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Lupinus angustifolius protein isolates: chemical composition, functional properties and protein characterization

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Abstract

Two types of protein isolates were prepared from *Lupinus angustifolius* defatted flour by alkaline extraction, with (Isolate B) and without (Isolate A) sodium sulphite, and acid precipitation of proteins at the isoelectric point (IEP 4.3). Chemical composition, main functional properties and protein composition of *L. angustifolius* defatted flour and protein isolates were determined. Isolate A and B have 93.9 and 84.6% protein content, respectively, and had a balanced composition of essential amino acids, with respect to the FAO pattern except for lysine. The in vitro protein digestibility ranged between 86.3 and 93.9% for isolate A and B, respectively.

Keywords: *Lupinus angustifolius*; Protein isolates; Chemical composition; Functional properties; Protein digestibility

1. Introduction

Legumes constitute a large family of plants, many of them cultivated, such as bean (*Phaseolus vulgaris*), chick-pea (*Cicer arietinum*), lentil (*Lens culinaris*), soya bean (*Glycine* max), pea (*Pisum sativum*), or lupin (*Lupinus* sp.). Nutritional value of these plants is related to the high protein, mineral and vitamin content of the seeds (Cubero & Moreno, 1983; Hebblethwaite, 1983).

Legumes are consumed in the majority of mediterranean countries as an essential component of their diet, due to agricultural, economic and nutritional reasons (Periago, Ros, Martínez, & Rincón, 1992). Among legumes, the use of lupin in human foods and livestock is limited, mainly due to the presence of toxic alkaloids in the seed. In this sense, the production of lupin protein isolates may overcome this problem because alkaloids are water soluble and removed during the preparation of protein isolates (Millán, Alaiz, Hernández-Pinzón, Sánchez, & Bautista, 1995).

In this sense, the production of plant protein isolates is of growing interest to industry because of the increasing application of plant proteins in food and non food markets. The use of plant protein isolates in foods as functional ingredients, to improve the nutritional quality of the products or for economic reasons, is in increase (Sánchez-Vioque, Clemente, Vioque, Bautista, & Millán, 1999; Zayas, 1997).

Technology of preparation of protein isolates include the solubilization of proteins in alkaline media (pH 10–12) and their subsequent precipitation at the isoelectic point (IEP). In this way, proteins are purified from non protein substances such as sugars, fibre, lipids and other non desirable components in the final product (Fernandez-Quintela, Larralde, Macarulla, Marcos, & Martínez, 1993; Kolar, Richert, Decker, Steinke, & Vander Zanden, 1985).

Most studies in lupin as a food source have been centred on the uses of the grain and flour. In this regard a few studies have been done on lupin protein isolates, mainly in *Lupinus luteus* (Sousa, Morgan, Mitchell, Harding, & Hill, 1996) and *Lupinus albus* (Kiosseoglou, Doxastakis, Alevisopoulos, & Kasapis, 1999). Although studies have been done in *Lupinus angustifolius*, these are centred on fibre, sugars and alkaloids compositions (Cheetham, Cheung, & Evans, 1993; Johnson & Gray, 1993; Takagi, Suzuki, & Saito, 1994).

In the present work, we describe the preparation of two different types of protein isolates from *Lupinus angustifolius* seeds. The chemical composition, functional properties and protein composition have been

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determined and discussed in relation to the possible use of the protein isolates in the food industry.

2. Materials and methods

2.1. Materials

Lupinus angustifolius seeds were a gift from Koipesol Semillas, SA (Sevilla, Spain). The seeds were ground and extracted with hexane in a soxhlet extractor for 9 h to remove the fat. The resulting defatted lupin flour was used as the starting material. Trypsin, α-chymotrypsin, peptidase, protease, amiloglucosidase and α-amylase were purchased from Sigma (St. Louis, Missouri, USA). All other reagents were of analytical grade.

