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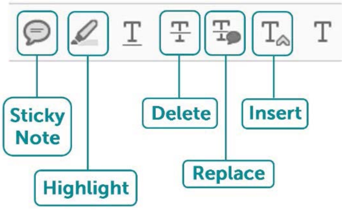

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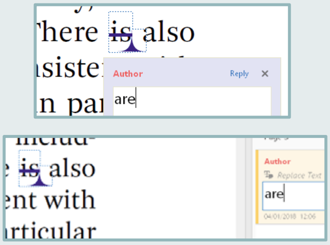

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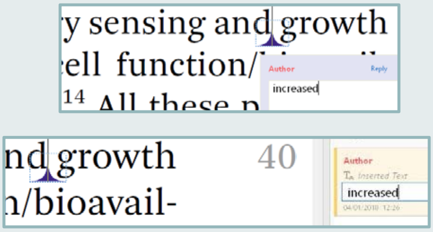

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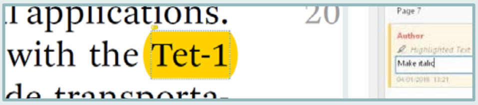

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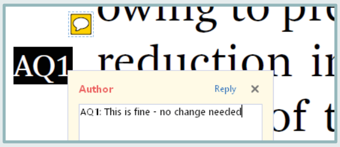

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Abstract

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Fermented foods are one of the indispensable components of the dietary culture of every community and among them fermented legumes have been a part of the human diet for centuries. As a staple food, legumes provide nutrients, vitamins and essentials minerals but also phytochemicals associated with non-nutritive attributes that are of interest for health. The merits of fermented legumes as naturally processed foods that go beyond basic nutrition, preservation and sensory properties are well documented. The microbial growth and the biochemical reactions occurring during fermentation
AQ1 are responsible for the final composition of legume-derived products. The enhancement of their nutritional value, taste and flavour, and the decrease in antinutritional compounds to levels where they can exert positive effects, confer a balanced equilibrium making fermented legume-derived products indispensable for nutrition and health promotion.

CHAPTER 9

Impact of Fermentation on the Nutritional Quality, Bioactive Compounds and Potential Health Properties of Legumes

J. FRIAS*, C. MARTÍNEZ-VILLALUENGA AND E. PEÑAS

Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), Department Characterization, Quality and Safety of Foods, Jose Antonio Novais 10, Madrid, 28040, Spain
*E-mail: frias@ictan.csic.es

9.1 Introduction

Fermented foods are one of the key components of the traditional cuisine of every community in the world with a preponderant position in contemporary food markets.[1] Although fermentation was developed by ancient populations to preserve perishable foods for consumption, the enhancement of the sensory attributes and the variety of nutritive benefits are globally acclaimed.[2] Wine, cheese, yogurt, bread, beers, miso, tempeh and idli are typical fermented foods that remain an important part of diets throughout the world. These foods are not only popular, but they are also considered healthy. Many of these claims revolve around the microbial and biochemical changes that occur during fermentation through positive modifications that

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give rise to nutritive and health benefits.[3] Among the attractive fermented foods that are consumed worldwide, legume-derived fermented food production has emerged as a strategy to achieve food security by providing rich nutrient-dense derivatives with several advantages over other conventional feasible methods of legume processing.[4]

Grain legumes, also called pulses, are considered staple food for a large part of the world population. They have been cultivated for centuries and consumed as part of the basic human diet. Pulses are nutrient-dense foods that provide not only valuable amounts of carbohydrates, proteins, low amounts of lipids (with the exception of soybean in which oil is the main product), fibre, B vitamins, tocopherols and minerals but also bioactive compounds with healthy attributes.[5,6]

The antiquity of fermented legumes has been documented precisely in ancient Chinese literature called *Shi-ji* written by Si-Ma-Qian in S. II.[7] Through the centuries, the transformation of legume dishes around the world has been mostly due to the migration of ethnic Chinese to Japan and other surrounding countries and the acceptance of legumes as a cultural food by non-Asiatic communities. Low-cost, high-value and culturally accepted legume fermented foods are consumed as staple foods in many countries of the globe. They form part of the diet as main course dishes, pastes and accompaniment, seasoning and, sometimes, as meat substitutes for vegetarian or vegan individuals worldwide. Although fermented soy products are widely consumed around the world, soybeans are not considered a legume crop,[8] therefore these products will not be included in this chapter.

