Chapter

# Fermentation as Strategy for Improving Nutritional, Functional, Technological, and Sensory Properties of Legumes

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## Abstract

Compared with cereals and other plant-derived food matrices, legumes can be considered as valuable sources of proteins with high biological value, dietary fibers, minerals, oligosaccharides, and phenolic compounds. Nevertheless, the presence of different antinutritional factors (ANFs) limited the large-scale use of such ingredients by the food industry. The potential of several biotechnological processes and enzymatic treatments in decreasing ANF in legumes and legume-derived ingredients was investigated. Among these options, fermentation is traditionally recognized as suitable tool to improve the overall quality of legumes in different areas of the world. The scientific community demonstrated the effectiveness of the use of selected lactic acid bacteria and biotechnologies inspired to sourdough fermentation in ANF degradation, improving technological and sensory profile of legume grains and flours as well as contributing to their safety in terms of spoilage or pathogenic microorganisms and toxic compounds. Apart from their consumption as they are, legumes are the main ingredient of many traditional food products, and fermentation allows them to be used as ingredients in innovative formulations of staple foods, such as baked goods and pasta with high nutritional and functional profile.

**Keywords:** fermentation, legumes, lactic acid bacteria, antinutritional factor, biotechnologies, sourdough, bread, pasta

## 1. Introduction

The challenge of feeding the growing world population and the necessity to provide a nutritionally balanced diet while reducing greenhouse gas emissions, as well as a transition to a diet higher in plant- rather than animal-derived proteins, require relevant increases in vegetables production. In this context, the fortification of foods and beverages has been identified as an effective, sustainable, and promising intervention capable of modulating the diet toward healthier choices, addressing environmental concerns, and meeting nutritional deficiencies and recommendations. To date, several studies investigated the nutritional value of



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additional ingredients to be used as wheat alternatives in cereal-based products, such as bread and pasta.

Legumes are considered as good source of high biological value proteins and dietary fibers. Moreover, they are rich in phenols, minerals, vitamins, and oligosaccharides. The optimal technological properties of the legume flours (e.g., high water-binding capacity and solubility) make them suitable ingredients for gluten-free foods.

Nevertheless, legumes contain part of their nutritional compounds under a nonbioavailable form and several antinutritional factors (ANFs) that may decrease digestibility of other nutrients or cause physiological discomfort or conditions. Furthermore, legumes have poor technological, rheology, and sensory attributes if compared with gluten-containing cereals. Hence, the full exploitation of such food matrices goes through the most suitable bioprocessing.

Lactic acid bacteria (LAB) are the group of microorganisms most largely used at food industrial level, having the status of Generally Recognized as Safe (GRAS). Used as natural (e.g., sourdough and spontaneous fermentation) or selected starters, LAB have the capability to conjugate desired functional activities, sensory properties, and microbiological safety.

Overall, bioprocessing including LAB fermentation is considered a safe, sustainable, and effective tool for improving the functional and nutritional features of many plant-derived matrices and to obtain suitable technological, sensory, and shelf-life characteristics of fermented foods and beverages (**Figure 1**). The positive effects of LAB fermentation are in part related to the acidification, although further effects can be observed, such as those related to the synthesis of metabolites and the activation of the flour endogenous enzymes. The properties of the fermented matrix are often profoundly different from the unfermented ingredients. Among the main nutritional advantages of the LAB fermentation, the increase of the protein digestibility and the decrease of the glycemic index have

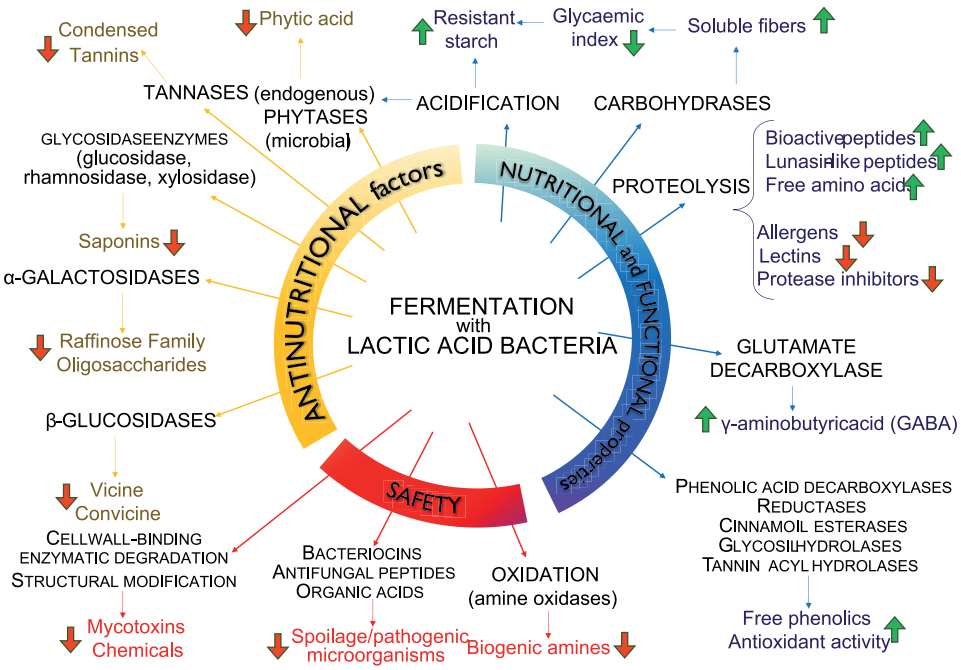


**Figure 1.**
*Main nutritional, functional, and safety properties deriving from LAB fermentation.*

been largely investigated. More recently, also the degradation of the antinutritional compounds (e.g., trypsin inhibitors, phytic acid, saponins, condensed tannins, and α-galactosides) and the synthesis of bioactive compounds have been described. Starting from the conventional application of the sourdough-inspired procedures, innovative biotechnological protocols, based on the use of selected starters, automatized bioreactors, and semiliquid formulations have been recently proposed to extend to a large-scale application the use of legumes in food industry.

Indeed, fermentation (both spontaneous or guided by selected LAB) has been recognized as the most suitable and sustainable process to exploit the potential of legumes to fortify staple foods such as baked goods, pasta, extruded snacks, and plant-based fermented beverages.

In this chapter, the scientific evidence confirming the nutritional, functional, rheology, sensory, and shelf-life improvements of fermented legumes and derived food products is described.

## 2. Nutritional insights

As recommended by global organizations, due to the growing concerns related to the environmental impact of animal breeding and the health risks associated with high meat intake, the decrease in animal-derived foods consumption led to the need for more plant-based foods in diet and more energy-efficient processing [1]. Simultaneously, the large market growth of foods designed for vegetarian, vegan, and gluten-free diets generated an increased consideration in improving the nutritional quality of grains-derived ingredients to be used in food preparation [2].