2.2. Analytical methods

Moisture and ash contents were determined using AOAC (1999) 945.39 and 942.05 approved methods, respectively. Total nitrogen was determined by the microKjeldahl method according to AOAC (1999) 960.52 approved method. Crude protein content was estimated using a conversion factor of 5.8. Total fibre was determined according to the procedure described by Lee, Prosky, and De Vries (1992). Lipids associated with the flour and protein isolates were extracted and measured following the method of Nash, Eldridge, and Woolf (1967). Soluble sugars and polyphenols were measured using standard curves of glucose (Dubois, Gilles, Hamilton, Reber, & Smith, 1956) and chlorogenic acid (Moores, Demott, & Wood, 1948), respectively.

2.3. In vitro protein digestibility

Samples containing 62.5 mg of protein were suspended in 10 ml of water and the pH adjusted to 8. An enzymatic solution containing 1.6 mg of trypsin (17.7 N-benzoyl-L-Arginine Ethyl Ester Units/mg), 3.1 mg of α-chymotrypsin (43 U mg^{-1}) and 1.3 mg of peptidase (50 U g^{-1}) per ml was added to the protein suspension in a 1:10 v/v ratio. The pH of the mixture was measured after 10 min and the in vitro digestibility calculated as a percentage of digestible protein using the equation: digestible protein = 210.464−18.103×pH (Hsu, Vavak, Satterlee, & Miller, 1977)

2.4. Determination of the IEP

For the determination of the IEP, 15 g of lupin flour were extracted twice with 300 ml of 0.2% NaOH pH 12 solution and centrifuged at 8000×***g*** for 20 min. Aliquots (40 ml) of the supernatant were titrated with 0.5 N HCl to various pH values, ranging from 2.0 to 6.5. The precipitate formed was separated by centrifugation as earlier. The percentages of nitrogen in the supernatants in relation to the total nitrogen extracted were plotted versus pH to determine the IEP.

2.5. Preparation of protein isolates

2.5.1. Isolate A

Lupin flour (20 g) was suspended in 200 ml of 0.2% NaOH solution pH 12, and extracted by stirring for 1 h. After centrifugation at 8000×***g*** and recovering of the supernatant, three additional extractions were carried out with half of the volume of alkaline solution. The supernatants were pooled and analyzed for nitrogen content. The pellet was dried in an oven at 50 °C, weighed and analyzed for nitrogen content. The pH of the soluble proteins was adjusted to the IEP (pH 4.3) and the precipitate formed was recovered by centrifugation at 8000×***g***. The precipitate was washed with distilled water adjusted to pH 4.3 and freeze-dried.

2.6. Isolate B

Lupin flour (20 g) was extracted as earlier but with 0.25% Na_2SO_3 at pH 10.5 to avoid the darkening of the final product. The precipitate obtained at the IEP was successively washed with 100 ml of distilled water adjusted to pH 4.3, ethanol and acetone, and dried at room temperature.

2.7. Gel filtration chromatography

Lyophilized samples (1 g) were dissolved in 10 ml of 0.1 M sodium borate, 0.2 M sodium chloride buffer pH 8.3. Gel filtration was carried out in a Fast Protein Liquid Chromatography (FPLC) system equipped with a Superose 12 HR 10/30 column from Amersham Pharmacia LKB Biotechnology. Volume injection and concentration of the samples were 200 μl and 1.6 mg of protein per ml, respectively. The eluent was the earlier mentioned borate buffer at a flow rate of 0.4 ml min^{-1}. Protein elution was monitored at 280 nm. The approximate molecular masses were determined using blue dextran 2000 (2000 kDa), β-amylase (200 kDa), bovine serum albumin (67 kDa) and ribonuclease A (13.7 kDa) as molecular weight standards. With these standards, the resulting calibration curve is V/Vo = −0.78 Log (MW) + 5.66.