A significant proportion of the world legume production is processed by fermentation prior to consumption. As a catabolic process, fermentation causes desirable biochemical changes in legume composition due to the metabolism of the microorganisms involved, and also the activity of hydrolytic endogenous legume enzymes that degrade macro- and micro-components. The microorganisms implicated in fermentation activate their enzymatic machinery to hydrolyse and metabolise legume constituents resulting in the production of valuable derived compounds of nutritive and functional interest. In addition, microorganisms have the ability to produce antimicrobial compounds to preserve fermented food from undesirable microflora and develop desirable organic acids that contribute to the improvement of legume flavour, appearance, texture, nutrient digestibility and nutritional quality as well as health properties.[9] As a consequence of the anabolic process, fermentation brings about a decrease in the non-nutritional components, *i.e.*, legume constituents considered as antinutritional factors in the past. These compounds show different bioactivities when they are consumed in certain doses, as antioxidants, hypolipidemic, hypoglycemic and chemopreventive agents, contributing to the desirable composition of fermented legume products and providing a potential health benefit for consumers.[10] All these features have been attributed to the occurrence of their nutrients and phytochemicals, as well as the presence of alive microorganisms involved in fermentation that can exert probiotic properties.[11] Therefore, fermented legume-derived products are gaining tremendous attention nowadays and they are considered natural, nutritive and healthy foods.[12]

In this context, the aim of this chapter is to collect the most recent and remarkable scientific literature describing the biochemical changes in nutritional and bioactive compounds of legumes that occur during fermentation and to highlight the beneficial effects to health of fermented foods using the available scientific evidence.

9.2 Traditional Fermented Legumes Around the World

Preservation and safeguarding of foods and beverages remain the principal objective of fermentation, with wholesomeness, acceptability and overall quality, having become increasingly valued features to consumers, especially in rural areas where old traditions and cultural particularities in food fermentations are generally well maintained. Fermented legume products have been traditional symbols of cuisines worldwide over the centuries. They have been the result of simple household procedures that generally require very few ingredients and minimal processing. Eventually, fermentation of foods has been developed into a skilled practice offering ~~enhanced~~ nutritional and sensory qualities with ~~health enhancing~~/therapeutic properties.[3]

A large variety of pulses are consumed around the globe as staple foods and part of the world legume production is processed by fermentation prior to consumption. Fermentation makes raw grain legumes into edible attractive products full of protein and energy as staple foods especially for low-income populations. It is usually pursued as a family business that becomes an art handed on from one generation to another. Diversity of legume grains and strains have created a wide variety of ethnic foods with unique sensory and nutritious features. Different countries in Asia, India, Africa and Latin America have traditional names for fermented legume food products, based on preparation and type of microbial strain involved in fermentation, resulting in differences in consistency, flavour, and nutritional attributes, and removal of antinutritional factors. Persistent references to health properties and therapeutic effects of artisan and traditional fermented pulses are gaining significance day by day for their preventative/alleviating effects on various degenerative and chronic diseases.[5,12] The alleged hypotensive and antidiabetic effects of *ugba*[13] a Nigerian traditional fermented food condiment, have gained attention. Similarly, *Dawadawa* from locust bean, *dhokla* and *khaman* from bengal gram or *dosa* and *idli* from black gram are some legume-based fermented foods of different countries whose mode of preparation has been extrapolated to other autochthonous legumes that form an important part of traditional diets.[14] Initially, the traditional manufacture of these fermented products included soaking of the seeds and fermentation initiated by the indigenous microorganisms present in the seed or inoculated during fermentation. Fermented legumes harbour a diversity of microorganisms responsible for their chemical changes and organoleptic properties, mostly mycelial *Rhizopus* ssp moulds, yeasts and bacteria including lactic acid bacteria (LAB) and *Bacillus* ssp.[5] *Tempeh* is one of the fermented legume products that initially originated in Central Java (Indonesia) and spread worldwide over the years. It is a well-known traditional fermented soybean food consumed as a staple source of protein in Asia, however other grain substrates such as chickpea, ground bean, horse gram, mung bean, lima bean and pea are used as a substitute for soybean. Therefore, *tempeh* is the collective name for cooked and fermented beans penetrated and bound together by the mycelium of *Rhizopus* mold that has been traditional in the diets of vegetarians in developing countries and nowadays is part of the diet of large populations around the globe.