Leguminosae family, belonging to the Dicotyledonae group, includes 18,000 different species. After cereals, legumes are the most important group of crops, and their consumption is widely distributed all over the world.

A large variety of legumes used for human diet are cultivated extensively or locally [3, 4]. The economic importance of the Leguminosae family is related to the low input required for their cultivation, the positive impact on the soil fertility, and the great adaptability to underrestrictive pedoclimatic conditions [4]. Moreover, the advantages of cereal-legume intercropping, also providing an efficient exploitation of natural resources, have been abundantly demonstrated [5].

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 [6]. The frequent consumption of legumes is effective to prevent or decrease risks of cardiovascular disease (CVD) [7], type 2 diabetes [8], some types of cancer [9], and overweight and obesity [10].

When cereals and legumes are combined in food formulations, protein efficiency improved thanks to complementary essential amino acid profiles [11]. Overall, compared with cereal, legumes contain less starch, more protein, and more fiber, whereas lipid content is either equal or higher. Starch content in wheat varies between 60 and 80%, whereas it ranges from 40 to 65% for legumes except for lupin, having a markedly lower starch content [1]. Proteins in legume flours vary between 20 and 30% and can reach up to 40% in faba and lupin flours, against the 9–18% in wheat and other cereals [1]. Fiber content is circa 2% (on dry matter) in wheat flour and semolina, while it can reach 10% in pea and faba flours, and even 20–40% in chickpea, lentil, and lupin flours [1]; however, legume flours are often obtained from whole grains (not dehulled) resulting in a higher proportion of fiber. Ultimately, lipid content varies between 1 and 3% (on dry matter) in wheat and legume flours except for chickpea and lupin flours in which it can reach 10–13% [1].

Besides nutritional composition, the main proteins contained in cereals and legumes also present several differences in terms of type and functionality. In wheat, for example, gluten proteins (gliadins and glutenins) are the most abundant, accounting for 80% of total protein fraction [12]. In legumes, globulins are the dominant group, accounting for 50–70% of total proteins [13]. Wheat gliadins and glutenins contain higher concentration of sulfur amino acids compared with legume globulins, meaning they have more reactive cysteine residues [13, 14]. Moreover, low-molecular-mass albumins are present in both cereal and legume grains, reaching, respectively, 15 and 15–40% of the total proteins content [13]. Just as for proteins, starch granules in wheat and legumes show differences. They both contain linear amylose and branched amylopectin organized in semicrystalline and amorphous structures; however, they differ in shape and amylose/amylopectin ratio [15]. Legume starches have a higher proportion of amylose than wheat starch, ranging from 24/76 to 40/60 for pea and lentil starches and from 23/77 to 35/65 for chickpea starch [16].

## 3. Antinutritional factors and microbial degradation

Legumes contain several ANFs, such as raffinose, phytic acid, condensed tannins, saponins, alkaloids, lectins, pyrimidine glycosides, and protease inhibitors [17]. Overall, ANFs decrease the bioaccessibility and bioavailability of other nutrients, and, in some cases, are responsible for adverse reactions to the ingestion.

The content of raffinose-family oligosaccharides (RFOs, raffinose, verbascose, and stachyose) in legumes ranges from 1 to 6% with stachyose as the most abundant compound [18]. While in cereals, it is commonly lower than 1.5%, with raffinose as the sole or the most abundant compound [19, 20]. RFOs are nondigestible oligosaccharides that may result in adverse digestive symptoms when about 15 g/person per day are exceeded [21], a threshold that is readily reached in legume-based diets. Raffinose and RFO are indeed fermented by the intestinal microbiota with abundant gas production, causing discomfort and flatulence.

Phytic acid is the main storage compound for phosphorous and minerals in cereal and legume seeds. In legumes, its concentration can reach 20 g/kg [22, 23]. Phytic acid and divalent minerals (e.g., $Ca^{2+}$, $Zn^{2+}$ and iron) form stable complexes (phytates) that are insoluble and not hydrolyzed in the gastrointestinal tract, thus reducing the bioavailability of minerals for the monogastrics. $Ca^{2+}$ and $Zn^{2+}$ deficiencies are commonly observed in developing countries, and complexation of dietary minerals by phytates in plant-derived foods contributes to the mineral deficiency [17]. Iron uptake from plant-derived foods is impeded not only by complexation with phytate but also by complexation with condensed tannins [24, 25].

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 [26]. Tannins impart bitter taste, reduce protein and starch digestibility by inhibition of pancreatic enzymes, and reduce iron uptake [26, 27]. The presence of tannins reduces the caloric content and the glycemic index of foods [28], but the abundance in diet reduces the supply of macro- and micro-nutrients.

Lectins and specific inhibitors of digestive enzymes (proteases and amylases) further reduce the digestibility of starch and proteins in legumes [26, 29].

Some ANFs are heat-labile (e.g., protease inhibitors and lectins) and easily removed by thermal treatments. Nevertheless, phytic acid, raffinose, tannins, and saponins are rather thermostable. Dehulling, soaking, air classification, extrusion, steaming, and pregelatinization are the main technological options for decreasing

the negative impact of ANF on legume consumption [30–32]. Nevertheless, biological methods such as germination, enzyme treatments, and especially, fermentation seem to be more efficient [30, 31, 33, 34].

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) are responsible for the inactivation of most ANFs.

Raffinose family oligosaccharides are hydrolyzed through the activity of α-galactosidases, levansucrase, and sucrose-phosphorylase activities of lactic acid bacteria [35, 36] or corresponding enzymes of fungal cultures; their removal in legume fermentations has been amply reported [37].

In cereal matrices [22], the phytase activity is often sufficient to degrade phytates, especially in acidic conditions [18, 38]. Therefore, phytate degradation in LAB-fermented matrices spontaneously occurs without microbial enzymes involvement [18]. The optimal pH for the activity of the cereal phytases corresponds to 5.5; nevertheless, phytases are still active at pH levels lower than those commonly reached by sourdough (3.8–4.2) [18]. Sourdough fermentation and other types of traditional bioprocesses involving LAB (e.g., fermentations for production of cereal porridges or beverages) allow the increase of the mineral bioavailability [39]. Compared with that found in cereals, the phytase activity in legumes is poor [22, 40]. Nevertheless, pretreatments and processing conditions including fractionation, germination, soaking, thermal treatments, and fermentation drastically decrease phytate levels in legumes [41]. In many spontaneously fermented legume products, substrate-derived phytases are inactivated, and phytate degradation is achieved by fermentation with bacilli or fungal cultures, for example, *Rhizopus stolonifer* or *Aspergillus oryzae*, which hydrolyze phytate with extracellular enzymes [42, 43].

