organisms at the time of consumption, fermented foods are a major contributor to the dietary intake of viable and non-pathogenic microbes [4, 62]. Species that comprise strains with well documented probiotic properties include *Lactobacillus acidophilus, Lactobacillus johnsonii, Lacticaseibacillus casei* (previously *Lactobacillus casei* [31]), *Lacticaseibacillus rhamnosus* (previously *Lactobacillus rhamnosus* [31]), *L. plantarum, Limosilactobacillus reuteri* (previously *Lactobacillus reuteri* [31]) and *L. fermentum* are also present in high cell counts in specific fermented foods [39, 63]. Lactobacilli that are part of fermentation microbiota in fermented foods were shown to exhibit probiotic properties [4, 64]. Whether or not probiotic properties should be attributed to fermentation cultures in fermented foods is discussed controversially [4, 65], however, the presence of viable and active microbes in fermented foods may address the reduced intestinal microbial diversity in developed countries.

Fermentation, FODMAPs and irritable bowel syndrome. The link of fructans, food fermentations and the irritable bowel syndrome or non-celiac wheat intolerance has been established relatively recently. Non-celiac wheat intolerance is poorly defined and is diagnosed by elimination of wheat allergies and celiac disease [66]. Non-celiac wheat intolerance, which affects approximately 6% of the North American population, largely overlaps with irritable bowel syndrome, which has a prevalence of about 11-15% [66]. A majority of individuals with irritable bowel syndrome or non-wheat intolerance are fructose malabsorbers [66]; i.e. fructose, which is a digestible sugar in most individuals, particularly when consumed in association with glucose, is non-digestible. A low FODMAP diet, which necessitates avoidance of wheat and onions, improves symptoms of the IBS [67] but also reduces the intake of dietary fibre and thus reduces the diversity of the intestinal microbiota, particularly by depleting the relative abundance of bifidobacteria [68]. Health benefits that are associated with ingestion of dietary fibre including non-digestible oligosaccharides are well documented [28, 69]. Low-FODMAP or gluten free diets may thus have long-term adverse health effects if the “fibre gap” is not addressed.

Does sourdough fermentation improve the tolerance of wheat products for individuals with non-celiac wheat intolerance or the irritable bowel syndrome? Anecdotal evidence for improved tolerance of sourdough bread is widely shared by sourdough bakers and social networks, however, scientific evidence for this claim is scarce. FruA mediates degradation of fructans in sourdough [27]; this enzyme is generally present in oral streptococci but present only in few swine-associated lactobacilli that also occur in one industrial sourdough fermentation [27, 63, 70]. FosE, the second extracellular fructanase in lactobacilli, is present only in *Lactocaseibacillus* and *Liquorilactobacillus* (previously *L. casei* group and part of the *L. salivarius* group [31]), organisms which do not commonly occur in commercial sourdough fermentations. Even conventional sourdough fermentation, which is typically carried out with *Fructilactobacillus sanfranciscensis* (previously *Lactobacillus sanfranciscensis*) in combination with *Kazachstania humilis* (previously *Candida humilis*) in type I sourdoughs or *Lactobacillus* and *Limosilactobacillus* species in type II sourdoughs (previously *L. delbrueckii* group and *L. reuteri* group [31, 71, 72]), reduces the FODMAP content by about 50% through elimination of raffinose-family oligosaccharides and fructans with a low degree of polymerization (Fig. 1) [27, 73]. The improved tolerance of low-FODMAP bread produced with FruA-expressing lactobacilli in individuals with the irritable bowel syndrome was demonstrated in clinical trials [74]. Because adverse effects are dose-dependent, however, it is likely that even conventional sourdough fermentation improves tolerance of wheat and rye bread for a significant portion of susceptible individuals.

Modification of immune-reactive dietary proteins. The prevalence of allergies in the population of affluent countries is increasing, in parallel with an increased prevalence of other auto-immune disorders [75]. Fermented foods were associated with a reduced allergenicity when compared to the corresponding non-fermented counterparts [76]. Fermented foods are not generally hypo-allergenic but specific allergens are degraded by fermentation of milk [77], soy [78], wheat [79] and eggs [80].

