Lupine (*Lupinus* spp.) proteins: characteristics, safety and food applications

Fatma Boukid[1*], Antonella Pasqualone[2]

[1]Institute of Agriculture and Food Research and Technology (IRTA), Food Safety Programme, Food Industry Area, Finca Camps i Armet s/n, 17121, Monells, Catalonia, Spain

[2]Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Via Amendola, 165/A, 70126 Bari, Italy

*corresponding author: fatma.boukid@irta.cat

Abstract:

Lupines (*Lupinus* spp.) have emerged as a cheap functional food with the advantages of being non-genetically modified crop able to adapt to harsh conditions and low-input farming. Lupines are rich in protein and poor in starch, similar to soy. The factor limiting the use of lupine is the presence of quinolizidine alkaloids especially in bitter species. Nevertheless, modern breeding programs ensured the selection of sweet lupine species with reduced alkaloid content (≤ 0.2 g/kg DM). Numerous techniques have been employed to produce lupine protein isolates, concentrates and hydrolysates. Lupine proteins are rich in bioactive peptides associated with health-related benefits and have reported with interesting techno-functional properties. Lupine protein isolates and concentrates are used mostly for developing healthy foods, while hydrolysates are more applied in nutraceutical and cosmetic industries. Further research is needed to ensure better safety and wider spectrum of application through adequate strategies for allergenicity mitigation and improving techno-functionality.

Keywords: sweet lupine, extraction, health benefits, nutrition, allergenicity

1. Introduction

The genus *Lupinus*, belonging to the legume family *Fabaceae*, includes almost 300 species. Traditional lupine species have bitter taste because of high content of alkaloids (from 1 to 3% of alkaloids in the dry weight of their seeds), where lupanine is the main alkaloid together with other minor alkaloids (*e.g.,* albine, hydroxylupanine, sparteine, anagyrine, lupinine, and angustifolin. High content of quinolizidine alkaloids is associated with severe intoxication (*e.g.,* trembling, shaking, excitation, and convulsion). EFSA considered the available data on the occurrence and consumption of these compounds still insufficient to accurately assess the risk of chronic exposure [1, 2]. Apart from their toxicity, alkaloids have pharmacological benefits such as alternative antibacterial and antifungal agents [3] and agronomic benefits by contributing in the defense function against predatory herbivores and microorganisms [4]. To ensure their safety, prior to use, bitter lupines traditionally go through successive soaking and cooking, able to reduce the content of alkaloids [5, 6].

In the twenties', modern breeding programs focused on selecting lupine varieties with low-alkaloid content to ensure the transition from the "wild bitter" to "cultivated sweet" lupine variety [7]. The mean total alkaloid content of sweet lupine seed ranges from 0.3 to 0.5% of alkaloids in the dry weight of their seeds, depending on the species, geography, and climate. Sweet lupines demonstrated high suitability for low input agriculture owing to their high adaptability to temperate and cold climates, low-fertile soils, harsh conditions and high nitrogen fixation ability [7–9]. For these reasons, sweet lupines are gaining traction as a more sustainable and non-genetically modified alternative to soy [8, 9]. The most economically important sweet lupines species are: *Lupinus albus* (white lupin), *L. angustifolius* (blue or narrow-leafed lupin), *L. luteus* (yellow lupin) and *L. mutabilis* (Pearl lupine or Tarwi) [8, 10, 11]. The maximum limit of alkaloids was fixed to not exceed 200 mg/kg by the Health Authorities of several countries [1, 2]. The four sweet species have alkaloid levels below the critical value (200 mg/kg for lupine -based foodstuffs, and thus are not toxic to humans [12]. As summarized in Table 1, the first three species of lupine (white lupine , blue lupine and yellow lupine) are originated from Mediterranean region and represent the majority of lupines cultivated worldwide [7]. *Lupinus albus* L., is grown in southern Europe and South America and it is widely used in the food industry compared to other species thanks to its white color [13]. *Lupinus angustifolius* L. is cultivated in central and eastern Europe, Australia, and New Zealand.. *Lupinus luteus* L. is mainly cultivated in the Mediterranean region and has shallow soil requirements (having less than 50 cm depth of solum). Its cultivated accessions have variable seed yields in Mediterranean environments [14]. On the other hand, Andean lupine (*Lupinus mutabilis* Sweet), also known as *chocho* or *tarwi,* is a native species domesticated by indigenous people of the Andean region of South America, where they use it as a food, feed and natural medicine [11]. Although these species are sweet, due to the high variability in

alkaloids contents depending on variety, species and environmental conditions, a debittering phase is required to ensure their safety for human consumption. Conventional and emerging pretreatments were applied to remove the bitterness of lupine such as soaking, cooking, fermentation, and ultrasound [15].