Metabolism of tannins or other polyphenols by LAB was deeply characterized only in a few fermented plant-derived matrices [44, 45]. *Lactiplantibacillus plantarum*, *Lactiplantibacillus paraplantarum*, *Lactiplantibacillus pentosus* have been identified, among the LAB, as the species that could decrease the tannins concentration through their tannases (tannin acyl hydrolase, EC 3.1.1.20) [46–48]. However, characterization of fermented cassava allowed identifying uncommon tannase producers such as *Weissella cibaria* and *Leuconostoc mesenteroides* ssp. *mesenteroides* [49]. Most of tannase producers were found in fermented vegetables but also in human feces. In *L. plantarum*, tannase is very well characterized. Its activity was demonstrated and characterized by Rodríguez et al. [47], and genetic analysis showed that it constitutes a novel family of tannases [50]. LAB tannases are intracellular. Genes involved in tannins degradation are regulated in a coordinated way and are inducible by tannin and other phenolic compounds [51].

The lactic fermentation of grass pea (*Lathyrus sativus*) with *L. plantarum* lowered the levels of phytic acid and trypsin inhibitory activity [52]. Selected strains of *L. plantarum* and *Levilactobacillus brevis* decreased the content of raffinose up to circa 64% during sourdough fermentation of different legume flours. Sourdoughs made with different legume flours (bean, lentil, pea, grass-pea, chickpea) contained an increased phytase activity compared with the unfermented controls [34]. The combination of legume sprouting and sourdough fermentation decreased the content of phytic acid, condensed tannins and raffinose, and trypsin inhibitory activity [53, 54].

Besides the abovementioned ANFs, faba 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 [55].

Divicine and isouramil trigger favism disease in susceptible individuals. Technological processes (air classification, roasting, and boiling) and selection of cultivars with low content of such compounds seemed to be only in part effective [55, 56]. On the contrary, β-glucosidase from LAB effectively degraded the pyrimidine glycosides from faba bean suspension and flour [30]. When used as starter to ferment fava bean flour, *L. 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 [57]. Similar results were obtained when flours from different faba bean accessions collected from the Mediterranean area were subjected to the LAB fermentation [58]. *Ex-vivo* hemolysis assays on human blood confirmed the lack of toxicity of the fermented fava bean [57].

## 4. Decrease of allergens, biogenic amines, mycotoxins, and chemicals through fermentation

Different legume proteins act in susceptible individuals as allergens. Their complex structures are difficult to degrade. The selection of legumes' natural variants or the use of specific biotechnological processes has been exploited to solve this issue. However, some side effects such as an increase in the protein synthesis pathways of the seed and the synthesis of other proteins that might be allergenic have been also reported [59–62]. Overall, plant proteins exhibit low digestibility compared with animal proteins. Poor protein digestibility can cause gastrointestinal disorder, and the increase in protein digestibility could reduce the level of immunoreactive proteins in their active forms, thus reducing the risk of food allergies symptoms [63]. Several studies showed that LAB fermentation increases the digestibility of plant proteins through the combined activity of microbial and endogenous proteases and peptidases [64, 65]. The use of fermentation to reduce or eliminate allergenicity of soy products represents an interesting opportunity to produce hypoallergenic food products from legumes [66, 67]. It was indeed shown that fermentation of soybean meal with *L. plantarum* or *Bifidobacterium lactis* allowed a significant increase in the total amino acids and a low immunoreactivity.

Besides allergens, many undesirable substances, contaminating foods and feeds, are harmful to human and animal health. These include mycotoxins, which are widely present in food and feeds commodities. The role of different microorganisms including fungi, yeasts, and bacteria in mycotoxins degradation has been investigated. Several studies extensively reported that mycotoxin degradation mechanisms are different and include cell wall binding, enzyme degrading, or structure modification. However, the degradative mechanisms are strain-dependent [68–73].

For example, patulin is a mycotoxin synthesized by different fungi, such as *Penicillium expansum,* able to colonize different fruits and vegetables [74]. Its toxicity is due to the high reactivity with thiols [75], which leads to the decrease of cellular glutathione levels. The capability of some yeasts or heterofermentative lactobacilli to release thiols during fermentation allows the patulin inactivation. Patulin degradation can also occur thanks to the conversion in inactive forms by *L. plantarum* esterase and reductase activities [76]. It was also reported that fermentation of legumes and cereals allows the decrease of aflatoxin concentration [77]; however, the mechanisms have not been completely clarified [78]. The mycotoxins absorption by the bacterial biomass has also been hypothesized [79].

Fermented foods often contain biogenic amines, derived from microbial metabolisms, and characterized by a dose-dependent toxicity. Biogenic amines (BAs) are

produced not only by Gram-positive and Gram-negative bacteria, but also by yeasts and molds [80]. Also LAB are considered as BAs producers in fermented foods and *Enterococcus*, species of the former *Lactobacillus* genus, *Streptococcus, Lactococcus, Oenococcus, Pediococcus, Weissella, Carnobacterium, Tetragenococcus, Leuconostoc, Sporolactobacillus* are the main genera showing this trait [81]. BAs production is a strain specific feature, and some studies revealed that the involved enzyme is encoded by unstable plasmids [82, 83]. Therefore, horizontal gene transfer is essential to disseminate this ability in LAB [82, 83].

Many intrinsic and extrinsic parameters affect the BAs production (e.g., pH, temperature, and water activity); nevertheless, their control is often difficult during food processes. The BAs production is strain-dependent; therefore, the starter selection is an efficient tool to decrease their accumulation in fermented foods. Another effective strategy includes the use of amine oxidizing selected starters [84].

Through their oxidases, such microorganisms catalyze the oxidative deamination of BAs and their conversion to aldehydes, hydrogen peroxide, and ammonia [85]. Kim et al. [86] isolated strains of *Bacillus subtilis* and *Bacillus amyloliquefaciens* from fermented soybean foods. They observed the ability of *B. subtilis* to degrade putrescine and cadaverine and of *B. amyloliquefaciens* to oxidize histamine and tyramine. Similarly, Kang et al. [87] showed the ability of *B. subtilis* and *B. amyloliquefaciens* strains to reduce tyramine in Cheonggukjang. Eom et al. [88] isolated from buckwheat sokseongjang, a Korean traditional fermented soybean food, three strains (belonging to *B. subtilis* and *Bacillus idriensis* species), which were able to degrade histamine and tyramine but also unable to produce them. Lee et al. [89] recently proposed the use of *L. plantarum* strains to reduce BAs content during Miso fermentation. The possibility to use amine oxidizing starter cultures is an effective tool to decrease the BAs concentration in fermented foods obtained with legumes, especially when traditional production methods are used.

