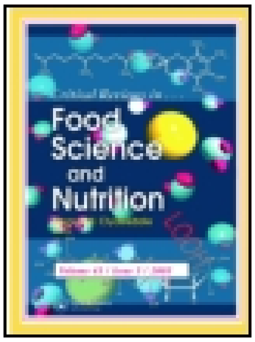

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REVIEW

The sourdough fermentation is the powerful process to exploit the potential of legumes, pseudo-cereals and milling by-products in baking industry

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ABSTRACT
In the era of fighting wastes and paying close attention to sustainability and new protein sources, legumes, pseudo-cereals and milling by-products deserve all the efforts for increasing their consumption. Even with obvious peculiarities, a common trait characterizes these heterogeneous matrixes: unquestionable nutritional and functional value combined with some technological, sensory and/or anti-nutritional weaknesses, which unfortunately limit the exploitation and consumption. With the perspective of their use to fortify staple baked goods, we reviewed the main technological, nutritional and functional features of various legumes and pseudo-cereals, and milling by-products. Notwithstanding the potential of other technological solutions, we reported numerous evidences that qualified the sourdough fermentation as the most sustainable and powerful process to exploit the technological, nutritional and functional features of these matrixes and to limit or eliminate weak attributes. Sourdough fermentations tailored for specific matrixes allowed the fortification of staple baked goods with abundant levels of legumes, pseudo-cereals or milling by-products while keeping high consumer acceptance.

KEYWORDS
Legumes; pseudo-cereals; bran; germ; sourdough; baked goods

Introduction

Current buzzwords in food processing are healthy diet to prevent diseases, food security and environmental sustainability. As the perception of the healthy diet has become more evident, industries and consumers have intensified the search and expectation for functional foods, which combine fewer calories, low glycaemic index and fat content, more fiber, plant proteins, low salt and fewer additives (Román et al. 2017). Inevitably, food security has pushed to search for new protein sources with focus on insects, protein-rich plants and agriculture by-products. Under the bio-economy umbrella and with a sustainable perspective, less conventional dietary ingredients (legumes, pseudo-cereals, and cereal bran and germ) have received an undoubtable interest because of the combination of nutritional and functional attributes. Baked goods have been, and still are, central components of the diet of most populations, and their fortification with nutritious, functional and protective compounds is an effective strategy to combine healthy foods and food security (Bartkiene et al. 2016). The preliminary selection of less conventional plants and by-products is crucial. Nevertheless, most of these matrices contain beneficial compounds under a non-bioavailable form and anti-nutritional factors that may decrease digestibility, and, inherently, have not completely satisfactory technological, rheology and sensory attributes (Bartkiene et al. 2016; Mironeasa et al. 2012). The full exploitation of such less conventional ingredients goes through the most suitable processing for making baked goods.

In particular, the use of legumes and pseudo-cereals is restricted due to poor rheological properties of their flours because of the gluten absence (Scarnato et al. 2016). Additives (e.g. modified starches, hydrocolloids, protein isolates), technological processes (e.g. hydrothermal and high-pressure treatments, extrusion cooking), and enzymes are the proposals to overcome this limit (Naqash et al. 2017). Microbial transgutaminase (mTG), which affects the dough microstructure by catalyzing protein cross-links, is effective also to enhance textural and sensory features of gluten-free matrices, in association with sourdough fermentation (Scarnato et al. 2016).

Lactic acid bacteria are the group of microorganisms most largely used at the industrial level, having the status of Generally Recognized as Safe (GRAS). Almost 25% of the European diet and 60% of that in third countries consist of fermented foods, prevalently based on lactic acid fermentation (Steinkraus 1983; Stiles 1996). Used as natural (e.g. sourdough) or selected starters, lactic acid bacteria have the capability to conjugate desired functional activities, sensory properties and microbiological safety (Juodeikiene et al. 2012). The demonstration of how the sourdough fermentation improves the conventional processing of several cereals has become incontrovertible during the last decade (Gobbetti et al. 2018).

Here, we reviewed how the sourdough fermentation and/or the use of selected lactic acid bacteria are the most

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suitable and sustainable processes to exploit the potential of legumes, pseudo-cereals and cereal by-products to fortify baked goods. Evidences focused on nutritional, functional, rheology, sensory and shelf life attributes.

Legumes

Leguminosae are the most important group of the *Dicotyledonae*, being one of the largest families of flowering plants with 18,000 species. After cereals, legumes are the second worldwide food crop. Legumes belong to the secular dietary habit of all countries, but their frequent consumption because of the nutritional and functional values has attracted only recently. Legumes are excellent sources of proteins with high biological value, providing many essential amino acids, contain carbohydrates and dietary fibers, and supply relevant levels of vitamins, minerals, oligosaccharides and phenolic compounds (Roy et al. 2010). The frequent consumption of legumes is effective to prevent or decrease risks of cardiovascular disease (CVD) (Widmer et al. 2015), type 2 diabetes (Jenkins et al. 2012), some types of cancer (Feregrino-Perez et al. 2008), and overweight and obesity (Mollard et al. 2012).

Baked good fortification

Apart from the direct consumption as dishes, legumes have the potential to become irreplaceable ingredients for making various baked goods and pasta. The use as baked good fortifiers should increase their consumption as strongly recommended in many dietary guidelines. In most of the cases, this fortification occurs as flour (also sprouted), which undergoes or not to a process of lactic acid fermentation. The use of lactic acid bacteria to ferment legumes is a traditional process because these bacteria are autochthonous in legume grains. Fermentation of legumes brings several advantages, which mainly concern the improvement of the protein digestibility and related nutritional values, and the biological availability of fibers and total phenols (Table 1). Selected strains of *Lactobacillus plantarum* and *Lactobacillus brevis* were the traditional sourdough starters to exploit the nutritional and functional potential of nineteen Italian legume flours. Compared to control doughs (without bacterial inoculum), the concentrations of free amino acids, soluble fibers, and total phenols increased. During sourdough fermentation, the level of γ-amino butyric acid (GABA) markedly increased and reached values up to 624 mg/kg. At the same time, almost all legume sourdoughs showed an increased antioxidant activity (Curiel et al. 2015). Lactic acid fermentation of cowpea (*Vigna unguiculata*), mottled cowpea, speckled kidney bean and small rice bean flours led to an increased antioxidant activity, which might had been associated with the biotransformation between soluble phenols and the release of bound phenols (Dueñas et al. 2005; Gan et al. 2016). Unlike most fruits and vegetables, legumes generally have a much higher percentage of bound phenols. Acidification and microbial enzymes bring about the bioconversion of polyphenols into more available and biologically active compounds (Lee et al. 2013). Recently, fundamental insights explained the metabolism of phenolic compounds by lactic acid bacteria (Figure 1). The bacterial capability to follow the metabolic paths is species-specific or strains-specific and depends on their portfolio of enzymes (Filannino et al. 2018). The physiological significance of the metabolism of phenolic compounds in lactic acid bacteria has been mainly explained as an efficient mechanism to detoxify such compounds (Sanchez-Maldonado et al. 2011; Reveron et al. 2012). However, the hypothesis that the metabolism of phenolic compounds by lactic acid bacteria might also have a role to preserve the cellular energy balance is gaining acceptance. Indeed, lactic acid bacteria use hydroxycinnamic acids as external acceptors of electrons, allowing the cofactor recycling, and gaining additional metabolic energy. Glycosyl hydrolase, phenolic acid decarboxylase and reductase, and esterase activities represent the main enzymes involved in the phenolic compounds conversion into biologically active metabolites (Filannino et al. 2018). Legume flours subjected to sourdough fermentation were suitable for using alone or better in mixture with cereals to fortify traditional baked goods, and as gluten-free ingredients for making novel and healthier products (Curiel et al. 2015). Type I sourdough, containing legumes or wheat-legume flour mixtures, were prepared according to traditional protocols. The lactic microbiota of wheat-legume sourdough comprised a very large diversity of *Lactobacillus* species. A mixture of legume flours, consisting of chickpea (*Cicer arietinum*), lentil (*Lens culinaris*) and bean (*Phaseolus vulgaris*), was used (15%, w/w) for making breads using selected wheat-legume sourdoughs. The fortification with fermented legumes increased the antioxidant activity and the *in vitro* protein digestibility (IVPD). According to the levels of carbohydrates, dietary fibers and resistant starch, the bread fortified with wheat-legume sourdough had a decreased value of starch hydrolysis index (Rizzello et al. 2014). GABA-producing strains such as *Lb. plantarum* and *Lactococcus lactis* subsp. *lactis* were other starters for sourdough fermentation of legume flours. Chickpea flour was the most suitable substrate for GABA enrichment. The sourdough fermentation of a blend of chickpea and pseudo-cereals allowed the synthesis of very high levels of free amino acids and GABA (ca. 4467 and 504 mg/kg, respectively) and the liberation of phenolic compounds, which increased antioxidant activity. Sensory analysis showed how the sourdough fermentation improved the palatability and the overall taste appreciation (Coda et al. 2010). The pairing between sourdough fermentation and legumes to accumulate GABA well performed also using adzuki bean flour (Liao et al. 2013) and extracts from kidney beans subjected to liquid state fermentation (Limón et al. 2015). Lupin (*Lupinus albus* L.) deserves an interest to replace genetically modified soya products and, more in general, as an ingredient to increase the protein content of baked goods. Compared to non-fermented flours, the protein digestibility of sourdough-fermented lupin markedly increased (Bartkiene et al. 2015). The incorporation of fava bean (*Vicia faba* L) flour into wheat-based products is a sustainable way to get protein-enriched baked goods. The