2.8. Amino acid analysis

Samples containing 2 mg of protein were hydrolyzed with 6 N HCl at 110 °C for 24 h and derivatized with diethyl ethoxymethylenemalonate. Amino acids were determined by reversed-phase high-performance liquid chromatography (RP-HPLC) using D, L-α-aminobutyric acid as internal standard (Alaiz, Navarro, Giron, & Vioque, 1992). The HPLC apparatus (Waters) consisted of a Model 600E multi-solvent delivery system, a

Wisp Model 712 automatic injector and a 484 UV–Vis detector. Data acquisition and processing were effected with Maxima 820 3.3 version software (Waters). Separations were attained with a 300×3.9 mm i.d. reversed phase column (Nova Pack C_{18}, 4 μm, Waters) using a binary gradient system with 25 mM sodium acetate pH 6.0 (A) and acetonitrile (B). The solvent was delivered to the column at a flow rate of 0.9 ml/min as follows: time 0.0–3.0 min, linear gradient from A:B (91:9) to A:B (86:14); 3.0–13.0 min, elution with A:B (86:14); 13.0–30.0 min, linear gradient from A:B (86:14) to A:B (69:31); 30.0–35.0 min, elution with A:B (69:31). The column was maintained at 18 °C by a temperature controller. Amino acid composition was expressed as grams of amino acid per 100 g of protein.

Tryptophan was determined after basic hydrolysis of proteins according to Aitken and Learmonth (1996).

2.9. Protease inhibitor activity

Porcine trypsin and chymotrypsin inhibitors activity of lupin flour and protein isolates were determined by assaying trypsin and chymotrypsin activities in the presence and absence of lupin flour and protein isolates using casein as the substrate for the proteases as described by Batra, Vasishta, and Dhindsa (1986) with modifications. The assay mixture contains 1 ml of phosphate buffer (0.1 M, pH 7.6), 0.5 ml protease solution (1 mg/ml 1 mN HCl), 0.5 ml 1 mN HCl, 2 ml of casein solution (2% in phosphate buffer, 0.1 M, pH 7.6), and 1 ml of inhibitory solution in phosphate buffer (0.1 M, pH 7.6). Before addition of the substrate, the mixture was incubated for 30 min to allow binding of the inhibitors to the proteases. Casein was added and the reaction incubated for 20 min at 37 °C. A blank set was prepared by adding trichloroacetic acid before the addition of the protease solution. A control set was also prepared in which inhibitory solution was deleted from the assay mixture. The reaction was stopped by addition of 6 ml of 5% trichloroacetic acid. The samples were filtered through Whatman No. 1 filter paper, and the released tyrosine was determined in an aliquot of the filtrate according to the method of Lowry, Rosebrough, Farr, and Randall (1951). The inhibitory activity was determined by subtracting the amount of tyrosine released in the experimental set from that in the control set. Percentage of inhibition was calculated by comparing the reduction in enzyme activity on the addition of inhibitory solution with that given by the same enzyme concentration in the absence of any inhibitor.

2.10. Determination of functional properties

2.10.1. Water absorption

It was determined using the method of Sosulski (1962). Samples (3 g) were mixed with 25 ml of water and stirred six times during 1 min at 10 min intervals. The mixture was centrifuged at 1000×***g*** for 25 min. The supernatant was removed and the pellet was dried at 50 °C for 25 min and weighed. The water absorption capacity was expressed as the number of grams of water retained by 100 g of material at pH 7.

2.10.2. Fat absorption

For the determination of fat absorption the method of Lin, Humbert, and Sosulski (1974) was used. Samples (0.5 g) were mixed with 6 ml of canola oil. After 30 min the mixture was centrifuged at 1600×***g*** for 25 min and the volume of the supernatant was weighed. The oil absorption capacity was expressed as the number of grams of oil retained by 100 g of material at pH 7.

2.10.3. Gelation

The least gelation concentration was determined by the method of Sathe, Deshpande, and Salunkhe (1981). Test tube containing suspensions of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20% (w/v) material in 5 ml distilled water were heated for 1 h in boiling water followed by cooling in ice and further cooling for 2 h at 4 °C. The least gelation concentration was the one at which the sample did not fall down or slip when the test tube was inverted at pH 7.