In general, microorganisms involved in legume fermentation are responsible in one way or another for (i) dietary enrichment through development of a diversity of flavours, aromas and textures of the legume matrix, (ii) preservation through lactic acid, alcohol, acetic acid and alkaline fermentations, (iii) biological fortification of fermentation derived foods with proteins, essential amino acids, essential fatty acids, vitamins, and minerals, (iv) detoxification by degrading undesirable compounds, (v) enhancement with antioxidant and antimicrobial properties, (vi) probiotic vehicles and (vii) decreasing cooking times and fuel requirements.[14] All of these features have made traditional fermentation-based pulses contributors to the nutrition and well-being status of large population groups around the world, mostly in developing countries, and are having large repercussions in developed ones.[13] Notwithstanding the transcendence in safety and nutrition of traditional fermented legume products, their importance in modern-day life is underlined by the wide spectrum of such foods marketed both in developing and industrialized countries. Fermented legume foods are treasured as major dietary constituents in numerous developing countries because they maintain their quality under ambient conditions – thereby contributing to food security – and because they add value, enhance nutrient-dense quality and digestibility, improve food safety, and are traditionally acceptable and accessible worldwide. Fermentation is a low-input enterprise and provides individuals with limited purchasing power access to safe, inexpensive and nutritious foods, contributing to food security in all corners of the planet.

9.3 Modifications in Legume Composition by Fermentation and Associated Health Benefits

Fermentation is a biological process in which the metabolic activity of the responsible microbiota, in addition to the endogenous enzymatic activity of pulses, can transform their nutritive and non-nutritive compounds resulting in positive benefits for human nutrition and health. Grain legumes are nutritionally attractive due to their high protein content (20% in chickpeas to 40% in lupins), starch (60% in cowpeas to 64% in lentils, and, exceptionally, low in lupins), dietary fiber (11% in cowpeas to 25% in dry peas) and low content of lipids (with the exception of lupins) providing around 300–400 kcal/100 g. They are important sources of minerals (Fe, Ca, Zn, Se), vitamins (mostly water soluble vitamins) and phytochemicals of physiological relevance (such as phenolic compounds or prebiotic oligosaccharides, among others). The whole composition of pulses contributes to their health benefits, such as prevention of chronic diseases including cardiovascular diseases, diabetes, obesity and metabolic syndrome and, even, as chemopreventive agents (see included chapters).

Nevertheless, in the complex composition of grain legumes, there are several compounds, called antinutritional factors in the past due to their detrimental effects, with the dual features of being non-nutritive compounds but conferring healthy properties when they are consumed in certain doses. The processing of legumes before consumption is generally recommended in order to remove those negative compounds that, although minor in content are large in chemical diversity and limit the full nutritive potential of pulses. This group is led by galacto-oligosaccharides (flatulence-causing compounds), protease inhibitors (reduce protein digestibility) and phytic AQ6 acid (limit mineral bioavailability). Additionally, some of these constituents might alter the sensory qualities, contributing to bitterness and astringency (tannins) or texture (dietary fibre may be hard to cook), reducing the acceptance of legume food products.[15]

The positive features in protein quality and composition during lactic-acid fermentation have been well documented. In the production of *idli*, protein digestibility and limiting amino acid scores improved.[16] During the fermentation of black gram (chickpeas), the proteinase activity increased, producing an increase in total nitrogen and available soluble proteins, with an enhanced balance of essential amino acids.[17] Similarly, fermentation of mung bean in a *dosa*-like product resulted in a higher nitrogen, protein and free amino acid content,[18] while kidney bean *tempeh*-like derivative was enriched in protein (+21.7%) and provided more sulphur amino acids (Met + Cys) as well as six other essential amino acids (score = 0.91 *versus* 0.76 in unfermented beans), improving the *in vitro* protein digestibility and the calculated protein efficiency ratio of the fermented pulse product.[19]

Fermentation of lentils by *Lactobacillus plantarum* causes the release of antihypertensive peptides and the biosynthesis of γ-amino butyric acid (GABA),[20] a non-protein amino acid with key physiological functions as an inhibitory neurotransmitter of the central nervous system with parallel actions as a hypotensive, antidiabetic, diuretic and tranquilizer.[21] *Udga* products from Nigeria local kidney beans provide essential amino acids to meet the FAO requirements, improving the nutritional status of a young population.[17] In AQ7 *tempeh*-like foods from common beans, chickpea and Bambara groundnut, higher *in vitro* protein digestibility, total amino acid content, available Lys and protein efficiency ratio were found. At the same time, the levels of the trypsin inhibitors included in the Bowman–Birk class of proteins were largely reduced during fermentation and, hence, the protein quality was enhanced.[22] Our group has led the initiative of reducing trypsin inhibitors during lactic acid fermentation of legumes.[23] LAB provide a complex and active proteolytic system that plays an important role in legume fermentation, degrading protein inhibitors leading to bioactive peptides in different fermented faba bean products.[24] Similarly, fermentation carried out by *Bacillus* ssp. in cowpea, chickpeas and ground beans led to a large decrease in trypsin inhibitors and, hence, an increase in protein digestibility.[25] On the other hand, fermentation-derived products from dry peas, cowpeas and kidney beans with *Saccharomyces cerevisiae* maintained the activity of trypsin inhibitors at more than 50%.[26] These results show the different effect of fermentation conditions on these kinds of proteins, which have to be considered as bioactive compounds with dual effects since recent research has shown the ability of Bowman-Birk proteins to inhibit the growth of different cancer cell lines *in vitro*, and hence increased the interest in this class of protein for use as nutraceutical products.[27,28]