Table 1. Main advantages with the application of sourdough biotechnology to legume flours and legume-fortified wheat bread.

Legume	Fermentation type	Effects	References
Bean, chickpea, grass pea, lentil, pea (local cultivars)	*Lactobacillus plantarum* C48 and *Lactobacillus brevis* AM7	Increase of phytase and antioxidant activity; increase of free amino acids, GABA, soluble fibers, and total phenols concentrations. Decrease of raffinose and condensed tannins concentrations.	Curiel et al. (2015)
Grass pea	*Lactobacillus plantarum*	Decrease of phytic acid concentration and trypsin inhibitory activity.	Starzyńska-Janiszewska and Stodolak (2011)
Faba bean	*Lactobacillus plantarum* DPPMAB24W	Decrease of vicine and convicine concentration, trypsin inhibitor activity, starch hydrolysis index. Increase of protein digestibility, and free amino acids and GABA concentrations.	Rizzello et al. (2016a)
Chickpea; mixture of chickpea and pseudocereals	*Lactobacillus plantarum* C48 and *Lactococcus lactis* subsp. *lactis* PU1	Increase of free amino acid and GABA concentrations; decrease of the starch hydrolysis index (HI); increase of antioxidant activity; increased palatability and overall acceptability of bread	Coda et al. (2010)
Soybean	Spontaneous fermentation	Improved nutritional quality, as estimated by Protein Efficiency Ratio (PER); Net Protein Ratio (NPR), Apparent Digestibility (AD), true digestibility	Peñaloza-Espinosa et al. (2011)
Lupine	*P. acidilactici* M1807	Decrease of the trypsin inhibitor activity and hydrolysis index; increase of protein digestibility and antioxidant activity.	Bartkiene et al. (2011)
Chickpea/lentil/bean (mixture)	Type I sourdough	Increase of free amino acid concentration; increase of antioxidant and phytase activities.	Rizzello et al. (2014)
Cowpea, mottled cowpea, speckled kidney bean, small rice bean	Spontaneous fermentation; inoculum with *Lactobacillus plantarum* (WCSF1 and ATCC 149170) and *Lactobacillus paracasei* ASCC 279	Increase of antioxidant activity	Dueñas et al. (2005); Gan et al. (2016)
Soybean	*Lactobacillus plantarum* NTU 102 and *Lactobacillus paracasei* NTU 101	Conversion of isoflavones into daidzein and genistein; Attenuation of the obesity in rats fed under high-fat diet	Lee et al. (2013)
Kidney beans	Spontaneous fermentation; inoculum with *L. plantarum* ATCC 14917	Increase of GABA concentration	Limón et al. (2015)
Adzuki bean	*Lactococcus lactis* subsp. *lactis* and *L. rhamnosus* GG	Increase of GABA concentration	Liao et al. (2013)
Lupin	*Lactobacillus sakei* KTU05-6, *Pediococcus acidilactici* KTU05-7 and *Pediococcus pentosaceus* KTU05-8	Increase of protein bioavailability and digestibility	Bartkiene et al. (2014)
Faba bean	*Weissella confusa* VTT E – 143403 (E3403) and *Leuconostoc pseudomesenteroides* DSM 20193	Increase of protein concentration. Increase of viscoelastic behavior, specific volume of bread. Decrease of crumb hardness of bread	Wang et al. (2018)
Faba bean	*Pediococcus pentosaceus* I02	Increase of protein digestibility and protein biological indexes. Increase of volume and hardness of bread. Decrease of glycemic index.	Coda et al. (2017)
Faba bean	*Lactobacillus plantarum* DPPMAB24W	Increase of protein digestibility, nutritional indexes and resistant starch. No detrimental effect on pasta texture and cooking loss.	Rizzello et al. (2017a)
Lentil	*Lactobacillus plantarum*	Release of bioactive peptides.	Torino et al. (2013)
Bean, chickpea, grass pea, lentil, pea (local cultivars)	*Lactobacillus plantarum* C48 and *Lactobacillus brevis* AM7	Release of lunasin-like polypeptides; inhibition of the proliferation of human adenocarcinoma Caco2 cells.	Rizzello et al. (2015a)

(*continued*)

Table 1. Continued.

Legume	Fermentation type	Effects	References
Chickpea, lentil	*Lactobacillus rossiae* LB5, *Lactobacillus plantarum* 1A7 and *Lactobacillus sanfranciscensis* DE9	Increase in the concentrations of peptides, free amino acids and GABA, increase of protein digestibility and decrease of starch availability. Decrease of phytic acid, condensed tannins, raffinose concentrations and trypsin inhibitory activity.	Montemurro et al. (2018)
Soybean and African breadfruit	Spontaneous fermentation	Increase of protein digestibility and improvement of the sensory properties	Ariahu et al. (1999)
Cowpea	Spontaneous fermentation	Increase of lysine concentration and essential amino acids concentration.	Hallén et al. (2004)
Bean	Spontaneous fermentation	Decrease of α-galactosides, phytic acid, trypsin inhibitors and condensed tannins concentrations.	Granito et al. (2002)

protein content increased from 11.6 to 16.5% of dry matter as well as the protein digestibility and related biological indexes, while the predicted glycaemic index decreased (Coda et al. 2017). Fava bean fortified semolina pasta also. The preliminary sourdough fermentation by *Lb. plantarum* led to a relevant level of substitution of semolina flour (30%, w/w), which did not negatively interfere with the pasta texture and cooking loss. Compared to traditional semolina pasta and pasta containing non-fermented fava bean flour, the protein nutritional profile (IVPD and other related indexes) and resistant starch improved (Rizzello et al. 2017a).

Lactic acid fermentation and microbial enzymes bring about the release of bioactive peptides from legume proteins (Torino et al. 2013). Legume hydrolysates had *in vitro* activities towards cancer and CDV or their physiological manifestations like oxidative damage, inflammation, hypertension and high cholesterol (López-Barrios et al. 2014). Lunasin is a 43-amino acid peptide with anticancer, anti-inflammatory, antioxidant and cholesterol lowering activities. Its main source is soybean and dietary supplements are already present into the market. Sourdough fermentation with *Lb. plantarum* and *Lb. brevis* liberated lunasin-like polypeptides in nineteen traditional Italian legumes. As shown by western blot analysis, the number and the intensity of lunasin-like polypeptides increased during sourdough fermentation because of the microbial proteolysis of native proteins. Extracts from these legume sourdoughs showed marked inhibition on the proliferation of human adenocarcinoma Caco-2 cells (Rizzello et al. 2015a).