Lupine crop has been recognized as a rich source of proteins similar to soy, where *Lupinus mutabilis sweet* had higher values than Mediterranean species (Table 1) [11, 16, 17]. Mediterranean species were characterized by high crude fiber (21-40 g/100 g of dry matter) [16, 17]. All lupines have low starch content (<10% of dry matter) similar to soy, and provide an average energy of 309 kcal/ 100g [18]. Besides macronutrients, lupine seeds are rich in polyphenols, carotenoids and phytosterols providing several health benefits [7, 10, 19]. Therefore, lupine seeds and flours are widely used in food systems as a nutritious plant protein source since this species has similar content to that of soy and higher than peas [10, 16]. Nevertheless, the incorporation of lupine flours above 10% induce detrimental effects on the food quality (compact structure and hard texture particularly in bakery products as well as dark color and bitterness) [20, 21]. Various techniques have been employed to isolate lupine proteins to be used as functional ingredient thereby avoiding the negative effects of fiber and oligosaccharides on quality [10].

Table 1

Lupine protein isolates and concentrates showed interesting physical and functional properties (e.g. solubility, water and oil absorption, emulsifying capacity, foaming capacity and gelation capacity), which make them valuable ingredients for food and beverage industry [10, 22, 23]. Besides food industry, the global demand for sweet lupine proteins is increasing due to its various applications in nutraceutical, cosmetic and feed applications [13]. Geographically, North America (United States, Canada and Mexico) holds the largest share followed by Europe (Germany, UK, France, Italy, Russia and Turkey), Asia-Pacific (China, Japan, Korea, India, Australia, Indonesia, Thailand, Philippines, Malaysia and Vietnam), South America (Brazil, Argentina and Columbia), and Middle East and Africa (Saudi Arabia, UAE, Egypt, Nigeria and South Africa) for the forecast 2020-2026 [24]. In the light of these considerations, this review addresses the processing technologies applied to extract lupine protein ingredients (isolates, concentrates and hydrolysates), the characteristics of these ingredients and their application in human nutrition.

2.2. Lupine proteins production

2.3. Pretreatment

Although breeding sweet lupine drastically reduced the amount of alkaloids, several approaches are applied to ensure further reduction to levels far below the maximal level allowed by international regulations (≤ 0.2 g/kg DM) [19]. Given the relevant interaction between the genetic and environmental factors, the content of alkaloids can vary, and therefore, sweet lupines can require debittering to ensure their safety for human consumption.

Traditional debittering of bitter lupine was based on the solubility properties of alkaloids in aqueous solutions [25]. The alkaloid level in bitter lupines (0.05–4 g/kg) can be easily decreased (less than 200 ppm) by successive washes with water (at room temperature or hot water) [26]. This process ensured the removal of 95% of total alkaloids. Nevertheless, this method requires large water quantity (almost 62 times the weight of the dry lupine) and long processing time (up to 6 days) [27]. As well, it results in relevant loss of total solids (around 22% loss of soluble proteins, minerals, flavonoids, monosaccharides and sucrose, fat and carbohydrates), and can deteriorate the microbiological quality of the product due to long processing time [27]. To reduce water and time, different solvents have been used such as ethanol, hexane or supercritical CO_2 but these treatments were mostly carried out at laboratory scale [28]. Compared to aqueous treatment, saline debittering -in water with 0.5% (w/v) sodium chloride is less time and water consuming. In particular, water treatment requires 10 h hydration, 10 h cooking, and 73 h washing, whereas saline debittering can be done with 8 h hydration, 1 h cooking and 49 h washing [6]. Combining fermentation (using *Rhizopus oligosporus* at 28 °C for 4 days) with thermal-aqueous debittering (hydration at 80 °C for 10 h; cooking in water at 91 °C for 1 h and washing) reduced alkaloids and enhanced the nutritional value as well as reduced water and processing time [29]. Likewise, combining soaking and germination improved nutrients availability and protein digestibility and reduced processing time by 32 h compared to traditional processing [30]. The fermentation (using *Rhizopus oligosporus*) of germinated lupine flour further decreased antinutrients and increased the phenolic contents and antioxidant potential [31]. Non-thermal treatment, such as ultrasound, reduced alkaloids (81% less than control) depending on sweet lupine species [25]. Freezing-cooling cycles were also used for lupine cell disruption by ice crystals prior to aqueous treatment to facilitate the leaching of alkaloids [15].