2.10.4. Emulsion activity and stability

The emulsion activity and stability were determined according to Naczk, Diosady, and Rubin (1985) with modifications. Sample (3.5 g) was homogenized for 30 s in 50 ml water using a model A Polytron homogenizer (Brinkmann, Wesbury, NY) at setting six (approximately 10,000 rpm). Canola oil (25 ml) was added, and the mixture was homogenized again for 30 s. Then another 25 ml of canola oil were added, and the mixture homogenized for 90 s. The emulsion was divided evenly into two 50 ml centrifuge tubes and centrifuged at 1100×***g*** for 5 min. Emulsifying activity was calculated by dividing the volume of the emulsified layer by the volume of emulsion before centrifugation×100 at pH 7. The emulsion stability was determined using the samples prepared for measurement of emulsifying activity. They were heated for 15 min at 85 °C, cooled and divided evenly into two 50 ml centrifuge tubes and centrifuged at 1100×***g*** for 5 min. The emulsion stability was expressed as the percentage of emulsifying activity remaining after heating at pH 7.

2.10.5. Foaming activity and stability

The activity and stability of foam were determinated by the method of Lin et al. (1974). Fifty millilitres of a 3% dispersion of material in distilled water were homogenized using a model A Polytron homogenizer at a setting of six (approximately 10,000 rpm). The mixture was immediately transferred into a 250 ml graduated

cylinder and the foam volume measured. The foaming activity was expressed as the percentage of volume increase at pH 7. Foam stability was expressed as foam volume remaining after 20, 40, 60 and 120 min at pH 7.

2.10.6. Protein solubility

Protein solubility was measured according to Morr et al. (1985). The samples (250 mg) were homogenized in 20 ml 0.1 M NaCl pH 7 during 1 h. The samples were centrifuged at 20,000×***g*** for 30 min and the nitrogen content determined in the soluble fractions. Solubility was expressed as the percentage of total nitrogen of the original sample that was present in the soluble fraction.

3. Results and discussion

3.1. Preparation of Lupinus angustifolius *protein isolates*

The yield of protein alkaline extraction ranged around 90%, being higher in isolate A (97.3%) than B (88.9%). This is probably due to the higher pH used in the protein extraction of isolate A (pH 12 versus pH 10.5). Higher pH, although more efficient in extracting the proteins, should be avoided due to the possibility of disruption of the protein structure and degradation of certain amino acids (Cerletti, Fumagalli, & Venturin, 1978; Sgarbieri, Antonia, & Galeazzi, 1978). Thus, protein integrity of isolate B is higher because the extraction conditions used are milder. Besides sodium sulfite used inhibits polyphenol oxidation and thus limits reactions between proteins and oxidized polyphenols, that are responsible of the colour of protein isolates obtained without sodium sulfite (Cater, Gheyasuddin, & Mattil, 1972; Gonçalves, Vioque, Clemente, Sánchez-Vioque, Bautista, & Millán, 1997).

After alkaline extraction proteins were precipitated at their isoelectric point. In this sense solubility of *Lupinus angustifolius* proteins was minimum at pH 4.3 (Fig. 1) as in chickpea (Sánchez-Vioque et al., 1999) and similar to cowpea (Rotimi & Rickey, 1993) and rapeseed IEP (pH 4.5; Gonçalves et al., 1997). The percentage of proteins recovered after precipitation at the IEP was 79.9 and 72.2% for isolates A and B respectively (Table 1). Soluble proteins remaining after IEP precipitation are essentially albumins (Berot & Davin, 1996).

3.2. Chemical composition of L. angustifolius *protein isolates*

Main components of the deffated flour are proteins (32%) and fibre (37.6%) (Table 2). Similar amounts of protein have been reported by Musquiz, Burbano, Rey, and Cassinello (1989) for *Lupinus hispanicus* seeds.