Beside the use after milling, flours from germinated legumes may contain increased amount or availability of nutrients. As an ancient practice, sprouting deserves an emerging interest because of the significant enhancement in bioactive components (e.g. vitamins, dietary fibers, peptides and amino acids, and phenols) and palatability. During germination, reserves within the storage tissues of the seed undergo hydrolysis in low molecular weight compounds and mobilize to support seedling growth (Bewley 2001). The degree of these changes depends on temperature, humidity, steeping (soaking) and length of germination (Koehler et al. 2007). The fortification of baked goods with flours from germinated legumes has been proposed recently (Mäkinen and Arendt 2015). Nevertheless, the integrate biotechnological approach that combines germination and sourdough fermentation seems to better exploit the nutritional modification of grains in terms of protein and starch hydrolysis, and mineral solubility (Katina et al. 2007). Sprouting and sourdough fermentation with *Lactobacillus rossiae*, *Lb. plantarum* and *Lactobacillus sanfranciscensis* enhanced the nutritional and functional features of chickpea and lentil by increasing the concentrations of peptides, free amino acids and GABA. Breads made with fermented sprouted flours had high protein digestibility and low starch availability, and appreciable sensory attributes (Montemurro et al. 2018). Germination followed by sourdough fermentation improved the IVPD and enhanced the sensory properties of soybean and African breadfruit seeds (Ariahu, Ukpabi, and Mbajunwa 1999). The

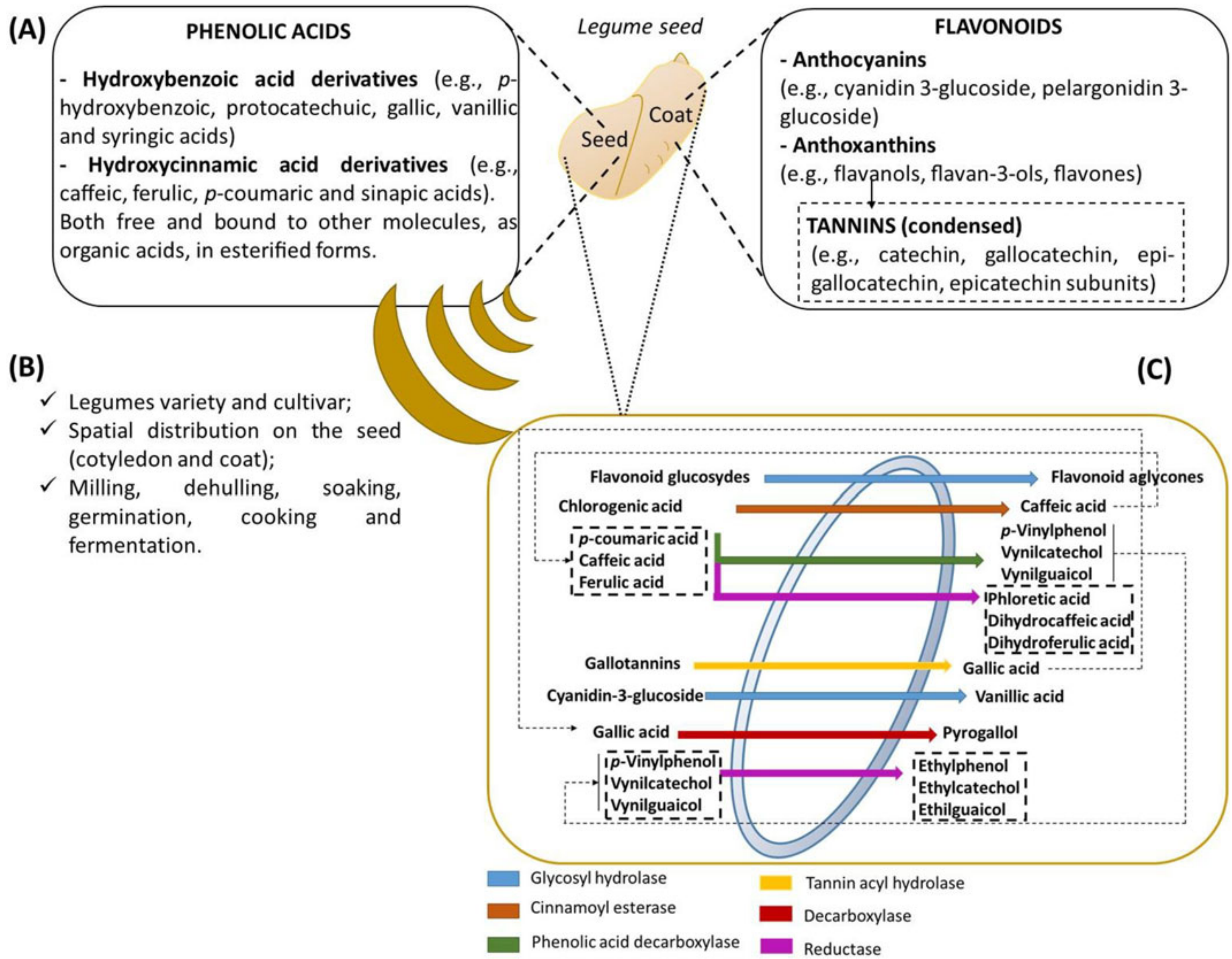

Figure 1. Schematic representation of the main classes of phenolic compounds found in legume seeds (A), potential factors affecting the inherent phenolic compounds level (B), and metabolic paths followed by lactic acid bacteria during legumes fermentation (C). Dotted boxes represent pathways where phenolic compounds are used as external acceptors of electrons allowing the cofactor recycling.

same occurred for the germinated and fermented cowpea flour, which fortified the bread formula with high lysine content and optimal essential amino acid balance (Hallén et al. 2004).

Removal of anti-nutritional factors

Legumes contain heterogeneous and species-dependent anti-nutritional factors (ANF), namely raffinose, phytic acid, condensed tannins, alkaloids, lectins, pyrimidine glycosides (e.g. vicine and convicine) and protease inhibitors (Coda et al. 2015a). ANF decrease the digestibility and reduce the bioavailability of other nutrients, and, exceptionally, are responsible for diseases. Some ANF are heat-labile (e.g. protease inhibitors and lectins) and easily removed by thermal treatments. Others (e.g. phytic acid, raffinose, tannins and saponins) withstand heating. Dehulling, soaking, air classification, extrusion or cooking are the main technological options for decreasing the negative impact of ANF on legume consumption (Coda et al. 2015a; Rizzello et al. 2014). Nevertheless, biological methods such as germination, enzyme treatments and, especially, fermentation seem to be more efficient (Coda et al. 2015a; Luo et al. 2009). The content of α-galactosides, phytic acid and tannins, and the trypsin inhibitory activity markedly decreased to the point of elimination during sourdough fermentation of several legume species (Granito et al. 2002; Curiel et al. 2015). The lactic fermentation of grass pea (*Lathyrus sativus*) with *Lb. plantarum* lowered the levels of phytic acid and trypsin inhibitory activity (Starzyńska-Janiszewska and Stodolak 2011). Selected strains of *Lb. plantarum* and *Lb. brevis* decreased the content of raffinose up to ca. 64% during sourdough fermentation of Italian legume flours. At the same time, almost all legume sourdoughs contained an increased phytase activity (Curiel et al. 2015). The combination of legume sprouting and sourdough fermentation decreased the content of phytic acid, condensed tannins and raffinose, and trypsin inhibitory activity (Hallén et al. 2004; Montemurro et al. 2018). Proteolysis, enzyme inhibition due to acidification, acid activation of flour endogenous enzymes (e.g. phytases) and/or microbial enzyme activities (e.g. α-galactosidase, β-glucosidase, phytases, tannases) were