Dehulling can be a dry or wet process. Dry dehulling consists in removing the hulls from the kernel using a mechanical dehuller or in separating milled whole flour in hulls and refined flour using a vacuum separator or a sieve; whereas wet dehulling follows soaking in water to loosen the hulls [16, 32]. Dry dehulling decreases the levels of several antinutrients such as phytic acid, trypsin inhibitors, tannins, raffinose, and stachyose [32]. However, wet dehulling was more efficient in reducing alkaloids since they will be leached in the soaking water [33]. Generally, dehulled seeds are ground or flaked and subsequently defatted with organic solvents, like hexane or petroleum ether [26]. The solvent can be eliminated by drying under controlled temperature or by extraction with supercritical CO_2 [34].

2.4. Isolation and concentration

Alkaline extraction (alkali solubilization and isoelectric precipitation extraction) is the most used method for lupine protein isolation and it is generally preceded by a defatting [25]. Defatted lupine flour is suspended in alkaline solution (pH 8.0–8.5 with a diluted NaOH solution). After centrifugation, the pellet is discharged, and the supernatant is precipitated under acid conditions (pH 4.5 with a diluted HCl solution) [35]. After centrifugation, the pellet contains conglutins α, β and δ. The supernatant is subjected to selective precipitation with Zn^{2+} or filtration at pH 7–8 to recover conglutin γ [20]. Alternatively, proteins collected after centrifugation can be reconcentrated by ultrafiltration followed by diafiltration resulting in protein concentrates (73% crude protein per dry matter, DM) [34]. Alkaline extraction, however, enables the recovery of proteins isolates with about 90% crude protein (DM). Beside protein isolation, the alkali-based extraction can further reduce residual alkaloids [25]. However, such treatment requires high energy and water consumption together with the use of chemicals [12].

Dry milling followed by air classification produces lupine protein concentrates with protein content from 35% to 43% [15, 36]. Prior defatting increases the protein purity of protein rich fraction and reaches 57% protein. As an alternative to air classification or as an additional post-treatment, electrostatic separation increases the purity of air-classified lupine fraction (59% protein) [15, 36]. Compared to alkaline extraction, dry fractionation resulted in lower purity, but it is a milder and more sustainable method for lupine proteins concentration [12].

2.5. Enzymatic hydrolysis

Following alkaline extraction, protein isolate can be subjected to enzymatic hydrolysis for several reasons including producing bioactive peptides, enhancing the techno-functionality and reducing allergenicity [37–39]. As a main drawback, protein hydrolysis can increase bitterness compared to isolates due to the release of bitter peptides [38]. Enzymatic hydrolysis can be carried out using different enzymes, mainly alcalase (protease from *Bacillus licheniformis*) and flavourzyme (protease from *Aspergillus oryzae*) have been reportedly used [37].

3. Lupine proteins characteristics

3.1. Structure

3.2. Lupine proteins consist mainly of albumins and globulins with a quantitative ratio of 1/9; and a minor fractions, including prolamins [40]. Albumins are a diverse group of functional proteins, mainly metabolic enzymes having biochemical functions linked to plant cells [8, 41]. Globulins are

high-molecular-weight storage proteins. Based on their electrophoretic mobility, globulins can subdivided into α-conglutin (35–37% of the total globulins), β-conglutin (44–45%), γ-conglutin (4–5%) and δ-conglutin (10–12%) [17]. Furthermore, α and β-conglutin of lupine resemble to legumins and vicilins as reported for other legume crops with some relevant differences in the glycosylation and the proteolysis of polypeptides [42]. Both γ -conglutin and δ-conglutin are rich in sulphur-containing amino acids [20]. **Amino acids profile**