What distinguishes food fermentations from other unit operations in food processing? Fermentation with lactic acid bacteria, particularly heterofermentative lactic acid bacteria, modifies protein in the fermentation substrate by proteolysis and by reduction of disulfide bonds (Fig. 1). Proteolysis is readily achieved by alternative (enzymatic) processes but sustained reducing power for modification of disulfide-linked immune-reactive proteins requires metabolism by living cells and is achieved only by germination of seeds or by fermentation. Proteolysis is a key selection criterion for cultures in dairy fermentations while substrate-derived or fungal proteases are relevant in cereal and legume fermentations [81, 82]. Low-molecular weight thiol compounds are accumulated particularly by the glutathione reductase and cystathionine-γ-lyase activity of heterofermentative lactobacilli including *F. sanfranciscensis* and *L. reuteri* [83], key organisms in cereal fermentations [71, 72]. A specific contribution of glutathione dehydrogenase of *F. sanfranciscensis* to protein modification was demonstrated for the hydrolysis of gluten proteins in wheat sourdoughs [82] and for reduced allergenicity of ovotransferrin [80].

The relevance of protein modifications during food fermentation also relates to proteins that are not allergenic but exhibit pro-inflammatory properties and may exacerbate or trigger auto-inflammatory diseases. Lectins including the wheat germ agglutinin are highly disulfide-bonded and immune-reactive proteins, however, their contribution to adverse health effects in humans is disputed [84] and their fate in food fermentations is unknown. Pro-inflammatory properties are more clearly established for the wheat amylase-trypsin inhibitor, which not only inhibits pancreatic enzymes but also induces intestinal inflammation through activation of Toll-like receptors [85]. The wheat amylase trypsin inhibitor is a hetero-tetramer which inhibits insect and mammalian amylases [25]. Owing to its pro-inflammatory activity, the wheat amylase-trypsin inhibitor was also hypothesized to contribute to non-celiac wheat intolerance and the irritable bowel syndrome [66]. Germination in combination with sourdough fermentation of wheat flour decreased the trypsin inhibitory activity [86], likely through degradation of amylase trypsin inhibitors. Sourdough fermentation of flour from resting grains also reduced ATI levels, and converted the dominant and biologically active multimeric form to the monomeric form [74]. However, detailed studies on the role of proteolysis, reduction of disulfide bonds, heat inactivation during baking, or other factors on the fate of ATI in (sourdough)-baking are currently lacking.

Food Fermentations – an essential *ex vivo* digestion step for agricultural societies?

Humans have mastered the skill of conversion of agricultural crops by fermentation to improve their nutritional value since the Neolithic Revolution. Whether the ability to control food fermentations was a prerequisite for this landmark transition in human history cannot be answered with currently available data, however, several beneficial nutritional aspects of fermented foods remain relevant at present. Can food fermentations be considered an essential *ex situ* digestion step to achieve improved digestibility of plant foods in agricultural societies? This question is partially inspired by the observation that many omnivorous or herbivorous monogastric animals including swine, poultry and rodents harbor lactobacilli as dominant members of the microbiota in their upper intestine, i.e. the esophagus, the crop and the forestomach [87]. The composition and the metabolic functions of these animal microbiota substantially overlap with the species-level composition and metabolic activity of lactobacilli in food fermentations [63]. The case can be made that humans compensated the lack of an organ for *in vivo* lactic fermentation by using the cognitive function of another organ, the brain, to employ food fermentation as an *ex situ* digestion step to improve the nutritional value of plant crops.

Is the case convincing? Probably not. Most humans can digest most plant foods or, in regions with high food security, are able to substitute offending foods with more appropriate choices.

Is the analogy relevant from a public health perspective? Probably yes. Gastrointestinal disorders including irritable bowel syndrome, non-celiac wheat intolerance and auto-immune disorders impact a substantial part of the population in developed countries. Even though an increased consumption of fermented foods may make only a small and incremental change in these disorders, an increased proportion of fermented foods in the diet, or a reversion to including more diverse fermentation microbiota, e.g. by reinstating sourdough fermentations in bread production, may increase the health and the quality of life in a significant proportion of the population.