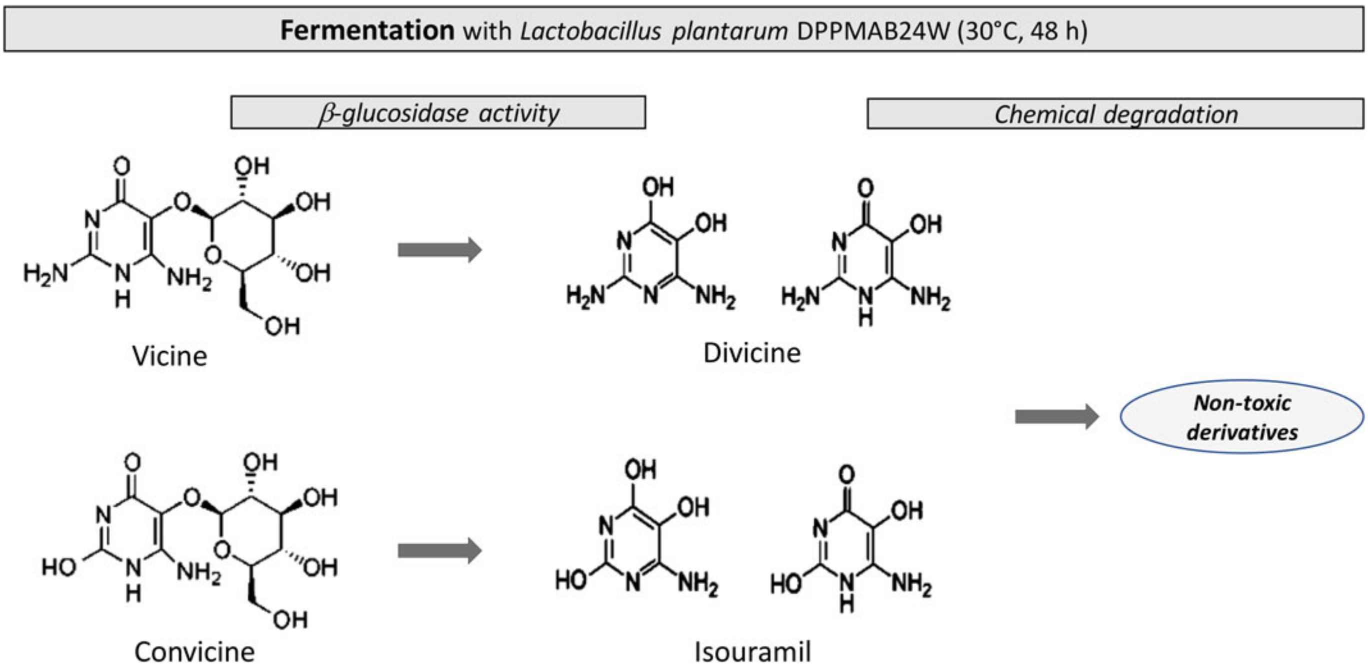

Figure 2. Degradative pathway of the glucosidic aminopyrimidine derivatives vicine and convicine during 48 h fermentation of fava bean flour by *Lactobacillus plantarum* DPPMAB24W, previously selected for the high β-glucosidase activity. The hydrolysis of the β-glucosidic bond led to the release of their respective aglycones, divicine and isouramil, that were not detectable in the fermented faba bean flour since subjected to a spontaneous chemical degradation in acidic conditions. Modified from Rizzello et al. (2016a).

responsible for the inactivation of most ANF. Fava bean is rich in two glucosidic aminopyrimidine derivatives, vicine and convicine, which, upon hydrolysis of the β-glucosidic bond generate the aglycones divicine (2,6-diamino-4,5-dihydroxypyrimidine) and isouramil (6-amino-2,4,5-trihydroxypyrimidine), respectively (Crépon et al. 2010) (Figure 2). Divicine and isouramil are the main drivers for favism disease. Technological interventions (air classification, roasting, harsher and boiling) and selection of cultivars with low content of such compounds seemed to be only in part effective (Crépon et al. 2010; Pulkkinen et al. 2015). On the contrary, β-glucosidase from lactic acid bacteria effectively degraded the pyrimidine glycosides from fava bean suspension and flour (Coda et al. 2015b). When used as starter to ferment fava bean flour, *Lb. plantarum* expressed β-glucosidase activity and decreased the content of vicine and convicine by more than 90%. The degradation was complete after 48 h of fermentation and aglycone derivatives were not detectable. *Ex vivo* assays on human blood confirmed the lack of toxicity of the fermented fava bean (Rizzello et al. 2016a).

Bio-preservation

Contamination by fungi is the most common source of microbial spoilage and is a costly problem in bakery processes. For several baked goods, fungal contamination is the major factor governing shelf life. Together with hygiene during processing and packaging techniques, chemical preservatives are currently the main antagonists to prevent the growth of fungi in baked goods (Dal Bello et al. 2007; Ponte and Tsen 1987). Nevertheless, the European directive on preservatives has decreased the allowed concentrations of organic acids and consumers expect preservative-free, label clean and bioprocessed baked goods. These concomitant circumstances have oriented the search for new preservatives, especially those derived from natural sources. In general, plants contain several proteins and peptides that play a pivotal role for *in situ* fungal resistance (Xia and Ng 2005). Seeds of many different species of leguminous plants are classical examples (Ng 2004; Van Etten 1973). At low minimal inhibitory concentration, the water-soluble extract of *Phaseolus vulgaris* cv. Pinto was inhibitory toward a large spectrum of fungal species isolated from bakeries. The water-soluble extract mainly contained phaseolin alpha-type precursor, phaseolin, and erythroagglutinating phytohemagglutinin precursor. Bread manufactured with the addition of this water-soluble extract (27%, v/w) did not show fungal contamination until at least 21 days of storage at room temperature, ensuring a level of protection comparable to that afforded by calcium propionate (0.3%, w/w) (Coda et al. 2008). The water/salt-soluble extract of a pea (*Pisum sativum*) hydrolysate, obtained by fungal protease, had high inhibitory activity towards *Penicillium roqueforti* and several fungi isolated from bakeries. The antifungal activity depended on pea defensins 1 and 2, nonspecific lipid transfer protein (nsLTP) and a mixture of peptides, identified as sequences encrypted in leginsulin A, vicilin, provicilin and nsLTP. Artificially inoculated slices of a bread containing this water/salt-soluble extract did not show contamination by fungi until at least 21 days of storage (Rizzello et al. 2015b). A water/salt-soluble extract from a mixture of legume hydrolysates, consisting of pea, lentil, and fava bean flours, inhibited *Aspergillus parasiticus*, *Penicillium carneum*, *Penicillium paneum* and *Penicillium polonicum*. Some native proteins and a mixture of peptides, identified as sequences encrypted in legume vicilins, lectins and chitinases, were responsible for the antifungal activity (Rizzello et al. 2017b).

Textural properties

The improvement of the technological properties of legume flours lacking of gluten proteins is one of the challenge in food research. Structuring agents (e.g. hydrocolloids) for manufacturing gluten-free products and wheat-based baked goods, due to gluten network weakening since wheat flour replacement, are under investigation.

The addition of legume flours to wheat flour worsens the dough properties proportionally to the amount added. Increased water absorption of wheat–legume composite flours may provide more water for dough starch gelatinization during baking and prevent stretching and tearing of gluten strands (Hallén et al. 2004). Overall, a number of reports demonstrated that substitution of wheat flour with legumes at levels higher than 20–30% caused detrimental effects on dough and bread properties, which resulted sticky and excessively compact (Hallén et al. 2004; Coda et al. 2017). Sourdough fermentation of legume flours, mainly interfering with starch gelatinization, and fibers hydration lead to the improvement of the structural characteristics of the fortified bread (Coda et al. 2010; Rizzello et al. 2014; Naqash et al. 2017).