Table 2 summarizes the amino acid composition of sweet lupine species in comparison with peas and soy. As well, the recommended levels of amino acids for human for single amino acids and total essential amino acids were reported [43]. Lupine species show one of the highest protein levels among legumes and an excellent amino acid profile, with a comparable amino acid score to that of soy protein and higher than peas. However, the amounts of essential amino acids in lupine species were slightly lower than soy, particularly threonine, valine and tryptophan. Nevertheless, lupine species show important amounts of leucine and lysine, particularly *Lupinus mutabilis sweet.* All lupine species are deficient in threonine and valine and do not reach the WHO/FAO/UNU requirements. tryptophan and lysine in *Lupinus luteus* show amounts that correspond to FAO/WHO requirements, similar to soy, and higher than lupine species and peas. *Lupinus luteus* shows the highest values of essential amino acids compared to other lupines. Overall, total essential amino acids of lupines are below the 36 g/16 gN recommended by FAO/WHO, computed based on nine amino acids (Lys, Met, Cys, Thr, Ile, Trp, Val, Leu and His).

Table 2

3.3. Health benefits

The consumption of lupine proteins has been reported by numerous studies to be associated with several health benefits (hypoglycemic, cholesterol-lowering, anti-oxidative, and prevention towards cardiovascular diseases) [44, 45]. Nevertheless, the mechanism of action is not completely understood yet.

Hypoglycemic effects of lupine proteins were reported by numerous studies [46, 47]. Lupine (*Lupinus albus* L.) proteins can decrease blood glucose and improve insulin sensitivity through the inhibition of dipeptidyl peptidase IV enzymatic activity in diabetic and insulin resistance-induced rats [46, 48]. Especially γ-conglutin (from *Lupinus mutabilis* L. and *L. angustifolius*) decreased serum glucose concentrations and hepatic neoglucogenic glucose-6-phosphatase (G6pc) gene expression, and increased insulin gene expression at mRNA level [44, 47]. Furthermore, γ-conglutin regulated *Slc2a2* gene expression in liver and normalized GLUT2 protein content in pancreas of streptozotocin-induced rats [44]. Oral administration of

γ-conglutin also reduced the glycemic peak in healthy rats subjected to glucose overloading [49]. Furthermore, β-conglutin from *L. angustifolius* increased mRNA and protein levels of genes involved in insulin modulation in the case of patients with type 2 diabetes [50]. A recent study revealed that lupine (*L. angustifolius*) hydrolysate contributes in the inhibition of Gαq mediated signal transduction (Gαq protein/phospholipase C/protein kinase C) in β-cells and the stimulation of insulin secretion [51]. Likely, lupine proteins increase intracellular Ca^{2+} and decrease K^{+} permeability, resulting in a glucose-dependent insulinotropic effect [52]. Specific bioactive peptides including LTFPGSAED, LILPKHSDAD and GQEQSHQDEGVIVR were reported to be effective in regulating insulin and glucose metabolism through the inhibition of Dipeptidyl peptidase IV [45]. Thus, lupine hydrolysates may have potential nutraceutical use in type 2 diabetes or incorporated into various foods to reduce glycemic load [51, 53].

Lupine proteins exhibited cholesterol-lowering effects. Lupine protein concentrates and isolates (30 g/day of protein) were reported effective in modulating plasma low-density lipoproteins cholesterol and reducing the inflammatory marker "high-sensitivity C-reactive protein" (hs-CRP) in hypercholesterolemic subjects [54, 55]. Noteworthy, high doses of lupine proteins (30 g/day of protein) used in clinical studies can hardly be consumed under normal physiological conditions [55]. As an alternative, proteins hydrolysates (obtained from *Lupinus albus* or *Lupinus angustifolius*) efficiently reduced plasma total, very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) cholesterol concentrations in human cells (Sirtori et al., 2004). These results suggested that lupine proteins can contribute in lowering blood pressure and reducing the risk toward cardiovascular diseases in individuals with high hypercholesterolemia [57]. This activity may be linked to the inhibition of the activity of angiotensin converting enzyme (ACE) by peptides generated through cleavage of lupine protein [39, 58]. Lupines peptides also improved the low density lipoprotein receptor protein levels via SREBP-1 activation, leading to a better ability of HepG2 cells to uptake extracellular low density lipoproteins [59, 60]. Hypocholesterolemic peptides obtained by enzymatic hydrolysis were identified such as YDFYPSSTKDQQS via the inhibition of 3-hydroxy-3-methylglutaryl CoA reductase (HMGCoAR) and the modulation of cholesterol metabolism in HepG2 cells [59, 60].