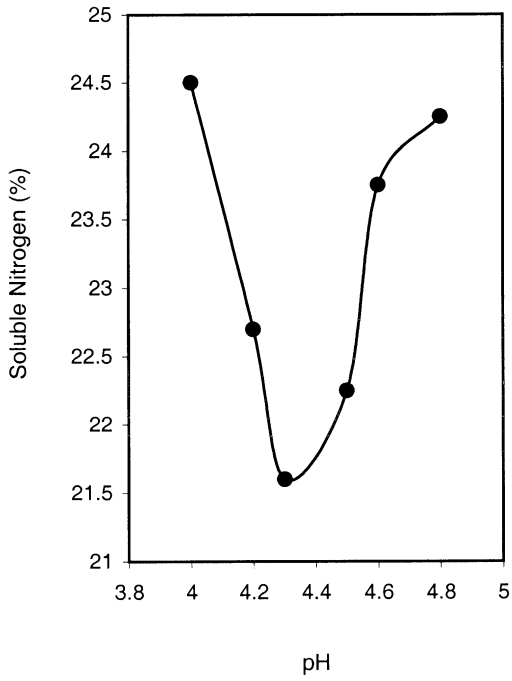

Fig. 1. Solubility curve for lupin proteins. Protein solubility is expressed as percentages of soluble nitrogen at various pH values.

Higher amounts of protein have been observed in other varieties of *Lupinus angustifolius* such as Unicrop with 37.4%, Borre with 36.4% (Oomah & Bushuk, 1983), or *Lupinus mutabilis* with 44.6% protein content (Millán et al., 1995). On the other hand, fibre content of *Lupinus angustifolius* seeds was very high compare with *Lupinus hispanicus* (17.2%) (Muzquiz et al., 1989), and Unicrop variety with 14.6% (Oomah & Bushuk, 1983). In protein isolates a decrease in fibre content and an increase of protein content in isolate A (93.9%) and B (84.6%) is observed. Also, a reduction of the contents of other components is observed in isolates with respect to the flour (Table 2).

Although the flour was defatted with hexane, lipids are not totally eliminated (Table 2), remaining in part associated with protein which affects the flavour (Rackis, Sessa, & Honing, 1979), and nutritional value of protein isolates (Kikugawa, Ido, & Mikami, 1981).

Polyphenol contents in the defatted flour and protein isolate were below 0.1%. In this sense low levels of polyphenols are desirable from a functional and nutritional viewpoint. The alkaline extraction allows removal of undesirable compounds in the protein isolate (fibre, sugars, polyphenols, lipids, alkaloids) and the preparation of a protein isolate for high-quality protein products. With this method Gonçalves et al. (1997) have eliminated, from rapeseed flour, more than 90% of undesirable compounds in the protein isolate.

Table 1
Nitrogen balance during the process of protein isolate obtention

Extractions	Isolate A			Isolate B		
	EN[a]	INIEP[b]	SNIEP[c]	EN	INIEP	SNIEP
First	76.7±1.8	63.0±1.5	13.7±0.3	67.7±2.3	56.0±1.8	11.7±0.5
Second	15.5±0.8	13.6±0.8	1.9±0.1	15.0±0.2	11.4±0.1	3.6±0.1
Third	3.8±2.0	2.7±1.4	1.1±0.6	5.4±1.4	4.4±1.3	1.0±0.1
Fourth	1.3±0.2	0.5±0.2	0.8±0.0	0.8±0.0	0.4±0.0	0.4±0.0
Total	97.3±4.8	79.9±3.8	17.4±1.0	88.9±3.9	72.2±3.2	16.7±0.7

Data are expressed as percentage and are the mean±S.D. of three analyses.
[a] Extracted nitrogen.
[b] Insoluble nitrogen after isoelectric point precipitation.
[c] Soluble nitrogen after isoelectric point precipitation.

3.3. Protein digestibility

Seed protein digestibility is limited by the presence of trypsin and chymotrypsin inhibitors, and the globular structure of proteins. The removal of protease inhibitors in protein isolates, increases the in vitro protein digestibility of isolate A (86.3%) and B (93.9%) with respect to the lupin flour (80%; Table 2). A 66% and 69% reduction in the activity of trypsin inhibitor has been observed in isolate A and B respectively in relation to the flour. Besides, lupin proteins are partially denatured during the preparation of the isolates being more accessible to digestive enzymes, and improving the hydrolysis (Lynch, Rha, & Catsinpoolas, 1977). In vitro protein digestibility of lupin protein isolates was similar to the observed in rapeseed (90%; Gonçalves et al., 1997) and chickpea protein isolates (90–94%; Sánchez-Vioque et al., 1999).