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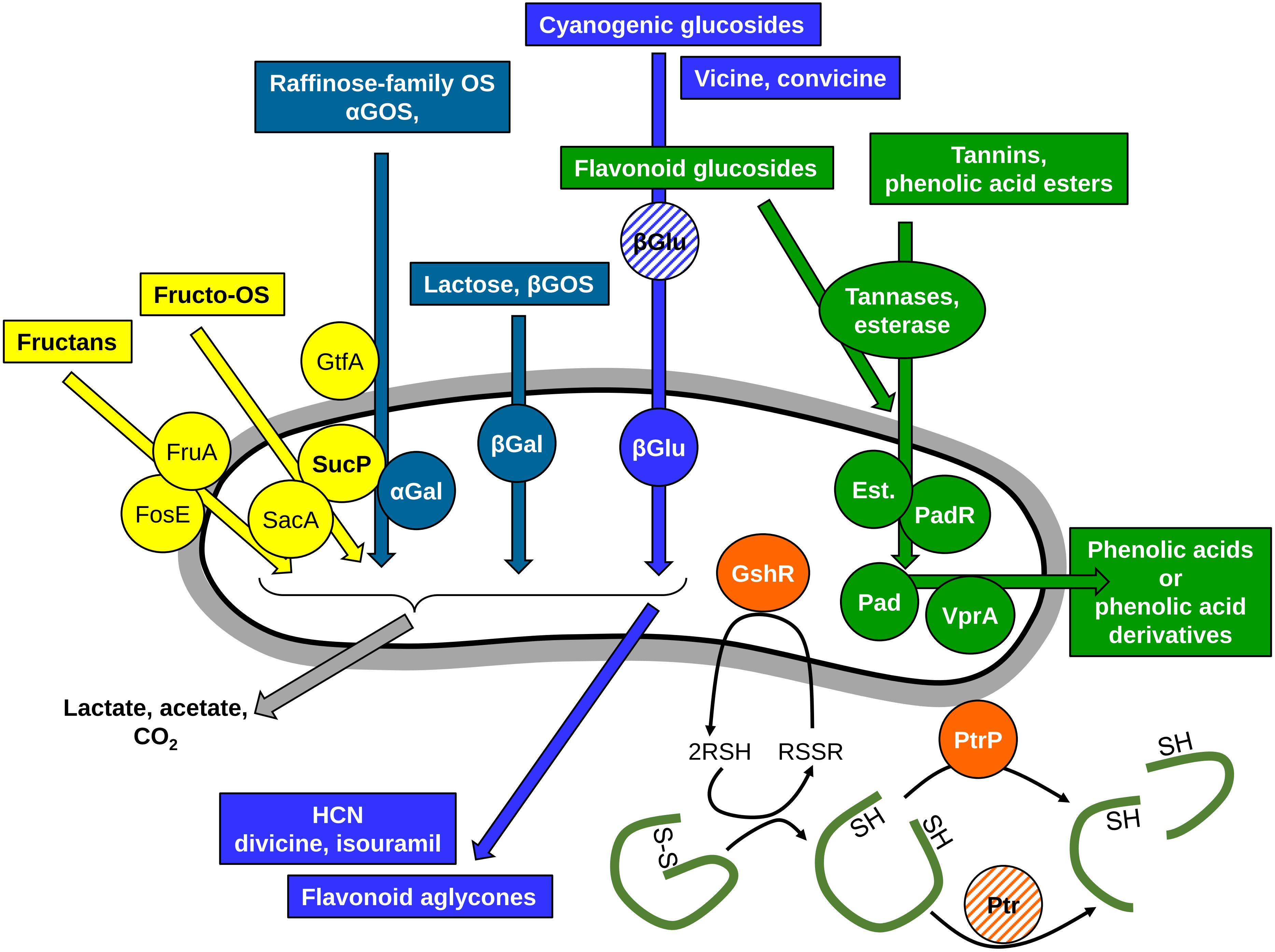
Figure legends