Another growing concern for the consumer is represented by the potential presence of chemicals and pesticides in foods, especially if correlated to the global recommendation to increase the dietary uptake of fruit and vegetables. It has been reported, for example, that the cumulative intake of pesticides by high consumers of fruits and vegetables in Brasil exceeds the Acute Reference Dose [90]. There is a consensus that the level of residual pesticides in foods needs to be decreased. However, the replacement of conventional pesticides in agriculture is a slow and difficult process. Therefore, the possibility to degrade pesticides through fermentation has been investigated. Several chemicals can be converted by microorganisms, but many of the most effective species characterize the environmental microbiota and are not easily usable in food processing.

The conversion of pesticides during food fermentation has been investigated in correlation, for example, to the large diffusion of contaminated soy (genetically resistant to the herbicide glyphosate). The degradation of organophosphorus insecticides was observed during the fermentation of Kimchi by *Leuc. mesenteroides, Lv. brevis, L. plantarum*, and *Latilactobacillus sakei* strains [91].

*Lv. brevis* was also seen as an active catalyst against the same family of compounds during the fermentation of milk products [92]. The degradation of organochlorine pesticides has also been investigated in milk during yogurt and cheese production showing the effect of starters [93]. Other examples refer to the capability of *Micrococcus varians* to degrade DDT (dichlorodiphenyltrichloroethane) to DDD (ddichlorodiphenyldichloroethane) and lindane to 2,4-, 2,5-, 2,6-, and 3,4-dichlorophenol and of *Lactococcus lactis subsp. lactis* to degrade dinitrotoluene isomers [94, 95].

## 5. LAB as biopreservation agents against pathogenic and spoilage microorganisms

Besides decreasing antinutritional factors and allergy, LAB can fulfill a task of biopreservation [96]. This word can be defined as the extension of shelf-life and food safety by means of natural or controlled microbiota and/or their antimicrobial compounds [97]. Overall, LAB fermentation is one of the most common methods of food biopreservation.

In South-East Asia, specific biopreservation strategies to limit pathogens and spoilage microorganisms contamination in foods have been proposed. Overall, the most common contamination of legumes in the field is represented by sporulating bacteria; then, fungi can develop and produce mycotoxins. Finally, different pathogens can occasionally derive from cross-contamination with other foods.

Phan et al. [98] studied LAB strains isolated from fermented products from Vietnam, including dua gia (bean sprouts), identifying *L. plantarum*, *Limosilactobacillus fermentum,* and *Lactobacillus helveticus* strains as dominant. In legumes, such as in other products, it is important to use bacteria that can grow rapidly to become dominant compared with the endogenous microbial contaminants.

The biopreservation mechanisms by which LAB inhibit spoilage organisms include the destabilization of cell membrane and subsequent interference with the proton gradient, inhibition enzyme activity, and creation of reactive oxygen species [96]. Moreover, LAB strains are able to produce antimicrobial compounds such as low-molecular-weight metabolites (reuterin, reutericyclin, diacetyl, fatty acids), hydrogen peroxide, antifungal compounds (propionate, phenyl-lactate, hydroxyphenyl-lactate, and 3-hydroxy fatty acids), and bacteriocins that may be exploited in the biopreservation of foods [99]. There is a wide number of bacteriocins produced by LAB that are classified into three classes: Class I (Lantibiotics), class II (Non Lantibiotics), and class III (Big peptides) depending on their chemical and genetic characteristics. The antibacterial activity of nisin, the most studied lantibiotics, has been demonstrated against *Listeria* spp., *Micrococcus* spp., and sporulating bacteria such as *Bacillus* spp. and *Clostridium* spp. [100]. Nguyen et al. [101] isolated the LAB from nem chua and determined their antimicrobial activity against pathogenic and sporulating strains such as *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella typhimurium*. Five strains NH3.6, NT1.3, NT1.6, NT2.9, and NT3.20 showed a broadened antimicrobial activity against both pathogenic Gram-positive *B. cereus* and *Ls. monocytogenes* and Gram-negative *E. coli* [101]. *L. plantarum* HA2, HA3, HA5, HA8, and HA9 and *L. fermentum* HA6, HA7, and HA10 isolated from Vietnamese fermented vegetables showed an intense antifungal activity against different indicator molds and yeasts (*Aspergillus terreus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Absidia corymbifera*, *Paecilomyces lilacinus*, *Geotrichum candidum*, *Fusarium sp*., *Scopulariopsis brevicaulis*, *Curvularia lunata*, *Penicillium* spp., and *Candida albicans*) [102]. These LAB strains are currently investigated for the specific use in legume-fermented products [96].

Fungi are the most common spoilage microorganisms of baked goods and represent a huge economic problem in bakery sector. The use of chemical preservatives is currently the only effective tool to prolong the microbial shelf-life of baked goods [103, 104]. Nevertheless, the European directive on preservatives has recently decreased the allowed concentrations of preservatives, and consumers require clean label and preservative-free baked goods. Therefore, the scientific and industrial research is now oriented toward the search for new preservatives, derived from natural sources. Overall, plants produce proteins and peptides involved in fungal resistance mechanisms, and seeds of many different species of leguminous plants are

rich in such active compounds [105]. It was reported that the water-soluble extract of *Phaseolus vulgaris* cv. Pinto showed inhibitory activity toward a large spectrum of fungal species isolated from bakeries. The antifungal proteins corresponded to 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 with that afforded by calcium propionate (0.3%, w/w) [106]. A pea (*Pisum sativum*) protein hydrolysate, obtained by a food-grade protease, showed high inhibitory activity toward several fungi isolated from bakeries. The antifungal activity was correlated to pea defensins 1 and 2, nonspecific lipid transfer protein (nsLTP), and a mixture of peptides, encrypted in leginsulin A, vicilin, provicilin, and nsLTP, and released by the enzymatic activity of the protease [107]. A mixture of legumes-derived protein hydrolysates inhibited *Aspergillus parasiticus*, *Penicillium carneum*, *Penicillium paneum*, and *Penicillium polonicum*. Several native proteins and a mixture of peptides, encrypted in legume vicilins, lectins, and chitinases, were identified as the compounds responsible for the antifungal activity [108].

More recently, a LAB-fermented chickpea flour was proposed as fresh pasta ingredients aiming at prolonging the shelf-life of the product, moreover, achieving different nutritional advantages [109].