Fermented legumes are a good source of carbohydrates.[29] During natural fermentation, microbial and endogenous amylases are activated to hydrolyze starch. Although hydrolysis of starch during fermentation led to a decrease in amylose and amylopectin, the remaining amount is still difficult to digest. Fermented legumes have shown low values of hydrolysis index and, hence low glycaemic index, an attractive property of legumes for diabetic patients.[30] Hydrolysis of starch during fermentation concomitantly increases reducing sugars that favour the fermentative process[31,32] promoting lactic acid growth and, hence, a drop in pH associated with lactic acid production.[1] An enhanced digestibility of starch has been reported in naturally fermented pulses[31] and in different *tempeh*-like products originating from *Bacillus* ssp.[33] while fermentation with *Rhizopus oligosporus* and *Aspergillus oryzae* led to lower sugar bioavailability.[34,35] In addition, fermented legumes provide larger resistant starch than the unprocessed seeds, contributing to the total content of dietary fiber whose consumption is recommended for intestinal health, and they also enhance satiety, reduce energy intake and control postprandial glycaemia.[29]

Dietary fiber is one of the attractions of fermented pulses. As well as resistant starch, insoluble dietary fiber increased significantly during natural and induced lactic acid fermentation of common kidney beans while minor changes have been reported for the soluble fraction.[36,37] In naturally fermented lentils, a decrease in soluble pectins and hemicelluloses was found while insoluble cellulose and lignin content remained almost constant.[38] Natural fermentation contributed also to drop in total dietary fibre in a bean cake, and this was mostly attributed to the decrease in the soluble fraction.[36,39] The content of dietary fiber is associated with benefits in lipid metabolism, the fiber acting as an efficient lipid lowering compound by trapping fatty acids and bile salts while passing through the small intestine without digestion, thus inhibiting fat absorption in the small intestine.[40] In this sense, *idli* products have been recognized as a dietary fiber source with concomitant cardiovascular disease prevention attributes in India.[41]

Pulses consumed by humans present low lipid content ranging from 2% in lentils and peas to 6% in chickpea. However, most of the 70% of lipids are constituted by polyunsaturated fatty acids (PUFA) of relevant physiological importance such as oleic (18:1), linoleic (18:2) and linolenic (18:3) acids.[42] Different results have been reported regarding lipid composition after legume fermentation. *Tempeh*-like chickpea presented 50% less lipid content than the original flour,[43] however in *tempeh*-like cowpea and canavalia products, lipid content increased slightly but PUFA were mostly represented by linoleic (Ω-3) and linolenic (Ω-6) acids.[44,45] It has been shown through a meta-analysis study that diets rich in these PUFA contribute to lower risk of cardiovascular diseases associated with lower levels of serum lipids and apolipoproteins[46] and thus fermented legumes could contribute to improved heart health status in the world population.

Legumes are recognized to be a source of low molecular weight carbohydrates (2–12%) so called α-galactosides, galacto-oligosaccharides as well as raffinose family oligosaccharides (RFOs) that are strongly associated with the flatulence, an issue that sometimes arises after legume consumption. Such carbohydrates are mostly represented by the trisaccharide raffinose, the tetrasaccharide stachyose and the pentasaccharide. RFOs are non-digestible sugars resistant to the digestive enzymes that pass entirely to the large intestine where they are fermented by colon microbiota producing gasses and, hence, abdominal discomfort. In contrast, α-galactosides exert prebiotic activity stimulating the growth and activity of beneficial intestinal bacteria potentially associated with gut health and wellbeing. Besides these positive effects, the presence of α-galactosides is one of the reasons why people limit their consumption of legumes. Fermentation is clearly one of the most studied processes to reduce the content of α-galactosides to relieve the associated abdominal discomfort and flatulence and, hence, directly convince people to increase their legume consumption.[47] Our group have made a significant contribution in studying the nutritional composition of naturally and controlled lactic acid fermented legumes and it was observed that these processes bring about a sharp reduction in the content of α-galactosides (70–100%) in lentils, kidney beans, cowpeas and pigeon pea.[48–55] As an example, lactic acid fermentation of kidney beans by naturally occurring bacteria and LAB-controlled fermentation by the addition of a commercial starter (a mixture of *Lactobacillus acidophilus*, *Bifidobacterium* and *Streptococcus thermophilus*) led to a significant reduction in galacto-oligosaccharides, but natural fermentation was more effective than induced fermentation in reducing the flatulence-producing factors (95% and 11%, respectively, after 96 h of fermentation).[56] Similar results have been published in *natto*-like cowpeas produced with *Bacillus subtilis*,[57] while the production of *tempeh*-like grass pea by fungal fermentation only reduced α-galactosides partially and it was less effective in the prevention of intestinal cramps.[58]