3.4. Functional properties of defatted flour and protein isolates

Functional properties are shown in Table 3. Protein isolates A and B showed higher solubility, water absorption and oil absorption capacity than the flour. However, emulsion and foaming properties of flour and protein isolates were similar. Thus, isolate B is more appropriate to be used in foods in which fat retention is desirable, such as meat and dairy products, whereas isolates A could be suitable in foods such as frankfurters or creams.

The least gelation concentrations of protein isolate A and B were respectively, 12 and 10% (w/v; Table 3), but lupin flour presents lower gelation concentration with 6%. In this sense, the gelation is not only a function of protein quantity but seems also to be related to the type of protein as well as to non-protein components, as suggested by Sathe et al. (1981), and Tjahjadi, Lin, and Breene (1988).

Table 2
Chemical composition of lupin flour (LF) protein isolate A (IA) and B (IB)

	LF	IA	IB
Lipids	13.6±2.0	3.2±0.5	1.0±0.0
Moisture	7.9±0.6	3.4±1.5	9.4±1.1
Ash	2.1±0.1	0.7±0.0	0.3±0.0
Protein content[a]	33.8±6.9	87.4±0.2	83.9±2.5
Polyphenols	>0.1	>0.1	>0.1
Fibre	39.9±6.1	4.0±0.3	4.8±0.3
Soluble sugars	2.7±0.1	1.3±0.1	0.6±0.1
In vitro protein digestibility[b]	80.0±0.2	86.3±1.1	93.9±4.7
Trypsin inhibitors activity[c]	28.1±1.4	9.4±0.4	8.6±0.2

Data are the mean±S.D. of three analyses.
[a] Total nitrogen×5.8.
[b] Grams of protein digested/100 g of protein.
[c] Percentage of inhibition.

Table 3
Functional properties of lupin flour (LF) protein isolate A (IA) and B (IB)

	LF	IA	IB
Solubility[a]	13.1±2.1	19.2±0.6	33.8±1.0
Gelation (%)[b]	6	12	10
Water absorption[c]	235.7±1.7	446.7±2.3	383.3±3.6
Fat absorption[d]	153.5±4.5	195.0±7.0	306.0±14
Emulsion capacity (%)	74.0±3.1	74.5±0.6	69.1±0.9
Emulsion stability (%)	69.4±4.1	71.0±1.5	66.7±0.8
Foaming capacity (%)	114.0±2.0	119.0±1.0	116.0±8.0
Foaming stability (%)			
at 20 min	96.3±1.4	97.8±1.2	96.5±1.3
at 40 min	93.8±2.7	96.8±2.0	95.3±1.8
at 60 min	92.5±2.3	96.0±2.0	94.3±2.4
at 120 min	90.3±1.1	94.8±3.2	93.3±3.0

Data expressed as g/100 g protein
[a] Percentage of soluble nitrogen in 0.1 M NaCl pH 7.
[b] Grams of material per 100 ml of water.
[c] Grams of water absorbed per 100 g of sample.
[d] Grams of fat absorbed per 100 g of sample.