## 6. Traditional and novel fermented legume products

### 6.1 Traditional foods

Legumes are used as food ingredients worldwide, but only in few geographical areas they are commonly used for the production of fermented foods (**Table 1**), such as Japanese natto, Nigerian dawadawa or iru, Nepalese kinema, and Thai thua nao. Fermented legumes are consumed directly or used as ingredients or flavoring agents [124]. Yukiwari-natto and hama-natto spontaneous microbiota are dominated by molds, while *Bacillus* spp. is commonly isolated in itohiki-natto. Molds are also responsible for meitauza, oncom, and sufu fermentation, while yeasts dominate the fermentation of the Indian papad/papadam. Tempe is characterized by a microbial consortium including molds and LAB. Also Indian idli, wadi, and dhokla are produced by the combined fermentation activities of LAB and yeasts. Complex microbiota including LAB, yeasts, and molds characterize the fermentation processes for obtaining inyu, kecap asin, kecap manis, meju, miso, soy sauce, and tauco [118, 125].

Fermentation has an important impact on the nutritional and sensory profile of legumes [2, 96]. However, production of traditional fermentation products is often managed empirically, with rudimentary equipment, and based on the activity of endogenous microorganisms [96]. The quality of raw materials as well as the biotechnologies is not standardized [96]. These products are characterized by the local cultural identity. Despite their important sensorial role in Asian food, bringing, for instance, the umami taste to the meals [126], the necessity to improve overall quality and to minimize food safety hazards has been recently highlighted [127].

LAB have an important role in some of the traditional fermented legume products (such as in vietnamese tuong and cambodian sieng), but many other microorganisms (bacteria, yeasts, and molds) are involved in spontaneous fermentation processes. Nevertheless, the advantages of legumes fermentation with LAB are gaining interest from the scientific and food industry community [2].

| Product | Main ingredients | Microorganisms | Area | Reference |
|---|---|---|---|---|
| Adai | Legume seeds and cereal grains | Lactic acid bacteria (*Pediococcus* spp., *Streptococcus* spp., and *Leuconostoc* spp.) | South India | [110] |
| Afiyo (okpehe or kpaye) | Mesquite bean (*Prosopis* sp.) | | Nigeria | [111] |
| Aisa | *Albizia saman* seeds | Bacilli (*Bacillus* spp.) and staphylococci (*Staphylococcus* spp.) | Nigeria | [112] |
| Amriti | Black gram dal (*Vigna mungo*) | Aerobic mesophilic bacteria | India | [113] |
| Bedvin roti | Black gram dal, opium seed or walnut flour | | India | [114] |
| Chungkokjang (cheonggukjang or jeonkukjang) | Soybean (*Glycine max*) | Bacilli (*Bacillus* spp.) | Korea | [115, 116] |
| Dawadawa (soydawadawa) | Soybean | | Nigeria | [117] |
| Dawadawa, kinda, iru, soumbala | Locust bean (*Parkia biglobosa*) | | West and Central Africa | [117] |
| Dhokla | Rice grains and bengal gram dal (*Cicer arietinum*) | | India | [118] |
| Dosa | Black gram dal and rice grains | | India | [118] |
| Douchi | Black soybean | | China | [117] |
| Gochujang/Kochujang | Soybean, red pepper, rice, barley malt powder | Bacilli (*Bacillus* spp.) | Korea | [115] |
| Idli | Black gram dal and rice grains | | India, Sri Lanka | [118] |
| Kinema, hawaijar, tungrymbai, aakhone, bekang, peruyyan | Soybean | | Darjeeling hills and North East of India, Bhutan, Nepal | [117] |
| Maseura (masyaura) | Black gram dal/ ricebean (*Vigna umbellate*) | Lactic acid bacteria, bacilli, and yeast | India, Nepal | [119] |
| Meitauza | Okara (soybean press cake) | | China, Taiwan | [117] |
| Meju | Soybean | Fungi and bacilli (*Bacillus* spp.) | Korea | [115] |
| Natto | Soybean | | Japan, Korea | [117] |

| Product | Main ingredients | Microorganisms | Area | Reference |
|---|---|---|---|---|
| Oncom: Hitam (black) and merah (red) | Peanut (*Arachis hypogaea*) press cake | | Indonesia | [118] |
| Oso | *Cathormion altissimum* seeds | | Nigeria | [117] |
| Otiru | African yam bean (*Sphenostylis stenocarpa*) | | Nigeria | [117] |
| Owoh | African yam bean | | Nigeria | [117] |
| Papad or papadam | Black gram, bengal gram, lentil (*Lens culinaris*), red gram (*Cajanus cajan*) or green gram (*Vigna radiata*) flour | | India | [118] |
| Pitha (chakuli, enduri, munha, chhuchipatra, podo) | Black gram dal and rice grain | Lactic acid bacteria | India | [110] |
| Sepubari | Black gram dal | | India | [120] |
| Soybean paste: Doenjang or jang, miso, tauco, tao chieo | Soybean, wheat or rice grains | | China, Indonesia, Japan, Korea, Thailand | [117] |
| Soy sauce: Jiang you, shoyu or tamari shoyu, kanjang, kicap, kecap, taosi, ketjap, inyu | Soybean/black soybean and wheat grains | | China, Japan, Korea, Malaysia, Indonesia, Philippines, Indonesia, Taiwan, Hong Kong | [117] |
| Sufu or furu | Soybean | | China, Taiwan | [118, 121] |
| Tempeh | Soybean | | East Java, Indonesia | [118, 122] |
| Thua nao | Soybean | | Thailand | [117] |
| Tuong | Soybean | Bacilli (*Bacillus* spp.), enterobacteria | North and central Vietnam | [123] |
| Vada | Legume and cereal | Lactic acid bacteria (*Pediococcus* spp., *Streptococcus* spp., and *Leuconostoc* spp.) | India | [110] |
| Ugba/ukpaka | African oil bean (*Pentaclethra macrophylla*) | | West and Central Africa | [117] |

| Product | Main ingredients | Microorganisms | Area | Reference |
|---|---|---|---|---|
| Wadi | Black gram dal | | Northern India | [118] |
| Yandou | Soybean | | China | [117] |

**Table 1.**
*Main traditional food products containing fermented legumes.*

## 6.2 Sourdough-inspired fermentation, sprouted flours, and baked good fortification

Besides the direct consumption as conventional dishes, legumes have a great potential as ingredients in various baked goods and pasta. Their use as fortifiers should increase their consumption as strongly recommended in many dietary guidelines. With this goal in mind, in the past decades, many researchers focused on using legume flours (also sprouted), fermented or not, as part of food formulations. Fermentation of legumes mainly determines improvement of the protein digestibility and related nutritional values and the biological availability of fibers and total phenols (**Table 2**). However, unlike cereal flour sourdoughs, very little is known about the microbiota of sourdough-type propagation, when only legume flour is used. Coda et al. [136] explored this topic investigating, through 16S rRNA gene pyrosequencing and culture-dependent analysis, the microbial ecology of faba bean sourdoughs obtained from an Italian and a Finnish cultivar, belonging respectively to *Vicia faba major* and *V. faba minor* groups. Among the LAB isolates, *Pediococcus pentosaceus, Leuc. Mesenteroides, and Weissella koreensis* had the highest frequency of occurrence in both sourdoughs. The presence of hulls and the different microbial composition reflected on biochemical characteristics of Finnish sourdoughs, including acidification and phenolic compounds [136].