Fermentation can also reduce the content of phytic acid in legumes, the main phosphorus storage compound in seeds responsible for chelating minerals of physiological relevance such as Ca, Fe and Mg and, therefore, reducing their bioavailability.[55] Phytic acid content of kidney beans underwent a significant decrease of 58% after solid state fermentation.[59] Native phytase enzymes from pulse flour are activated during fermentation and, in addition to those provided by the microorganisms involved, are able to hydrolyse the reactive phosphate groups and thus enhancing the bioavailability of essential minerals. Lactic acid fermentation caused a large reduction of phytic acid in Indian *idli* and *dosa* breakfast and, consequently, the bioaccessibilities of Ca, Fe and Zn were improved.[60] With this premise, the traditional consumption of these staple foods is recommended for Indian children to prevent mineral malnutrition and anaemia among the young populations.[61]

Fermented legume products are a rich source of B group vitamins.[62] *Idli* and *dosa* products provide larger amounts of thiamine, riboflavin, niacin, folic acid and cobalamin than the unfermented black gram flours, and fortification with mung bean led to higher content of these water-soluble vitamins.[63] Similarly, *tempeh*-like Bambara groundnut products contain higher levels of riboflavin, folic acid, niacin and biotin than the raw seeds.[64] In addition, naturally fermented cowpea products are a source of vitamin E, the most active natural liposoluble antioxidant.[55]

Well-known bioactive components produced and made available through fermentation include phenolic compounds, which may act as natural antioxidants.[65,66] In legumes, polyphenols are present in free form and conjugated with other organic acids or sugars groups as soluble phenolics and covalently bound with cell wall macromolecules such as polysaccharides and proteins as bound phenolics.[66] The former can be easily hydrolysed and absorbed in the upper intestinal tract while the latter reach the colon and are fermented and released by the colonic microbiota, exerting versatile health effects.[67] Phenolic solubilization has been described during lactic acid fermentation of chickpeas, faba beans, grass pea, lentils and black bean[68] and an enhancement of phenolic bioavailability by the release of free soluble forms from the esterified bound compounds in pigeon peas, Bambara groundnut, African yam bean and kidney beans has been also reported.[69] Natural and LAB-mediated fermentation significantly enhanced the phenolic content in the soluble and bound fractions of black gram, mottled cowpea, speckled kidney bean, lentil and small runner bean compared with non-fermented seeds. Among them, mottled cowpeas showed the highest phenolic content and their fermentation-derived products contained increased amounts of catechin, quercetin, ferulic acid and *p*-coumaric acid, while protocatechuic acid was identified in both soluble and bound fractions.[70] These authors explained that phenolic compounds in mottled cowpea could be transformed or metabolised differently depending on the naturally present microorganisms and LAB-inoculated strains involved in the fermentation process since they produce different enzymes such as β-glucosidase, decarboxylases, reductases and esterases that can metabolise, transform and release free forms of polyphenols and other products that are more easily absorbed.[71] Similar results were found in naturally fermented lentils where the content of *p*-hydroxybenzoic acid, protocatechuic acid and catechin increased, while hydroxycinnamic acid and procyanidin dimers decreased.[72] Lentils contain large amounts of proanthocyanidins, many of which are catechin polymers and derivatives that throughout the fermentation process could result in an increase in catechin monomers. The release of free shikimic acid, chlorogenic acid, rutin, daidzein, genistein and biochanin A has been also reported in fermented chick peas.[73]

The improvement of legume antioxidant activity due to the fermentation process has been widely reported and it has been mostly attributed to an increase in phenolic compound content.[74] Naturally fermented cowpeas brought about an increased inhibition of lipid oxidation and Trolox equivalent antioxidant capacities that were correlated to the rise observed in total phenolic compounds and vitamin E.[75] Tyrosol, vanillic, *p*-coumaric and ferulic acids increased significantly in fermented cowpeas, highlighting the large amount of quercetin (11–22 $\mu g\ g^{-1}$), a flavonoid with antioxidant implications that was not identified in unprocessed seeds.[74] In this line, fermented chickpea products presented an increased antioxidant capacity which correlated positively ($r = 0.88$, $P \leq 0.05$) with the total phenolic content.[76] Similarly, kidney bean *tempeh*-like products exhibited more antiradical activity (+43%) and antioxidant capacity (+38%) than unfermented seeds, values that were correlated with the large phenolic content.[77] A significant higher free radical scavenging activity and inhibition of lipid oxidation power during fermentation of underutilized varieties of pigeon pea, Bambara groundnut, African yam bean and kidney beans was associated with the content of free soluble phenolics.[69] Similarly, an enhanced antioxidant activity in LAB-mediated fermentation of chickpeas, faba beans, grass pea, lentils and black bean was also correlated with an increase in phenolic solubilisation.[68]