The use of a legume sourdough including chickpea, lentil and bean flours (15% wheat replacement) allowed the manufacture of a bread with a significantly higher specific volume than the control bread made with the same percentage of unfermented legume flours (Rizzello et al. 2014). Compared to native legume flours, texture instrumental analysis demonstrated that lactic acid fermentation improved the bread softness (hardness decreased of ca. 30%), and crumb elasticity (significant increase of fracturability). Resilience, springiness, cohesiveness of breads fortified with fermented legume flours were comparable to those of conventional wheat flour bread (Coda et al. 2010; Rizzello et al. 2014). Exopolysaccharides (EPS)-producing lactic acid bacteria further contributed to improve the structural properties of fortified baked goods. Indeed, the replacement of wheat flour with the 30% of a sourdough fermented with *Weissella confusa*-synthesizing dextran (Wang et al. 2018) compensated the gluten dilution, and improved bread volume and crumb softness. Most likely, the gluten-dextran interactions strengthened gas cells and, hence, prevented their collapse during proofing and baking (Bárcenas and Rosell 2005). This, combined with water binding capacity, led to higher loaf volume and softer crumb. Hydrocolloids have a weakening effect on starch structure because the inhibition of amylose leaching and crystallization, and amylopectin retrogradation. This modifies the water distribution and moisture retention in the bread crumb (Biliaderis et al. 1997). Accordingly, dextran-enriched faba bean sourdough could be incorporated at high level (up to 43% of the dough weight) in the formula, which allowed an increased protein content (Wang et al. 2018).

Pseudo-cereals

The last decade pseudo-cereals gained popularity among industries and consumers because of the combination of various attributes. Most pseudo-cereals adapt to variable agro-ecological conditions, supply relevant crop yields, and have important nutritional and functional potential, and the gluten-free status. Based on these attributes, quinoa (*Chenopodium quinoa* Willd.), amaranth (*Amaranthus hypochondriacus*) and buckwheat (*Fagopyrum esculentum*) are those species, which have found the largest application in baked good formulas. Although not routinely classified within this group, hemp (*Cannabis sativa*), whose seeds are milled into flour and used for human consumption, also gained the status of an emerging pseudo-cereal. Because of the absence of gluten, and the need to exploit the peculiar sensory attributes and nutritional potential, the choice of the technique for processing pseudo-cereals becomes crucial (Figure 3).

Quinoa

Native of South America, the cultivation of quinoa currently occurs under a wide range of environmental conditions. The estimation is that that quinoa will contribute to food security in the 21st century (Jacobsen et al. 2003). Compared to common cereals, quinoa has a better-balanced nutritional profile. Non-gluten proteins (up to 23% of dry matter), encompassing essential amino acids, lipids (mainly unsaturated), vitamins (e.g. folate and tocopherols), minerals and other phytochemicals (e.g. steroids, phenols and flavonoids) are the distinguishing nutritional traits (Kuljanabhagavad et al. 2008). Quinoa also deserves a technological interest because it forms emulsions, has high water holding capacity and high solubility, and reaches gelatinization temperatures in short time (Bolívar-Monsalve et al. 2018). Nevertheless, its use as raw material has some disadvantages because of the rheology attributes and the presence of triterpenic saponins, which generate bitter taste and prevent the intestinal absorption of some nutrients (Gómez-Caravaca et al. 2014). The main use of quinoa flour (or seeds in some cases) as an ingredient (6 to 50%, w/w) concerned blends of flours for making bread or other baked goods. Fortification with quinoa flour per se enhances the protein quality and content, increases the total phenolic and flavonoid levels and antioxidant activity, but, at the same time, worsens the rheology (decreased specific volume and increased hardness) and sensory characteristics of the dough and bread (Stikic et al. 2012; Wolter et al. 2014). An abundant literature converged on the sourdough fermentation of quinoa flour or blend of flours as the most suitable option to promote technological and nutritional advantages (Dallagnol et al. 2013). The sourdough fermentation was suitable to decrease the level of saponins, thus improving rheology and sensory attributes (Bolívar-Monsalve et al. 2018). *Lb. plantarum* and *Lb. rossiae* were isolated and identified from quinoa flour, and selected for preparing sourdough. Compared to non-fermented flour, free amino acids, soluble fibers, total phenols, phytase and antioxidant activities, and IVPD markedly increased during fermentation. The bread made using 20% (w/w) of quinoa

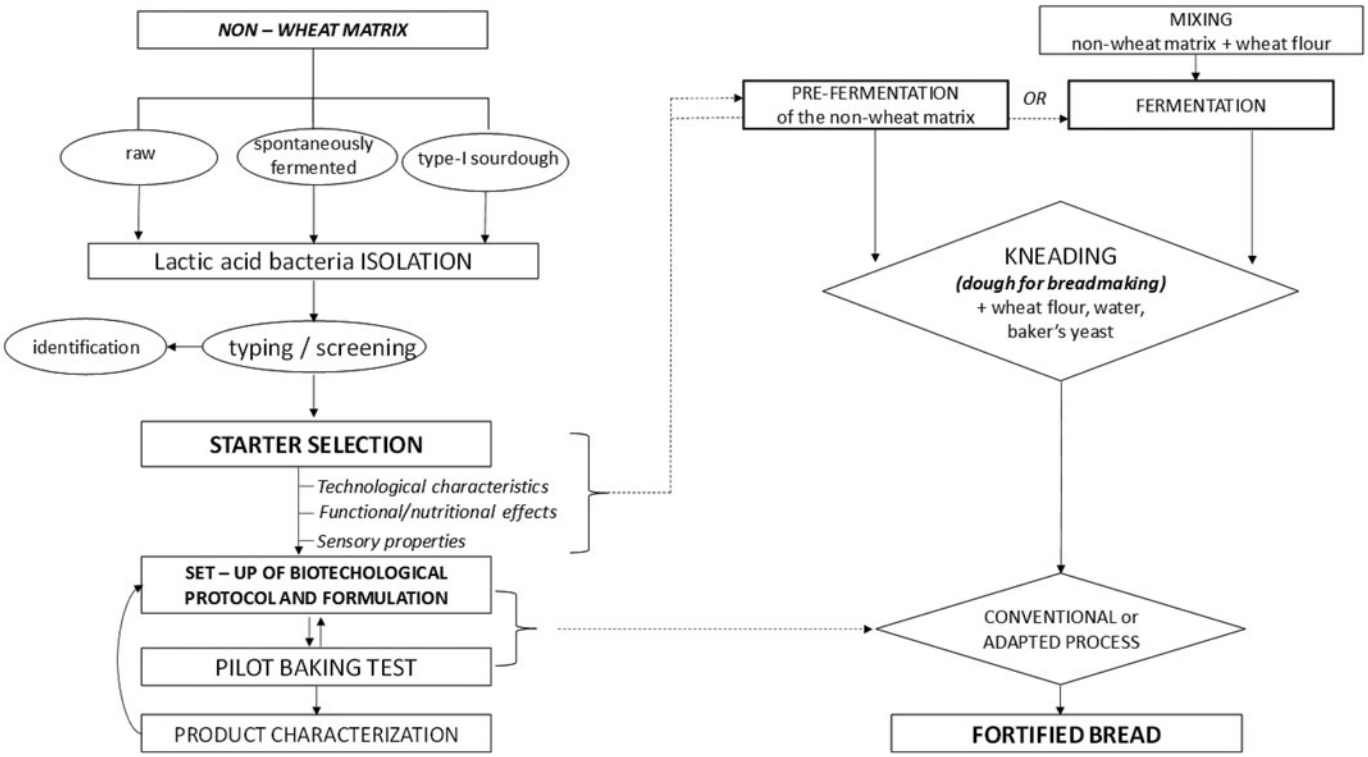

Figure 3. Use of fermented pseudo-cereals for bread fortification. On the left: starter selection, setup of biotechnological protocols and processing scale up. On the right, the industrial bread making. Starter selection is based on technological characteristics (growth, acidification, proteolytic activity) and on the capability of the lactic acid bacteria strains to affect the nutritional/functional profile of the fermented matrix (e.g. decrease of the glycemic index, increase of protein digestibility, degradation of antinutritional factors); selection is carried out on isolates deriving from raw or spontaneously fermented non-wheat flours to be used as pre-fermented ingredient. The biotechnological protocol including the fermentation conditions is set-up at laboratory level and tested at pilot plant scale, performing the characterization of the experimental products. Then, the protocol is transferred at industrial scale, in which fermentation conditions and the bread recipe are defined. The optimization of the production process is carried out based on the achievement of the desired product characteristics