Traditional varieties and biotypes, often replaced by modern cultivars selected for improved agronomic and commercial traits, can also be rediscovered and valorized through fermentation [34, 58, 130, 133]. Nineteen Italian legume flours, fermented with selected strains of *L. plantarum* and *Lv. brevis*. and compared with doughs without bacterial inoculum, had higher concentrations of free amino acids, soluble fibers, and total phenols. During sourdough fermentation, the level of γ-aminobutyric acid (GABA) markedly increased reaching up to 624 mg/kg [34]. GABA-producing strains of *L. plantarum* and *Lc. lactis* subsp. *lactis* were employed as starters for sourdough fermentation of a blend of chickpea and pseudo-cereals resulting in sourdough bread with very high levels of free amino acids and GABA (up to 504 mg/kg) [131]. The pairing between sourdough fermentation and legumes to accumulate GABA was performed also using adzuki bean flour [128] and extracts from kidney beans subjected to liquid state fermentation [129]. Type I sourdough, containing wheat-legume flour mixtures, was also used (15%, w/w) in bread making. The fortification 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 [32].

Nevertheless, either considering gluten-free products or wheat-based baked goods, the lack of gluten is one of the challenges deriving from the use of legumes. The addition of wheat-legume flours increases water absorption providing more water for dough starch gelatinization during baking and preventing stretching and tearing of gluten strands [53]. Substitution of wheat flour with legumes at levels higher than 20–30% causes detrimental effects on dough and bread properties, which results in sticky and excessively compact [53, 140]. Hence, maintaining good

| Legume | Fermentation type | Effects | References |
|---|---|---|---|
| Bean (Adzuki bean) | *Lactococcus lactis* subsp. *lactis* and *Lacticaseibacillus rhamnosus* GG | Increase of GABA concentration | [128] |
| Bean | Spontaneous fermentation | Decrease of α-galactosides, phytic acid, trypsin inhibitors and condensed tannins concentrations | [33] |
| Bean (Kidney beans) | Spontaneous fermentation; inoculum with *L. plantarum* ATCC 14917 | Increase of GABA concentration | [129] |
| Bean, chickpea, grass pea, lentil, pea (local cultivars) | *Lactiplantibacillus plantarum* C48 and *Levilactobacillus brevis* AM7 | Increase of phytase and antioxidant activity; increase of free amino acids, γ-aminobutyric acid (GABA), soluble fibers, and total phenols concentrations. Decrease of raffinose and condensed tannins concentrations | [34] |
| Bean, chickpea, grass pea, lentil, pea (local cultivars) | *Lactiplantibacillus plantarum* C48 and *Levilactobacillus brevis* AM7 | Release of lunasin-like polypeptides; inhibition of the proliferation of human adenocarcinoma Caco2 cells | [130] |
| Chickpea | *Lactiplantibacillus 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 | [131] |
| Chickpea | *Weissella confusa Ck15* | Synthesis of linear dextran from sucrose | [132] |
| Chickpea (black chickpea) | *Lactiplantibacillus plantarum* T0A10 | Increase of free amino acids, resistant starch, and protein digestibility; release of bound phenolic compounds; decrease of raffinose, condensed tannins, trypsin inhibitors, and saponins. Decrease of HI, increase of antioxidant potential and overall acceptability of fortified pasta | [133] |
| Chickpea, lentil | *Furfurilactobacillus rossiae* LB5, *Lactiplantibacillus plantarum* 1A7 and *Fructilactobacillus 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 | [54] |
| Cowpea | Spontaneous fermentation | Increase of lysine concentration and essential amino acids concentration | [53] |
| Cowpea, mottled cowpea, speckled kidney bean, small rice bean | Spontaneous fermentation; inoculum with *Lactiplantibacillus plantarum* (WCSF1 and ATCC 149170) and *Lactobacillus paracasei* ASCC 279 | Increase of antioxidant activity | [134, 135] |
| Faba bean | *Lactiplantibacillus 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 | [57] |
| Faba bean (*Vicia faba* var. *major* and *minor*) | Type I sourdough | Increase of free amino acid content and antioxidant activity. Decrease of α-galactosides and condensed tannins concentrations | [136] |

| Legume | Fermentation type | Effects | References |
|---|---|---|---|
| Faba bean (Mediterranean accessions) | *Lactiplantibacillus plantarum* DPPMAB24W | Increase in the concentrations of peptides, free amino acids, and GABA, increase of protein digestibility; decrease of α-galactosides, trypsin inhibitors, condensed tannins, and vicine concentrations | [58] |
| Faba bean (high protein content) | *Leuconostoc citreum TR116* | Improved amino acid profile, increased nitrogen utilization rate and PER of bread; decrease of anti-nutritional compounds and increase antioxidant potential in bread | [137] |
| Faba bean | *Propionibacterium freudenreichii DSM 20271* | Synthesis of vitamin B12 | [138] |
| Faba bean | *Weissella confusa* VTT E–143,403 (E3403) and *Leuconostoc pseudomesenteroides* DSM 20193 | Increase of protein concentration. Increase of viscoelastic behavior, specific volume of bread. Decrease of crumb hardness of bread | [139] |
| Faba bean | *Pediococcus pentosaceus* I02 | Increase of protein digestibility and protein biological indexes. Increase of volume and hardness of bread. Decrease of glycemic index | [140] |
| Faba bean | *Lactiplantibacillus plantarum* DPPMAB24W | Increase of protein digestibility, nutritional indexes, and resistant starch. No detrimental effect on pasta texture and cooking loss | [141] |
| Grass pea | *Lactiplantibacillus plantarum* | Decrease of phytic acid concentration and trypsin inhibitory activity | [52] |
| Lentil | *Lactiplantibacillus plantarum* | Release of potentially bioactive peptides having antioxidant and angiotensin I-converting enzyme (ACE) inhibitory activities | [142] |
| Lentil | *Lactiplantibacillus plantarum* CECT 748 and Savinase® 16L | Release of bioactive peptides showing ACE-inhibitory properties | [143] |
| Lentil (native and sprouted) | *Weissella confusa* SLA4 | Synthesis of dextran from sucrose. Increase of total and soluble fiber content, specific volume and decrease of crumb hardness and staling rate in wheat bread supplemented with 30% of lentil sourdough | [144] |
| Lentil, bean, chickpea, and pea flours, raw and gelatinized | *Lactiplantibacillus plantarum* MRS1 and *Levilactobacillus brevis* MRS4 | Increase of free amino acids and protein digestibility; degradation of phytic acid, condensed tannins and raffinose; decrease of trypsin inhibitory activity and starch hydrolysis index | [31] |
| Lupin | *Latilactobacillus sakei* KTU05–6, *Pediococcus acidilactici* KTU05-7 and *Pediococcus pentosaceus* KTU05–8 | Increase of protein bioavailability and digestibility | [145] |
| Lupin | *Saccharomyces cerevisiae* and *Saccharomyces boulardii* w/o probiotic lactic acid bacteria | Increase of protein content; degradation of anti-nutritional factors (α-galactosides, phitic acid and alkaloids) | [146] |