Anti-oxidation properties of lupine proteins are gaining interest due to the increasing focus on finding novel antioxidant compounds for protecting against oxidative stress and reducing the impact of various chronic diseases [61, 62]. Lupine protein hydrolysates (obtained via proteolysis using alcalase, trypsin and pepsin) demonstrated higher antioxidant and angiotensin converting enzyme inhibitory activities compared to lupine proteins [61, 63]. The fraction with low molecular weight (MW< 3 kDa) had the highest antioxidant and ACE inhibitory activities compared to other fractions (MW 3–10 kDa) and (MW < 3 kDa), which was associated with increased activities of super oxide dismutase and glutathione peroxidase [61]. The most active hydrolysates were obtained by hydrolyzing lupine α and β conglutin [39]. Peptides containing the

sequences LLPH and PHY showed important angiotensin converting enzyme inhibitory activity and antioxidant properties [64]. More studies are required to understand the promising potential of lupine peptides as functional ingredient to develop healthy foods [61]. Lupine protein hydrolysates can exert anti-inflammatory effects on both cell-free *in vitro* systems and cultured macrophages derived from THP-1 human monocytic cell line [62]. A patented peptide (GPETAFLR) (Patent number WO 2016051000A1), was identified as an anti-inflammatory agent in THP-1 derived macrophages [65]. Lupine protein hydrolysates were found to decrease the inflammatory response and improve the oxidative status in human peripheral lymphocytes [62].

3.4. Allergenicity and mitigation strategies

Lupine sensitivity is an emerging food allergy, more prevalent in Mediterranean countries and Australia, where lupine is more consumed, and less in North America and Northern Europe (Sanz et al., 2010). Allergic reactions to lupine proteins were reported either as primary lupine allergy or because of cross-reactivity to other legumes (e.g. soybean, pea, lentil, chickpea) or/ and peanut [38, 66]. The prevalence of sensitization and allergic reactions to lupine in the general population is still unknown, while it was estimated in Europe to be around 0.3-8% [67]. Lupine allergy has been observed in 15–20% of individuals who have already peanut allergy [68]. In 1994, lupine allergy was reported for the first time in the case of 5-year-old girl with peanut sensitivity after the ingestion of spaghetti-like pasta fortified with sweet lupine seed flour [69]. In 2004, the European Food Safety Authority (EFSA) recognized lupine as a food allergen underlining the severity of lupine allergy and its cross-reactivity with peanut [70]. In 2006, lupine and products thereof were included in European regulations (Directive 2000/13/ EC) as a food whose presence must be declared on the food labelling [71]. In 2007, the European Union Regulation No. 1169/2011 included lupine in the list of products causing allergies or intolerances that must be declared on food labels [72].

The increasing prevalence of lupine allergy as a consequence to the functional characteristics of a growing number of sweet lupine-derived foods consumption makes the imperious necessity to identify the lupine proteins involved in allergy reactions [67]. Most of the allergenic proteins of lupine belong to globulin particularly α- and β-conglutins, with a lesser presence of γ- and δ-conglutins [73]. β-conglutin was reported as the major lupine allergen (Lup 1) by the International Union of Immunological Societies [74]. Also, α-conglutin (Lup-2) was reported highly allergenic and involved in cross-reactivity [75]. Lup-1 and Lup-2 bound specific epitopes (Ara h1 and Ara h3) of other legumes [66, 76], while Ara h2 cross-reacted with δ-conglutins [77].

Several approaches have been used to mitigate the allergenic potential of lupine proteins. Enzymatic hydrolysis of globulin of lupine using pepsin and trypsin lead to epitopes degradation thereby immune-reactivity weakening [78]. A recent study confirmed alcalase, papain, and pepsin were the most effective proteases in the degradation of the α- and β-conglutin [38]. Thermal treatments [boiling (up to 60 min), autoclaving (121 °C, 1.18 atm, up to 20 min and 138 °C, 2.56 atm, up to 30 min), microwave heating (30 min), and extrusion cooking] were also tested to mitigate lupine flour allergenicity [79]. Results showed an important reduction in allergenicity after autoclaving (138 °C for 20 min) and absence of IgE-binding after prolonged autoclaving (138 °C for 30 min) [79]. However, high-pressure homogenization, thermal treatment and mechanical process did not completely mitigate the allergenic potential of epitopes deriving from α-, β-, and δ-conglutin [80]. This suggests that lupine allergens have variable resistance towards processing (biological, thermal, and mechanical) and more research are required to produce stable hypoallergenic lupine proteins, not excluding breeding strategies.