sourdough maintained superior nutritional features, which combined with agreeable rheology and sensory attributes (Rizzello et al. 2016b). The use of quinoa in mixture with other non-conventional flours (amaranth, chickpea and buckwheat) and the fermentation with *Lb. plantarum* led to relevant contents of free amino acids, GABA and phenolic compounds, high antioxidant activity and decreased the rate of *in vitro* starch hydrolysis (Coda et al. 2010). Short chain peptides with typical antioxidant compositional features liberated during quinoa sourdough fermentation, being protective on human keratinocytes artificially subjected to oxidative stress (Rizzello et al. 2017c). Sourdough fermented quinoa flour replaced (20%, w/w) semolina flour for making pasta. Compared to semolina pasta, this addition enhanced the nutritional value with higher fiber, protein and free amino acid contents, and antioxidant activity, which emphasized through fermentation. Only the use of fermented quinoa improved the pasta tenacity and elasticity (Lorusso et al. 2017). Under a different perspective, the sourdough fermentation of quinoa flour by *Lactobacillus reuteri* and *Lb. brevis* led to the synthesis of a mixture of carboxylic acids, mainly 3-phenyllactic and 2-hydroxyisocaproic acids, with promising antifungal activity (Axel et al. 2016).

Amaranth

Originating from Mexico, amaranth has changed over time the status from niche product to popular natural resource. Outstanding nutritional characteristics, appreciated flavor impression and absence of gluten are the main attributes behind this popularity (Jekle et al. 2010). Amaranth has high protein content (up to 16.5%), with an optimal amino acid composition, and is rich in vitamins and minerals (Olusegun 1983). Several health benefits have attributed to amaranth consumption: decrease in levels of cholesterol and blood glucose, attenuation of hypertension and anemia symptoms, and stimulation of the immune system and anti-tumor activity (Caselato-Sousa and Amaya-Farfán 2012). Amaranth seeds contain biogenic compounds such protease inhibitors, antimicrobial peptides, lectins, antioxidant and anticancer compounds, and other phytonutrients, which inhibit both free radicals and oxidative chain reactions within human tissues and membranes (Nsimba et al. 2008; Silva-Sànchez et al. 2008). In spite of these features, it remains an underutilized crop. Amaranth flour may replace fully or in part wheat flour in bread, pastries, cookie, cereal flakes, tortillas and pasta (Silva-Sànchez et al. 2004). Composite recipes include amaranth flour at percentages of 10–40% either in gluten containing or in gluten-free preparations (Onyango et al. 2013; Schoenlechner et al. 2010). The best option for processing amaranth flour is lactic acidification because of the positive repercussion on technological, sensory and nutritional attributes. Autochthonous lactic acid bacteria (*Lb. plantarum*, *Lactobacillus sakei* and *Pediococcus pentosaceus*) were the starters for sourdough fermentation of amaranth flour (Sterr et al. 2009). The lactic acidification changes the rheological behavior of amaranth flour. The sourdough fermentation led to doughs with viscosity and elasticity similar to that found in pure wheat flours, also decreasing the typical elasticity of the amaranth flour (Houben et al. 2010). Gluten-free breads fortified (10%, w/w) with amaranth sourdough were the most appreciated by consumers (Różyło et al. 2015). Baked goods made with a mixture of amaranth and wheat flours were suitable for the growth of probiotic strains (e.g. *Lactobacillus rhamnosus* GG), which synthesized biogenic compounds during processing (Matejčeková et al. 2016). When added to the recipe, water-soluble extracts of amaranth seeds extended the shelf life of gluten-free and wheat flour breads. Novel antifungal peptides, encrypted in amaranth agglutinin sequences, were responsible for the prolonged storage behaving as the chemical preservatives (Rizzello et al. 2009). Overall, antifungal peptides from amaranth seeds had Defense activity towards pathogenic fungi (Lyapkova et al. 2001).

Buckwheat

Buckwheat is rich in proteins, lysine, antioxidants (e.g. rutin and quercetin), dietary fiber and micronutrients, and possesses cholesterol-lowering and prebiotic activities (Alvarez-Jubete et al. 2009; Li et al. 2010; Préstamo et al. 2003). Nonetheless, the fortification of baked goods with buckwheat is challenging because of technological issues and the presence of phytate and tannins, which decrease the digestibility of buckwheat proteins and confer bitterness (Li and Zhang 2001). One way for enhancing palatability, digestibility and baking performances of buckwheat flour relies on the manufacture of buckwheat sourdough. Amaranth flour is an important reservoir for autochthonous lactic acid bacteria and yeasts, which show diversity and various technological performances (Moroni et al. 2010). When incorporated (10–20%, w/w) into wheat dough, buckwheat sourdough strengthened the gluten network and decreased the dough elasticity. The resulting bread had high specific volume and softer crumb. The fermentation increased the level of soluble phenols, dietary fiber and magnesium, decreased the content of phytic acid and tannins, enhanced the antioxidant activity and extended the shelf life of bread (Alvarez-Jubete et al. 2010; Lin et al. 2009; Moroni et al. 2012). Innovative muffins obtained from fermented buckwheat flour showed high content of macro (potassium and magnesium) and microelements (zinc and manganese) (Ciesarová et al. 2016). Sourdough fermentation of buckwheat flour improved the protein and microelement contents of gluten-free products, also positively interfering with the overall sensory quality (Saturni et al. 2010, Hager et al. 2012; Wronkowska et al 2010, 2013). Fermentation with selected lactic acid bacteria strains increased the functional benefits of buckwheat through the release of GABA (Coda et al. 2010). The manufacture of a baked good with antihypertensive activity has combined sprouting and fermentation. This preparation decreased both systolic and diastolic blood pressure in spontaneously hypertensive rats, showing an effect comparable to that of antihypertensive drugs. Orally administered, the fermented buckwheat sprouts markedly decreased the angiotensin I-converting enzyme (ACE) activity in the lung, thoracic aorta, heart, kidney and liver of spontaneously hypertensive rats (Nakamura et al. 2013). Buckwheat rutin and related phenols (orientin and isoorientin) inherently possess vasorelaxant effects (Fu et al. 2005; Fusi et al. 2003), and the potent ACE inhibitor 200-hydroxynicotianamine is also present in buckwheat seeds and plants (Aoyagi 2006). The osbeckic acid from tartary buckwheat extract is a newly discovered non-rutin vasorelaxation factor (Matsui et al. 2010).