| Legume | Fermentation type | Effects | References |
|---|---|---|---|
| Mixture of soybean and African breadfruit | Spontaneous fermentation | Increase of protein digestibility and improvement of the sensory properties | [147] |
| Mixture of chickpea/lentil/bean | Type I sourdough | Increase of free amino acid concentration; increase of antioxidant and phytase activities | [32] |
| Mixture of chickpea and pseudo-cereals | *Lactiplantibacillus 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 | [131] |

**Table 2.**
*Main advantages of the LAB fermentation on legume flours and legume-fortified bread.*

technological properties is a key factor in the success of products that go beyond laboratory-scale levels. 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 [32, 128, 148].

Fermentation can further contribute to improving the structural properties of fortified baked goods if exopolysaccharides-producing LAB are selectively employed. Indeed, the replacement of wheat flour (up to 43%) with a faba bean sourdough fermented with *Weissella confusa* strains [132, 139] compensated the gluten dilution and improved bread volume and crumb softness. The gluten-dextran interactions might have strengthened gas cells and, hence, prevented their collapse during proofing and baking [143]. This, combined with water-binding capacity, led to higher loaf volume and softer crumb.

The increase of the antioxidant activity during fermentation was largely documented in legume flours most likely associated with the biotransformation between soluble phenols and the release of bound phenols [31, 34, 132–135, 143]. The bioconversion of phenolic compounds into more available and biologically active forms mainly relies upon acidification and microbial enzymes. In LAB phenolic compounds metabolism comes from the need to detoxify such compounds but also have a role in preserving the cellular energy balance [149–151]. Fermentation of black chickpea with *L. plantarum* T0A10 enabled the release of 20% of bound phenolic compounds and the conversion of free phenolic acids leading to high scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radicals and intense inhibition of linoleic acid peroxidation [133]. Caffeic, coumaric, ferulic, phenyllactic, and 4-hydroxibenzoic acids were found in high amount in faba bean flour subjected to air classification and fermented with *Leuconostoc citreum* TR116, resulting in a bread having better nutritional and technological performances compared with bread obtained with unfermented faba bean [137]. Indeed, phenolic acids are not only appreciated for their potential antioxidant activity after ingestion, they can also be advantageous with regard to the microbial shelf-life of food products [152].

Fermentation can also be used to enhance the content of compounds lacking in vegetable matrices such as vitamin B12. Species of the former *Lactobacillus* genus were found to produce pseudo-vitamin B12, an inactive form for humans, whereas *Propionibacterium freudenreichii* DSM 20271 was effectively used to singly ferment faba bean, soy bean, and lupin flours [138], increasing vitamin B12 content up to 400 ng/g.

The release of bioactive peptides showing *in vitro* activities toward cancer, cardiovascular diseases, oxidative damage, inflammation, hypertension, and high cholesterol [142, 153] is also an appealing trait of lactic acid fermentation. Lunasin is a bioactive peptide (43-amino acid residues) already characterized for anticancer, antioxidant, anti-inflammatory, and cholesterol-lowering functional activities. Lunasin is mainly recovered from soy and used as dietary supplements and for pharmaceutical formulations. The fermentation of different legumes with selected strains of *L. plantarum* and *Lv. brevis* allowed the enrichment of the matrices in lunasin-like polypeptides, released from native proteins in which they are encrypted in nonactive form. Extracts from these legume sourdoughs showed marked inhibition on the proliferation of human adenocarcinoma Caco-2 cells [130]. Fermentation with *L. plantarum* CECT 748 and treatment with a commercial protease of lentil flour led to the release of several antihypertensive peptides showing angiotensin-converting enzyme inhibitory activity (up to 85%) [143].

As an ancient practice, germination of legumes is becoming an emerging process because of the significant enhancement in bioactive components (e.g., vitamins, dietary fibers, peptides and amino acids, and phenols) and palatability. The fortification of baked goods with flours from sprouted legumes has been proposed recently

[154]. During germination, reserves within the storage tissues of the seed undergo hydrolysis in low-molecular-weight compounds and mobilize to support seedling growth [155]. Parameters such as temperature, humidity, steeping (soaking), and length of germination determine the degree of these changes [156]. Nevertheless, the combination of germination and sourdough fermentation seems to better exploit the nutritional modification of grains in terms of protein and starch hydrolysis and mineral solubility [157]. Sprouting and sourdough fermentation with *Furfurilactobacillus rossiae*, *L. plantarum*, and *Fructilactobacillus sanfranciscensis* enhanced the nutritional and functional features of chickpea and lentil by increasing the concentrations of peptides, free amino acids, and GABA.

Fermented sprouted flours were used to make breads with high protein digestibility and low starch availability and appreciable sensory attributes [54]. Germination followed by sourdough fermentation improved the IVPD and enhanced the sensory properties of soybean and African breadfruit seeds [147]. The same occurred for the germinated and fermented cowpea flour, which fortified the bread formula with high lysine content and optimal essential amino acid balance [53]. While more recently, sprouted lentil sourdough, added with 25% sucrose, and fermented with *W. confusa* SLA4, led to the synthesis of dextran up to 9.7% [144]. Wheat bread supplemented with 30% of this sourdough showed increased specific volume and decreased crumb hardness and staling rate, compared with the control wheat bread, as well as increased total and soluble fibers content [144]. Attempts to enhance the nutritional properties of legumes were also made combining gelatinization to fermentation with lactic acid bacteria. Fermentation of gelatinized flours (red and yellow lentils, white and black beans, chickpeas, and peas) with *L. plantarum* MRS1 and *Lv. brevis* MRS4 led to the further degradation of the antinutritional factors (condensed tannins, raffinose, phytic acid, and trypsin inhibitors), increased the protein digestibility, and reduced the starch hydrolysis index [31].