The comprehensive scientific evidences of fermentation-derived legumes and how they may provide health benefits had led to targeted identification of certain vitamins, minerals, amino acids, essential fatty acids, dietary fiber, probiotics and phytochemicals that distinguish fermented legumes from their unfermented forms, as described above. However, only a few recent studies have explored the role of fermentation-derived foods and their ingredients in health promotion such as antihypertensive activity,[20] glucose management,[78] lipid-lowering properties and hepatic activity of antioxidant enzymes,[79,80] chemoprotective agents,[27,81] antiinflammatory and healthy aging[82] and probiotic properties.[83] These findings for fermented legumes substantially advance the existing evidence and identify the consumption of fermented legume-derived products as a dietary strategy to fight non-communicable diseases.

9.4 Fermentation of Legumes is an Ancient Technology for Modern Times

Fermentation as a diversification enterprise offers many opportunities as a result of the global popularity of legumes. Fermented products are part of many social, cultural and consumption patterns. Despite the nutritional benefits of legumes being well recognised, the downward trend in their consumption in developed countries is a matter of concern nowadays. This fact seems to be associated with long cooking time, modern life style, low consumer acceptance of the flavours and textures of legumes, flatulence and adherence of consumers to calorific and ready to eat food products, among others. Nevertheless, the potential health benefits of consuming more pulse foods is challenging and new pulse-based products are being extensively studied.

It is firmly recognized that fermentation can serve as a valuable technology to produce natural, traditional and healthier foods of nutritional interest. Furthermore, in recent years, fermented legumes have emerged with significant strength as functional ingredients to develop new and desirable foods attractive for consumers. Sourdough fermentation has a well-established role in improving texture, flavour and palatability of breads, one of the foods about which consumers around the world are most demanding, and, hence, there is a growing interest in improving its nutritional quality and bioactive compounds profile.[84] Wheat sourdough fermentation and inclusion of legumes carried out with LAB is becoming one of the most efficient tools to improve the quality of breads and their healthiness by the supplementation or replacement of wheat flour with legumes. The sourdough legume fermentation can provide higher protein amounts of better nutritional quality with improved amino acid profile (lysine, glutamic and aspartic acids, among others) compared to cereal grains, the products produced are fibre-rich and gluten-free, retard starch digestion (low glycaemic index products), improve mineral bioavailability and also provide aromatic and pleasant flavours.[85] Beyond the nutritional benefits, legume sourdough can serve as a source of probiotic and bioactive compounds with protective effects against hypertension, cardiovascular diseases, diabetes, overweight, obesity and highly cardiometabolic diseases.[86]

The Italian group headed by Dr M. Gobbetti has performed extensive research work during the last decade exploring the suitability of different species of common and underutilized legumes such as kidney beans, chickpeas, *Lathyrus*, lentils, faba beans, and peas for application in sourdough fermentation with LAB. Besides the mentioned enhancement of the nutritional value, α-galactosides were degraded, pyrimidine glycosides from faba bean almost eliminated, trypsin inhibitor activity reduced, condensed tannins hydrolysed and phytic acid degraded by the action of pulse endogenous and microbial enzymes.[85,87–92] In addition to the reduction of non-nutritive compounds, large amounts of phenolic compounds and soluble fibre were reported.[89] Legume sourdough includes hydrolysed proteins with improved digestibility, bioactive peptides and free amino acids, especially essential amino acids and GABA.[91] As a result of sourdough fermentation of nineteen Italian legumes, Rizzello *et al.*[93] found an increase in the number and intensity of lunasin-like polypeptides as a consequence of the proteolysis of the native proteins by selected LAB. Nine of them exhibited similarity to lunasin, a 43-amino acid peptide with anticancer, anti-inflammatory, antioxidant and cholesterol lowering activities.[94,95]