3.5. Techno-functionality

Lupine protein techno-functionality (solubility, water/oil absorption capacity, emulsification, foaming and gelation concentration) is strongly influenced by the isolation procedure (e.g. solvent, temperature, pH value and NaCl concentrations) [23, 81]. Compared to isolates, lupine protein concentrates have low foaming capacity, low viscosity, but high emulsification capacity (particularly at low pH) [82]. Lupine protein isolates have higher solubility values than soy protein isolate and a similar emulsification capacity and satisfactory foaming activity [10]. Nevertheless, lupine protein isolate form weaker, deformable and less consistent gels compared to soy protein isolates [83, 84]. Batista et al. (2005) associated the gelling ability of lupine protein isolates to their resistance to thermal unfolding. Compared to soy protein, lupine proteins form a weaker gel due to higher resistance to thermal treatment, related to higher tendency to intramolecular crosslinking rather than intermolecular bonding. The thermal stability of lupine protein could offer opportunities to develop protein rich foods with low final viscosity [83]. Gelling properties also varied based on the species of lupine, where white lupine proteins resulted in stronger gels compared to those deriving from blue lupine [10].

Different post-processing strategies (chemical, enzymatic and physical) were applied to improve and to tailor the functional properties of lupine proteins [23, 35]. Enzymatic hydrolysis of lupine protein isolates improved solubility, emulsifying, and foaming activity [38]. Noteworthy, these changes were almost independent of the enzyme preparation used [85]. Lactic fermentation and extrusion of lupine proteins increased soluble protein water absorption capacity and solubility, and changed surface protein hydrophobicity, but worsened emulsifying properties [86]. High-pressure homogenization enhanced gel

strength and the emulsifying capacities of proteins deriving from *L. albus* L; whereas the functional properties of *L. angustifolius* did not change [22].

The mechanisms of improving functional properties of proteins are not fully understood, where more insights on the association between protein fractions and functional properties are required. Although fractionation processes enable to obtain ingredients with specific properties, they are energy and time consuming and require the use of chemicals [8, 87]. As an alternative, ultrafiltration showed great potential in reducing processing time and enhancing lupine functionality such as separating fractions with high foaming capacities [88]. Lupine protein fractionations have been related to specific properties, where type E (rich in α- and β-conglutins) has good emulsification properties, while type F (rich δ-conglutins) has good solubility and foaming properties [23, 35]. Fractionation also enables to obtain fraction with interesting solubility, foaming and emulsifying capacities and staabilities [8]. The effect of the drying on protein functionality depends on the drying method and on the type of protein. Spray drying leads to thermal damage of lupine protein isolates [89], while freeze-drying leads to the formation of large thermally stable protein particles without affecting the functionality [87]. Overall, understanding the behavior of lupine proteins in association with processing conditions can enable the tailoring of their functional properties to meet specific requirements of food products [8, 23].

4. Food applications

The supplementation of food products with lupine protein ingredients can be a powerful approach for improving their nutritional value through increasing protein content and delivering the health benefits associated with related bioactive peptides [62, 90]. The consumption of pasta enriched with γ-conglutin increased satiety of rats fed, while pasta enriched with α, β and δ-conglutin limited the body weight increase in rats. In addition, a reduction in glycaemia was recorded following glucose overloading; especially after the intake of the γ-conglutin concentrate supplemented pasta (45 mg per kg body weight) [90]. From a technological standpoint, spaghetti fortified with 5% of lupine protein isolate have color attributes, rheological properties and cooking loss comparable with the control spaghetti (100% semolina). Nevertheless, at higher level of addition (15%, 20% and 50%), the dough becomes very weak (low stability and development time, extensibility and resistance) resulting in high cooking loss (17% in 20% addition level versus to 8% for 5% addition level). Likely the high inclusion of protein isolate resulted in gluten dilution thereby weakening the overall structure of the spaghetti [91].