### 6.3 Use of fermented legumes in pasta making

Just like bread, pasta is considered a staple food worldwide with the potential to modulate the diet, and the addition of fermented legumes accounts for a further step toward this goal. Regardless, the biotechnology used for the production, higher content of proteins and fibers, and lower starch content characterize legume-containing pasta. Nonetheless, fermentation contributes to improving not only the nutritional profile, but also the technological features of fortified pasta [158].

Faba bean flour, either raw or fermented (spontaneously or with selected starters), used as dough or freeze-dried material, is among the most reported legume flours in pasta-making [141, 159–161]. The percentage of semolina replacement mostly ranges from 10 to 50% [141, 160, 161], reaching up to 100%, as in the case of gluten-free faba bean pasta described by Rosa-Sibakov and colleagues [159].

Besides the increase in proteins and dietary fibers content, which is directly proportional to the percentage of semolina replacement with both raw and fermented faba bean, as consequence of the proteolysis occurred during fermentation, a higher content of peptides and FAA was observed in pasta containing faba bean fermented by *L. plantarum* DPPMAB24W [141]. The proteolysis occurring during the LAB fermentation also allowed the increase of the protein digestibility. Moreover, essential amino acids (EAAI), biological value (BV), and protein efficiency ratio (PER) indexes increased when 30 and 50% of the semolina was replaced by fermented faba bean flour [141]. The Nutritional Index (NI) of the pasta fortified with 30% of fermented faba bean flour was twofold higher than that of the conventional semolina pasta. This parameter is commonly considered as a global predictor of the protein quality of foods, since qualitative and quantitative factors are included in its

calculation [162]. Replacement level higher than 30% led to the decrease of the NI, as a consequence of a weakening of the gluten network, unable to retain the soluble protein fraction during cooking [141]. The use of fermented faba bean flour as ingredient allowed a marked reduction of the starch hydrolysis index (HI) and, consequently, of the glycemic index (GI) [30, 141, 160]. As previously demonstrated, [163], this decrease can be correlated to the high level of dietary fibers and resistant starch and also to the effect of biological acidification [163].

Experimental pasta was also produced using exclusively fermented faba bean flour [159]. Whereas protein and starch content were similar between fermented and unfermented faba bean pasta (circa 35% and 43%, respectively), RS was found progressively higher in fermented fava bean pasta suggesting the possibility to use fermentation as a mean to decrease GI of commercial gluten-free products [164], usually higher than that of conventional foods [165].

Similar effects to those obtained in pasta fortified with fermented faba bean were obtained when spontaneously fermented pigeon pea (*Cajanus cajan*) (presumably due to LAB growth) was also used in pasta making [166–168]. Compared with semolina pasta, true protein digestibility (TD) and PER markedly improved (6 and 73%, respectively) in pasta fortified with fermented pigeon pea as consequence of the complementarity of amino acids composition deriving from legumes and cereal proteins [167, 168].

A Mediterranean black chickpea flour was fermented with *L. plantarum* T0A10 in semiliquid conditions and used (15% replacement level) to fortify a semolina pasta (116). Fermentation with the selected starter enabled the release of 20% of bound phenolic compounds and the conversion of free compounds into more active forms (dihydrocaffeic and phloretic acid) in the dough. Moreover, fortified cooked pasta, showing scavenging activity against DPPH and ABTS radicals and intense inhibition of linoleic acid peroxidation, was appreciated for its peculiar organoleptic profile [133].

Despite all the nutritional advantages deriving from the use of fermented legumes in pasta making, good sensory and textural properties remain a necessary foundation to achieve products approved by consumers. Differences in sensorial attributes and textural properties between pasta fortified with prefermented ingredients and the conventional one are often perceived unpleasant by trained assessors especially when semolina replacement exceeds 50% [169]. Increased chewiness, sourness, flavor, and off-flavor intensity were observed when fermented faba bean was added to pasta [159], as well as the onset of the red color, as the consequence of Maillard reaction [170]. However, fermentation also showed an important role in the improvement of sensory and textural characteristics of legume flours since it allowed the elimination of beany flavor [171]. Since the balance between flavors and off-flavors often lies in the amount of fortifier added [167], the right compromise between higher nutritional and functional properties and acceptable sensory and rheological ones should be addressed.

## 7. Conclusion and future perspectives

The rising demand for healthier plant-based food lies in the increasing awareness of the adverse risks associated with the consumption of animal proteins as well as the environmental impact animal farming entails. In this evolving agricultural system, legumes play a fundamental role in regard to both the support of good and sustainable agronomical practices and the maintenance of healthier diets.

Apart from their consumption as they are, legumes are the main ingredient of many traditional food products. Nevertheless, their consumption is often limited by antinutritional compounds and poor sensory and technological properties. Recently, the effectiveness of sourdough fermentation-inspired biotechnologies has proved to

be pivotal in improving legumes and legume-based foods acceptability and safety. Through the release of bioactive peptides, phenolic compounds, and soluble fibers or the degradation of antinutritional compounds, fermentation with selected starters proved to be able to improve the nutritional and functional properties of legumes. By synthesizing exopolysaccharides, better rheological properties can be obtained while microbiological safety can be achieved through the degradation of biogenic ammines, mycotoxins, or activity toward spoilage or pathogenic microorganisms.

Fermentation allows overcoming the issues that hold back legumes' potential and intensifies their use as ingredients in innovative formulations of staple foods, such as baked goods and pasta with a more balanced nutritional and functional profile.

The underlining idea behind functional foods is to reduce the prevalence of diet-related diseases by modulating the consumption of commonly eaten foods fortified with high-value ingredients. Fermented legumes fit the profile of such ingredients, but educating consumers on their health benefits, so that they can make an informed choice, is of paramount importance. It is necessary to get rid of the stigma of legumes as "poor man's meat" and recognize their value not only in agricultural practices but also their pivotal role in healthy and sustainable diets. Furthermore, there is growing recognition that changes in nutrition are critical to achieve several of the Sustainable Development Goals developed by the United Nations to promote prosperity while protecting the planet. In order to meet the global food demands, focus should be put into promoting the cultivation and utilization of local or underutilized legume crops often neglected and underexploited, which yet have a great impact on the biodiversity as well as in enhancing food and nutrition security. Whereas, from an academia point of view, those mechanisms, which are still unclear or need more exploiting, behind the advantages of fermentation in terms of biopreservation and safety in general, should be pursued as research topics, since they can further unleash legumes' potential.

## Conflict of interest

The authors declare no conflict of interest.

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