Hemp

Hemp seed contains 25–35% oil, 20–25% protein, 20–30% carbohydrates and 10–15% fiber, along with an array of trace minerals (Deferne and Pate 1996). The oil fraction is unique, being rich of 80% polyunsaturated fatty acids, mainly linoleic acid and α-linolenic acids at an optimal ratio (ca. 3:1) (Da Porto et al. 2015). Proteins contain high levels of sulphur-containing amino acids, arginine and glutamic acid and have consistent digestibility (House et al. 2010). The seed is not entirely free of Δ-9 tetrahydrocannabinol, but, depending on the variety, its content is less than 0.5 mg/g. In spite of the undoubtable nutritional value, hemp is still an underutilized crop. Poor knowledge about the technological and functional potential and the presence of ANF (phytic acid, condensed tannins, trypsin inhibitors, cyanogenic glycosides and saponins) are the main factors responsible for the limited use (Russo and Reggiani 2015). After a screening based on pro-technological, nutritional and functional attributes, hemp flour autochthonous lactic acid bacteria (*Lb. plantarum*, *Pediococcus acidilactici* and *Leuconostoc mesenteroides*) composed a mixed starter to prepare hemp sourdough. The fermentation led to marked decreases of the contents of phytic acid, cyanogenic glycosides, condensed tannins and total saponins. The IVPD increased up to 90%. Hemp sourdough (5–15%, w/w) fortified the recipe of wheat breads. It improved the rheology features without adversely affecting the sensory profile. Proportionally to fortification, the protein digestibility of the breads increased, and the predicted glycaemic index markedly decreased (Nionelli et al. 2018). The fermentation with *P. acidilactici* and *P. pentosaceus* of hulled and not hulled hempseed positively influenced the level and ratio among polyunsaturated fatty acids (Bartkiene et al. 2016). Lactic acid bacteria have the capability to transform the cis-9,cis-12-diene structure of C18 fatty acids into the conjugated diene structures cis-9,trans-11 and trans-9,trans-11 (Kishino et al. 2010).

Milling by-products

In the course of milling, cereal kernels separated into flour, germ and bran. Germ corresponds to 2–3% of the total weight of kernel. Bran contains the outer layers and accounts for a relevant amount (ca. 15%) of the grain mass

Table 2. Main advantages related to the application of sourdough biotechnology to bran and bran-fortified wheat bread.

Bran source	Fermentation type	Effects	References
Wheat	*Lactobacillus brevis* E95612 and *Kazachstania exigua* C81116 with enzymes	Increase of peptides and free amino acids concentration; increase of protein digestibility, soluble fiber concentration. Decrease of pungent flavor and bitter taste of fortified bread.	Coda et al. (2014b)
Wheat	Commercial baker's yeast and *Lactobacillus brevis* L62	Improvement of the texture and sensory properties of fortified bread; increase of starch gelatinizaton.	Salmenkallio-Marttila, Katina, and Autio (2001)
Wheat	*Lactobacillus sanfranciscensis* DE9 and *Lactobacillus plantarum* 3DM	Increase o textural and sensory properties of fortified bread. Increase of antioxidant and phytase activities; increase of free amino acids concentration and protein digestibility. Decrease of starch digestibility and glycemic response.	Rizzello et al. (2012)
Wheat	Spontaneous fermentation	Increase of folates and phenols bioavailability; pentosan solubilization.	Katina et al. (2012)
Wheat	*Lactobacillus plantarum* DSM 32248 and *Lactobacillus rossiae* DSM 32249	Increase of fiber content, protein digestibility and nutritional indexes of fortified bread. Decrease of glycemic index.	Pontonio et al. (2017)
Oat	*Streptococcus thermophilus, Lactobacillus rhamnosus, Saccharomyces cerevisiae* and *Candida milleri*	Folic acid fortification	Korhola et al. (2014)
Oat	*Candida milleri*	Increase of fiber solubility	Degutyte-Fomins et al. (2002)
Rye	*Weissella confusa*	Exopolysaccarides synthesis	Kajala et al. (2016)

(Hemery et al. 2007). Germ and bran are unavoidable and poor commercial by-products, mainly used as livestock feed. Reintegration of cereal germ and bran into baked good formulas combines economic interests and dietary recommendations.

Bran

Bran is an irreplaceable source of dietary fiber, vitamins, minerals and many bioactive compounds (e.g. phenolic acids, tocopherols, proteins and folates) (Kamal-Eldin et al 2009). Epidemiological studies (Anderson et al. 2009; De Munter et al. 2007; Slavin 2003;) have shown how the consumption of foods rich in bran decreases risks of obesity, diabetes, inflammation, CVD and some types of cancer. Despite these evidences, the intake of bran and, consequently, of dietary fiber is still less than the dose (25 g/day) recommended by WHO/FAO (WHO 2003). Two major problems underlie this state: the low attractiveness (colour, texture and taste) of baked goods supplemented with bran and the need for the full exploitation of the nutritional value. Bran supplementation weakens the dough structure, and decreases bread volume and crumb elasticity because of the partial disruption and/or dilution of the gluten network, which worsens the dough gas-holding capacity (Delcour et al. 2012). Bran per se contains cellulose, lignin, phenols and alkylresorcinols, which negatively affect taste and mouthfeel, also causing bitterness (Bin et al. 2012). Technological pretreatments such as washing, grinding, oxidation and heat may decrease the bran particle size and in part remove harmful components (de Kock et al. 1999; Lai et al. 1989a, 1989b; Nelles et al. 1998; Rao and Rao 1991; Rasco et al. 1991). Nevertheless, these processes are high-energy challenging, and negatively interfere with the activity of health beneficial metabolites (Prückler et al. 2015). Sourdough fermentation of cereal bran is the most sustainable and promising option for improving texture and taste attributes of whole grain and fiber-rich products (Graf 1992; Hartikainen et al. 2014) (Table 2). In comparison with the native, fermented bran did not confer pungent flavor or bitter aftertaste to bread (Coda et al. 2014b). Bran pre-fermentation with yeast and lactic acid bacteria (*Lb. brevis*) added flavor, and good and homogenous crumb structure to fortified bread. Spontaneous fermentation of bran did not behave similarly. Bran pre-fermentation had also a marked effect on gelatinization and swollen of starch granules (Salmenkallio-Marttila, Katina, and Autio 2001). Debranning prior milling wheat grains is also advantageous. Bran from debranning of durum wheat underwent to micronization and air fractionation, obtaining coarse and fine fractions. Soft wheat flour doughs, containing 5% (w/w) of these fractions, underwent fermentation by *Lb. sanfranciscensis* and *Lb. plantarum*. Compared to wheat flour, without supplementation, micronized bran fractions enhanced bread textural and sensory properties (Rizzello et al. 2012). Bran has a very complex structure, with multiple layers mainly containing arabinoxylans and β-glucans. These layers trap dietary fibers, phenols and other chemical compounds, thus decreasing the release from the natural matrix. Further, most of the dietary fiber in bran is insoluble, which also reduces the digestibility and bioavailability of nutrients and phytochemicals (Delcour et al. 2012). Sourdough fermentation, especially in combination with hydrolytic enzymes (e.g. xylanase, β-glucanase, α-amylase, cellulose), influences the bran structure (partial disruption of the different layers) and allows specific targeting or modifications of components, which enhance the health-promoting features. Overall, the fermentation enhanced the bioactive potential of bran and increased the concentrations