Figure 1. Overview on metabolic activities of lactic acid bacteria that contribute to the conversion of anti-nutritive, noxious or toxic compounds in food fermentation. **Fructans.** High DP inulin- and levan type fructans are degraded by the cell-wall bound enzymes FosE (*Liquorilactobacillus* spp. and few strains of *L. casei* and *L. paracasei*) and FruA (oral streptococci and swine-associated *Lactobacillus* spp.). Fructo-oligosaccharides with a DP of 2 – 4 are metabolized by the intracellular fructanases SacA and sucrose phosphorylase SucP. **Raffinose-family oligosaccharides and galacto-oligosaccharides.** Raffinose-family oligosaccharides are converted to αGOS by extracellular levansucrases (*Limosilactobacillus* spp., *Liquorilactobacillus* spp. and few *Lactobacillus* spp.) or metabolized by intracellular α-galactosidase and sucrose-phosphorylase. αGOS and βGOS including lactose with DP 2 – 4 are metabolised by intracellular α-galactosidase and β-galactosidase, respectively. **Cyanogenic glycosides, vicine and convicine** are converted by substrate-derived or intracellular microbial β-glucosidases; the resulting aglycones are rapidly detoxified or volatile. **Phenolic compounds.** Flavonoid glucosides are converted by substrate-derived or intracellular microbial β-glucosidases glycosyl hydrolases. Tannins and esters of phenolic acids are hydrolysed by extracellular tannases and extracellular or intracellular phenolic acid esterases; phenolic acids are converted by phenolic acid reductases (HcrB, HcrF or PadR1), decarboxylases (Pad) and vinyl reductases (VprA). **Protein modification and hydrolysis.** Glutathione reductase activity of *F. sanfranciscensis* or other thiol-accumulating enzymes in *Limosilactobacillus* spp. reduce intra- and intermolecular disulfide bonds, increasing the susceptibility of proteins including ovotransferrin and gluten to hydrolysis. Cell-wall bound proteases of lactic acid bacteria – mainly found in *Lactobacillus* spp. and lactococci, or substrate-

derived and fungal proteases hydrolyse proteins to peptides and amino acids. Drawn with information from [27, 32, 33, 44, 45, 48, 49, 53, 82, 83, 88].

Table 1. Toxic, conditionally toxic, antinutritive or noxious compounds in plant foods and milk.

Adverse Compound	Food involved	Adverse effects
Cyanogenic glycosides	Cassava, flaxseed, Lima beans, others	Release of cyanide after ingestion; chronic disease (konzo) leading to motor neuron damage or acute intoxication [15]
Vicine, convicine	Faba beans	Favism (hemolytic anaemia) in susceptible individuals with glucose-6-phosphate dehydrogenase deficiency [16]
Phytate	Cereals, legumes	Reduced mineral absorption [13]
Tannins	Sorghum, legumes	Bitter taste, inhibition of digestive enzymes [23]
Amylase trypsin inhibitors, lectins	Wheat, rye, legumes	Inhibition of digestive enzymes, inflammatory effects; potential contribution to non-celiac wheat sensitivity and irritable bowel syndrome [25]
Allergens	Wheat, legumes, eggs, milk	Allergic reactions, potential anaphylactic shock [76]
Raffinose-family oligosaccharides; Fructans	Legumes; wheat, rye	Flatulence, bloating, osmotic diarrhea; contribution to non-celiac wheat sensitivity and irritable bowel syndrome [28, 66]
Lactose	Milk	Lactose intolerance (Flatulence, bloating, osmotic diarrhea) [29]


Cyanogenic glucosides
Vicine, convicine
Raffinose-family OS αGOS,
Flavonoid glucosides
Tannins, phenolic acid esters
βGlu
Tannases, esterase
Lactose, βGOS
Fructo-OS
Fructans
GtfA
FruA
SucP
FosE
SacA
αGal
βGal
βGlu
Est.
PadR
GshR
Pad
VprA
Phenolic acids or phenolic acid derivatives
Lactate, acetate, CO_2
2RSH
RSSR
PtrP
SH
SH
SH
SH
S-S
Ptr
HCN divicine, isouramil
Flavonoid aglycones