Fermented legumes have promising potential as functional ingredients. This concept has been fueled by the concern of informed consumers who demand added-value food products providing components with potential health benefits. As health concern is increasing and the functional food industry is growing steadily worldwide, fermented legume ingredients can be tailored to maximize biofunctional activities. Water soluble fractions of fermented lupins, lentils and beans both naturally or by LAB exhibited larger proteolysis and were proven as particularly promising functional ingredients in preventing hypertension.[20,96] Our research group is one of the leading teams showing the effectiveness of the serine-protease enzyme *Savinase*® 16L, a proteolytic subtilisin from *B. subtilis* acting under pH-controlled fermentation conditions, that simultaneously releases multiactive peptides and phenolic compounds from legumes.[97,98] In pinto beans, identified bioactive peptides derived mainly from vicilin, convicilin and legumin showed high dual antioxidant and angiotensin converting enzyme (ACE) inhibitory activity.[97] In addition, the combined esterase and protease activities of *Savinase* released phenolic compounds bound to polysaccharides and proteins, increasing their bioaccessibility in pinto bean.[98]

In lentils, just recently, we have explored the potential of pH-controlled fermentation by LAB combined with *Savinase* as a tailored strategy for the production of multifunctional ingredients containing bioactive peptides and phenolic compounds, both of which are involved in the prevention of ~~MetS~~, and made efforts to elucidate the mechanisms of action by which changes in the biological activities occur. As mentioned previously, natural LAB fermentation reduces the pH to acidic conditions making proteins, peptides and phenolic compounds almost insoluble.[20] Moreover, the proteolytic system of *L. plantarum* does not encode the extracellular protease *prtP*, that is involved in the primary breakdown of proteins into oligopeptides, within the cell envelope proteinases (CEPs).[99] However, LAB are equipped with specific oligopeptide transport (*Opp* and *Dtp*) systems and intracellular proteases that easily degrade oligopeptides to peptides and amino acids that are transported further on into the bacterial cell allowing growth.[100] Therefore, our fermentation strategy was to provide neutral and soft-alkaline conditions to enhance protein solubility (enzymes included) so that, throughout the *Savinase* proteolytic system, an enrichment in peptides in the soluble fraction (ingredient) can be achieved.[101] In addition, the metabolic system of *L. plantarum* produces extracellular carbohydrolases, β-glucosidases and a wide range of esterases which are highly specific and effective in releasing bound phenolic compounds that are cross-linked to cell-wall components in legumes and are also soluble at pH 7–10 [102,103] and, hence, is responsible for the bioaccesibility and enhanced biological activity of the phenolic compounds. The feasibility of these combined fermentation conditions with the addition of *Saviase* enzyme reinforces the proteolytic activity of ~~BAL~~ (with reduced proteolytic activity in comparison with *Bacillus*)[104] providing, at the same time, larger total phenolic content and composition.[105] Our outcomes demonstrated that combined fermentation with *L. plantarum* CECT 748 along with *Savinase*® 16 L in alkaline conditions (FLPS) increased peptide content and some flavonoids as well as phenolic acids like *p*-hydroxybenzoic acid, vanillic acid, isorhamnetin glucuronide and kaempferol glucosides,[98,101] possibly due to microbial enzymatic activities ~~more~~ to exogenous enzyme activity from *Savinase*. These results match with those reported by Khaw *et al.*,[106] in fermented mulberry juice. Free *p*-coumaric acids may be decarboxylated or reduced by *L. plantarum* phenolic acid decarboxylases or reductases to the corresponding phenol or vinyl derivatives that are more active biologically.[107]

Both peptides and released phenolic compounds display antioxidant activities contributing to the delay of different pathologies, including MetS and numerous non-communicable diseases.[79] Lentil ingredients FLPS, obtained by the combined pH-controlled fermentative process with *Savinase*, exhibited antioxidant and chemopreventive effects as radical peroxyl scavengers through hydrogen donating mechanisms attributed to bioaccessible compounds generated during processing.[108] *Savinase* seems to contribute to a greater extent to antioxidant activity of FLPS due to the generation of small peptides able to donate hydrogen atoms[19,97] whilst pH-controlled alkaline fermentation with *L. plantarum* promotes the bioconversion of native phenolic compounds with antioxidant activity.[20,96] The lentil ingredient also exhibited cytoprotective effects in stressful conditions tested on RAW 264.7 macrophages, reducing ROS overproduction induced by ~~*t*-BOOH a~~ (+) catechin, *trans-p*-cumaric acid, and flavonols and flavon glucosides ~~are~~ the main contributors to such activity.[109,110] Xuan *et al.*[111] demonstrated that lentil soluble fraction attenuated angiotensin II-induced cardiomyocytes hypertrophy by decreasing the ROS level, which was attributed to phenolic compounds that provide parallel protection by ROS quenching and activation of the cell antioxidant defense system. In addition to antioxidant activities, lentil FLPS ingredients exhibited antihypertensive effects through the inhibition of angiotensin converting enzyme, an efficient strategy to reduce blood pressure, as well as inhibition of α-glucosidases and pancreatic lipase, with multiple positive health implications related to control of the glycemic index and hyperlipidaemia, respectively.[112–115] These effects were also attributed to bioactive peptides released by *Savinase* hydrolysis, those with specificity for aromatic and hydrophobic residues at position P1 of ACE[99] with low IC_{50} (140 μg mL^{-1}), and *L. plantarum* also contributes to the ACE inhibitory activity through the generation of ACE inhibitory peptides as reported previously.[96,113,116] Peptides released during the FLPS production contribute also to the inhibition of intestinal α-galactosidase enzyme, as do phenolic compounds such and kaempferol and quercetin glycosides previously described are good inhibitors of related enzymes.[110] Simultaneously, it has been observed that individual phenolic compounds are selective inhibitors of mucosal α-galactosidase subunits,[114] as reported for (+) catechin, a phenolic compound identified in FLPS.[108]