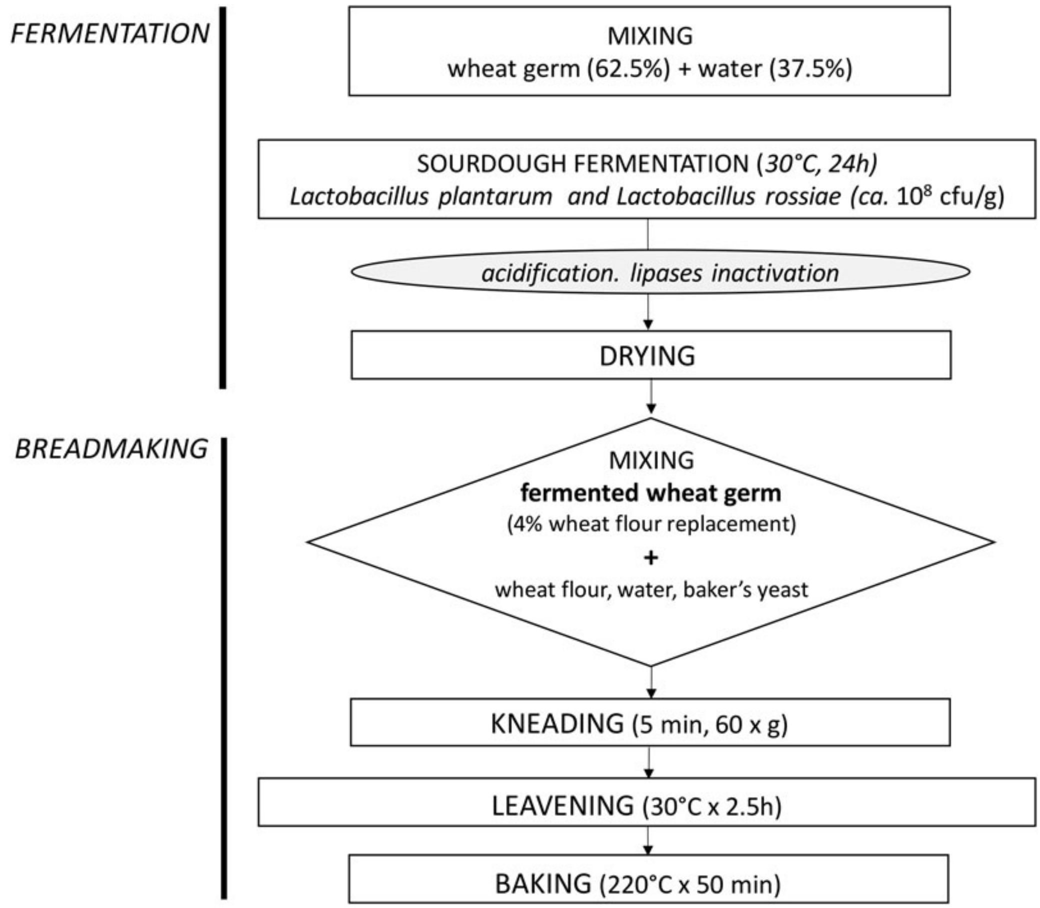

Figure 4. Schematic representation of bread making with fortification by fermented wheat germ. Wheat germ is mixed with water, inoculated with selected autochthonous strains of *Lactobacillus plantarum* and *Lactobacillus rossiae* and fermented for 24 h at 30 °C. Acidification lead to the lipase inactivation (Rizzello et al. 2010a). After freeze-drying, fermented wheat germ is mixed with wheat flour and water, and the dough leavened with baker's yeast (2%), before baking.

of folates and phenols compounds, and solubilized pentosans (Coda et al. 2015a; Katina et al. 2012). Bioprocessing of wheat bran with *Lb. brevis* and *Kazachstania exigua* improved the antioxidant and phytase activities, increased the content of peptides and free amino acids and the IVPD, and decreased the starch digestibility and the glycaemic response (Coda et al. 2014a; Poutanen et al. 2009; Rizzello et al. 2012). Bran dietary fiber per se affects the starch state and the water distribution in the matrix, and increases the viscosity of the digest (Poutanen et al. 2014). The low pH achieved during sourdough fermentation creates a barrier to starch digestion, thus improving the role of bran dietary fiber in the glucose metabolism (Scazzina, Siebenhandl-Ehn and Pellegrini 2013). The biochemical changes occurring during fermentation decreased the levels of alkylresorcinols and improved the bioavailability of ferulic acid, both *in vitro* and *in vivo* (Mateo Anson et al. 2009, 2010). Selected strains of *Lb. plantarum* and *Lb. rossiae*, previously isolated from wheat germ, were the starters to ferment a mixture of milling by-products, including bran. Wheat breads, manufactured using 15% (w/w) of fermented (and non-fermented) milling by-products, showed better protein digestibility, nutritional indexes, and rate of starch hydrolysis. The enriched bread also had a high content of dietary fiber and a low glycaemic index as determined *in vivo* (Pontonio et al. 2017).

Germ

Cereal germ has excellent compositional traits. It is a source of vitamins E and B, dietary fiber, essential amino acids, and functional phytochemicals such as flavonoids and sterols. Most of the essential amino acids from wheat germ proteins are present at concentrations higher than in the reference egg protein (FAO 1995; Ge et al. 2001). Nevertheless, the germ is quickly subjected to rancidity during storage because of the high levels of unsaturated fatty acids (mainly oleic, linoleic and α-linoleic acids) and the concomitant presence of hydrolytic (lipase) and oxidative (lipoxygenase) enzymes (Sjövall et al. 2000). For long time, this susceptibility has almost totally excluded the use of the germ for making baked goods. Most of the efforts (e.g. microwave and thermal treatments, removal of the oil fraction and oxygen elimination) aiming at germ stabilization have focused on lipase and lipoxygenase inactivation. Despite some effectiveness, these treatments are expensive, not fully resolving, impoverishing the nutritional value, and, more in general, in contrast with the consumer expectations (Paradiso et al. 2008). Sourdough fermentation has the capacity to decrease the pH at values, which inactivate the germ endogenous enzymes (Figure 4). These results accounted for the decrease in rancidity, as shown monitoring the hexanal marker during storage. Fermentation markedly decreased the content of glutathione, which is responsible for the deterioration of the dough structure in the presence of high levels of germ (Marti et al. 2014). Sourdough fermented wheat germ increased by ca. 50% the concentration of free amino acids, converted glutamic acid into GABA, markedly decreased the content of raffinose, and enhanced total phenols, and phytase and antioxidant activities (Rizzello et al. 2010a). These main nutritional features almost fully translated to wheat bread when enriched with fermented wheat germ. Besides, this fortification favored high specific volume, low values of hardness, resilience and fracturability and agreeable sensory properties of the bread (Rizzello et al. 2010b). Sourdough fermented wheat germ had also a promising antifungal activity. Its water/salt-soluble extract showed inhibition towards various fungi isolated from bakeries, with the antifungal activity attributed to a mixture of organic acids (mainly formic acid) and peptides synthesized during fermentation. Slices of bread made by addition of 4% (w/w) of freeze-dried sourdough fermented wheat germ did not show fungal contamination until 28 days of storage and behaved as the calcium propionate (Rizzello et al. 2011). A number of studies have reported the promising cytotoxic activity of fermented wheat germ towards cancer cell lines and during *in vivo* clinical trials, mainly attributing this activity to quinones (Mueller and Voigt 2011; Mueller et al. 2011). Selected strains of *Lb. plantarum* and *Lb. rossiae* favored the release of the non-glycosylated and physiologically active 2-methoxy benzoquinone, and 2,6-dimethoxybenzoquinone during 24 h of wheat germ fermentation. Sourdough fermented wheat germ had an *ex vivo* anti-proliferative activity towards cell lines of human cell tumor, colon carcinoma and ovarian carcinoma. The raw wheat germ did not show effect (Rizzello et al. 2013).

Conclusion

As recommended by dietary guidelines, legumes, pseudo-cereals and milling by-products are undoubtedly new nutrient resources to fortify staple baked goods. The simple addition under conventional processes does not alleviate the main counter indications (poor rheology and sensory attributes, and presence of anti-nutritional factors), which prevent the large-scale fortification. As an ancient and sustainable process, tailored sourdough fermentations are the most suitable option not only to improve rheology and sensory attributes and to limit or eliminate anti-nutritional factors but also to exploit fully the nutritional and functional potential of legumes, pseudo-cereals and milling by-products. Compared to the use of purified enzymes or technological treatments, fermentation would be a more sustainable and mild process. Time is suitable for a more concrete technological transfer and application on a wider large-scale.

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