In bread, the addition of lupine protein isolates (5%) increased the dough development time and stability due to lupine protein entrapment within gluten network as well as improved the volume, internal structure and texture of the breads [9]. However, incorporating 10% lupine protein isolates resulted in less resistant, less manageable and stickier dough compared to the control [92, 93]. As a result, the fresh bread made with lupine proteins (10%) had lower specific volume and higher crumb firmness due to the dilution of gluten and mechanical disruption of the gluten network structure by the lupine particles [9, 92]. The crumb alveoli were also smaller and heterogeneous compared to the control, which can be attributed to high water absorption of lupine protein resulting in the reduction of the generated steam during baking [9]. Regarding the volatile profile, bread enriched with lupine proteins was characterized by "green/vegetable-like", "earthy/mushroom-like", "fatty" and "roasted" notes predominated, mainly due to the oxidative degradation of fatty acids or thermal reactions [94]. Even though lupine proteins increased yellowness, it negatively impacted the odor and the texture resulting in low sensory scores [92, 93]. After storage (3 days), enriched breads (with 10% lupine protein) had more moisturized crumbs since lupine proteins retain more water than gluten [93]. The color of stored bread remained stable in breads made with lupine isolates whilst it faded in the control [93]. It was found that lupine isolates had an anti-staling effect and delayed bread firming [9].

Lupine protein was applied for developing protein rich gluten-containing and gluten-free cookies. The incorporation of lupine protein (10% addition level) resulted in golden-brown cookies and did not affect the shape parameters of the cookies industry [95]. During storage, these cookies maintained low water activity/content suggesting a potential anti-staling effect, as previously reported in bread [9]. Hence, the addition of lupine proteins improved the final product quality which aligns with the trends in the food industry [95].

Lupine proteins contributed to the stabilization of fat particles and reduced cooking loss in meat products owing to their emulsifying properties [96]. Additionally, these proteins strengthened the structure of meat products (*e.g.* patties, frankfurters and sausages) through increasing the viscosity and reducing the jelly separation due to their gel-forming ability, and reduced the lipid oxidation during storage due to their inhibitory effects [96–98]. The color also was improved and more stable in fresh and stored products than the control [97]. Overall acceptability of products enriched with up to 2% lupine proteins scored similar to the control, while those made with 3% were judged unacceptable due to the low scores of odor and taste [97, 98]. Incorporating 1% of lupine isolates improved the processing characteristics, color, texture and overall acceptability of meat products [96].

Preliminary formulation of yogurt using lupine proteins was performed due to the promising techno-functional properties of these proteins such solubility and emulsification [99]. As a result, a yogurt alternative enriched with lupine isolates (2%) showed acceptable rheological (apparent viscosity, hysteresis loop area, flow point, elastic, viscous and complex modulus), textural properties (firmness, consistency, cohesiveness and index of viscosity) and low tendency to syneresis [99]. During lactic acid fermentation, lupine concentrates give the possibility to reduce the fermentation time [100]. These products (enriched with 10% lupine protein concentrate) were perceived as having homogeneous consistency, sour-sweet taste and fruity smell, without negative flavor [100]. Noteworthy, lupine isolates performance is closely related to thermal treatment conditions and the lactic acid bacteria used for the fermentations [99, 101]. Lupine protein enriched yogurts can be classified as low-calorie dietary foods (energy <70 kcal per 100 g). However, the use of lupine flour or hydrolysates resulted in low structural and sensory qualities [102, 103]. Yogurt with 5% soy protein was characterized by dark color, firm and dense texture as well as low digestibility [104, 105]. Thus, lupine proteins can be a promising alternative for soy protein to produce soy-free and lactose-free yogurts and other dairy products. For instance, dairy-free ice cream enriched with 1.5% lupine proteins is available in the market since 2015 [106].

5. Conclusion and future perspective

Global population growth poses challenges to sustainable development. Legumes, such as lupine, are increasingly being explored as alternatives to animal protein, being adaptable to harsh conditions and low-input farming. Lupine species show one of the highest protein levels among legumes and an excellent amino acid profile, with a comparable amino acid score to that of soy protein and higher than peas. Lupine proteins have interesting techno-functional properties and can be effectively used for reformulating several types of food, such as pasta, bread, cookies, meat products and yogurt, improving their nutritional value. In addition, lupine proteins are rich in bioactive peptides associated with health-related benefits, such as hypoglycemic, cholesterol-lowering, and anti-oxidative effect, as well as prevention towards cardiovascular diseases.