AQ19 The composition of the ingredient~~s of FLPS~~ responsible for the bioactivity behind the prevention of cardiometabolic diseases and their identity and characterization justified further studies. For this purpose, fractionation was performed and the most active soluble fraction was screened for its peptide and phenolic profile as well as bioactivity involved in health promotion potential.[108] Fraction F1 was the elective fraction, remarkable for having the highest antioxidant potential, measured as inhibition of ROS generation in stressed RAW 264.7 macrophages and as radical scavengers, as well as the highest ACE, pancreatic lipase and intestinal α-galactosidase inhibitory activities. An exhaustive search to gain insight into the content and composition of this fraction showed a total of 30 peptides with molecular masses within the range of 1100 to 3200 Da derived mainly from the major lentil proteins convicilin, vicilin (allergen Len c 1.0101 and 1.0102) and lectin. Some of them exhibited strong antioxidant and ACE inhibitory activities, as previously observed in hydrolysed lentils and recorded in bioactive peptide databases.[117] Among them, three main peptides (DLAIPVNNPGQLESF, LVNEGKGNLELVGF and ITPEKNPQLQDLDIF) were recently reported to have dual antioxidant and ACE inhibitory activities.[117] Analysing the amino acid sequences, proline, histine and tyrosine were contained within most sequences, and N-terminal and/or C-terminal hydrophobic amino acids were determinants for antioxidant activity. Antioxidant features of histidine residues seem to be due to the hydrogen-donating and radical-trapping mechanisms, whereas tyrosine residues can donate hydrogen to reduce free radicals and hydrophobic amino acids may increase interactions between peptides and fatty acid radicals.[118] FLPS fraction also contained aromatic (phenylalanine), hydrophobic (valine, leucine, glycine, proline, phenylalanine) and positively charged (arginine and histidine) amino acids in the C_t-tripeptide that has been ascribed as the determinant residue for ACE inhibitory activity.[118] Additionally, lentil F1 fraction exhibited α-galactosidase inhibition activity attributed to the presence of peptides whose structure contained hydroxyl or basic side chain amino acids and alanine or methionine at the C_t position,[119] relevant positions found in peptide REQSPGQWRPSHGKEEDEEEKEQKEAQ derived from lentil convicilin.[108] In F1 fractioned from FLPS ingredient, five flavonoids were identified as prodelphinidin dimer, (+)-catechin-3-*O*-hexoside, kaempferol rutinoside-hexoside, isorhamnetin glucoronide and kaempferol rutinoside-rhamnoside (II). The kaempferol rutinoside-hexoside was the most abundant compound ($1\ \mu g\ g^{-1}$) accounting for 58% of the total phenolics. Flavonoids are well known antioxidants that exert biological action through several mechanisms. Lentil flavonoids seem to neutralize radicals and chelate metal ions through hydrogen, electron donating and mixed mechanisms.[110] Kaempferol exhibits protective effects against oxidative stress through activation of nuclear factor erythroid 2 (Nrf2)-mediated defensive response in lung tissues.[120] Additionally, kaempferol glucosides and aglycone have proven to be good inhibitors of intestinal α-glucosidase and pancreatic lipase activities.[110,121] Moreover, prodelfinidin dimers (0.33 $\mu g\ g^{-1}$ in FLPS ingredient) exhibited binding and inhibition activity on pancreatic lipase[122] and (+) catechin (0.25 $\mu g\ g^{-1}$ in FLPS ingredient) inhibited intestinal sucrose-isomaltase more potently than maltose-glucoamylase subunits.[114] All this information retrieved from the literature supports the idea that fermented legume-derived products can be a source of nutritious novel ingredients in the design of healthy functional foods to benefit modern society, contributing to the reduction of highly prevalent diseases and improving quality of life.

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