Several methods are available for preparing protein isolates, concentrates, and hydrolysates, however further research is needed to improve sustainability of the majority of these processes, particularly in terms of water consumption. Moreover, lupine sensitivity is an emerging food allergy, and biotechnological approaches have been considered to mitigate allergenicity of lupine proteins. There is, however, still work to be done to further reduce allergenicity by breeding strategies.

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Conflict of interest

None.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

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720 **Table 1:** General characteristics of sweet lupine species compared to pea and soy

Latin name	***Lupinus albus***	***Lupinus angustifolius***	***Lupinus luteus***	***Lupinus mutabilis sweet***	***Pisum sativum***	***Vicia faba* L.**	***Glycine max***
Common name	White lupine	Blue lupine	Lupine beans, annual yellow-lupine, European yellow lupine or yellow lupine	"lupino", "tarwi", "chocho"	Yellow peas	Faba bean	Soy
Origin	Mediterranean basin	North Africa	Southern Europe	Latin America	Southwestern Asia	Iran	Southeast Asia
Color	White	Blue	Yellow	Pearly white to black	Yellow	Pale yellow	Yellow
Proteins [g/100 g DM]	35.1-37.6	29.5-35.6	44.7-48.2	32–53	23.2-25.2	25–35	47.8-52.1
Crude fiber [g/100 g DM]	28.4-32.5	36.7-40.1	21-34.33	8.3-9.4	9.1-18.9	11-18	13.6-23.6
Fat [g/100 g DM]	10.4-12.6	5.5-8.6	4.5-6	13–25	2.4-12.1	1.1-3.2	15–25
Ash [g/100 g DM]	4.4-5.1	3.4-4.2	4.3-5.1	4.80-5	2.8-3.2	2.7-3.4	6.3-7
Starch [g/100 g DM]	2.8-3.27	4.6-5.5	4-4.5	2-3	46-49	30-35	10
References	[16, 27, 107, 108]	[16, 83, 107, 109][16, 107, 109–111][16, 107, 109–111][16, 107, 109–111]	[16, 107–110]	[11, 27, 112]	[16, 107, 113, 114]	[115, 116]	[16, 107, 117]

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722 **Table 2:** Amino acid profile of sweet lupines, yellow pea, faba bean and soy proteins (g AA per 16 g N)

Amino acids	***Lupinus albus***	***Lupinus angustifolius***	***Lupinus luteus***	***Lupinus mutabilis sweet***	***Pisum sativum***	***Vicia faba* L.**	***Glycine max***	**FAO/WHO requirements**
Aspartic acid	10.52	10.35	12.8	10.36	11.10	11.51	10.3	
Threonine	3.65	3.76	3.52	3.61	3.80	4.11	4.70	4
Serine	4.63	4.05	6.54	4.04	4.30	6.54	5.10	
Glutamic acid	21.66	21.20	24.58	22.45	16.60	15.13	16.2	
Proline	4.38	4.43	4.77	4.23	3.50	5.52	4.60	
Glycine	4.07	4.65	4.58	4.25	4.40	7.16	3.40	
Alanine	3.45	3.89	4.30	3.73	4.40	5.58	3.60	
Cysteine	1.74	1.77	2.88	1.46	1.40	1.21	1.80	
Valine	4.35	4.56	3.78	4.32	4.90	5.93	4.80	5
Methionine	0.76	0.80	0.57	0.73	1.00	0.67	1.80	
Isoleucine	4.71	4.56	6.22	4.82	4.40	4.81	4.30	4
Leucine	7.74	7.52	10.08	6.87	7.20	8.34	6.70	7
Tyrosine	4.32	3.59	2.32	4.20	3.60	2.96	3.60	
Phenylalanine	4.07	4.22	4.40	3.97	4.90	4.95	4.80	
Lysine	5.02	5.62	3.80	5.93	7.30	5.83	5.70	5.5
Histidine	2.38	2.91	3.80	2.95	2.40	2.48	2.50	
Arginine	10.86	10.56	18.40	10.67	8.50	7.81	6.90	
Tryptophan	0.76	1.06	0.60	0.97	0.81	0.30	1.30	1
Total essential amino acids	31.11	32.56	35.25	31.66	33.21	37.42	33.60	36
Amino Acid Score	0.96	0.99	0.98	0.84	0.74	0.68	1.00	
References	[25]	[25]	[118]	[25, 107]	[119, 120]	[121, 122]	[119, 120]	[43]

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