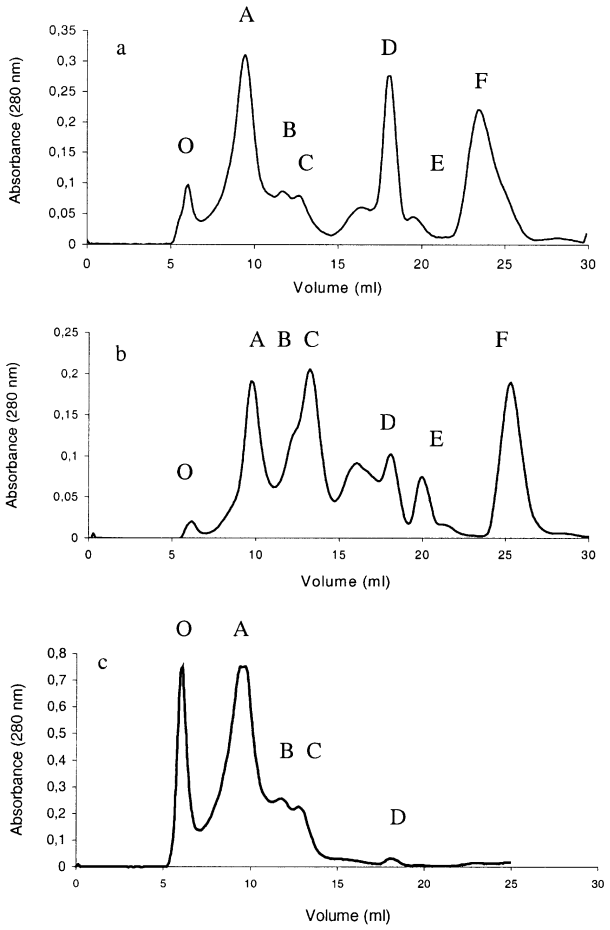

Fig. 2. (a) Gel filtration chromatography of protein extracted from (a) defatted flour (b) protein isolate A and (c) protein isolate B.

Table 4
Amino acid composition of lupin flour (LF) and protein isolate A (IA) and B (IB)

	FAO[a]	LF	IA	IB
Aspartic acid[b]		11.6	6.9	11.8
Glutamic acid[c]	1.9	25.6	19.9	25.8
Serine		7.1	7.7	6.8
Histidine	3.4	2.7	2.7	2.3
Glycine		4.6	5.3	4.2
Threonine		4.9	6.4	4.2
Arginine		11.5	13.5	12.2
Alanine		3.8	3.0	3.4
Proline		4.6	8.8	5.1
Tyrosine	6.3[d]	5.9	7.5	6.5
Valine	3.5	3.9	4.8	4.0
Methionine	2.5[e]	1.3	0.7	0.7
Cysteine		3.5	2.9	3.3
Isoleucine	2.8	5.5	5.9	5.6
Leucine	6.6	8.7	9.4	9.2
Phenylalanine		5.2	6.1	5.3
Lysine	5.8	5.4	4.3	5.2
Tryptophan		0.6	0.6	0.6

Data expressed as g/100 g protein
[a] FAO/WHO/ONU. Energy and protein requirement, 1985.
[b] Aspartic acid + asparagine.
[c] Glutamic acid + glutamine.
[d] Tyrosine + phenylalanine.
[e] Methionine + cysteine.

3.5. Protein composition of defatted flour and isolates

On gel filtration chromatography, lupin protein flour was fractionated into seven main fractions (Fig. 2a). Fraction O corresponds to aggregation or polymerization of proteins. Fraction A was the main one and corresponds to trimers of conglutin-α and to conglutin-β with approximately 180 kDa molecular weight. Fractions B and C correspond probably to other subunits of conglutin-α and conglutin-β (Cerletti et al., 1978). Fraction D with 3.5 kDa molecular weight corresponds to peptides and small proteins, and fraction F are probably secondary compounds such as phenols that are eliminated with ethanol in isolate B. Protein profiles of isolates A and B on gel filtration were different (Fig. 2b and c). Isolate B showed an enrichment in fraction A as was also observed in the flour (Fig. 2a). In isolate A, fraction A was not the major one but C with 29 kDa molecular weight that corresponds to other subunits of conglutin-α. The different pH values used in the preparation of isolates is probably the reason of the differences observed between both gel filtration profiles and the degree of dissociation of conglutin-α that may also explain the discrepance observed in the functional properties between both isolates (Guegen, Chevalier, Barbot, & Schaeffer, 1988).

The amino acid composition of defatted flour and protein isolates satisfied the FAO requirements for the essential amino acids (FAO/WHO/ONU, 1985) except for lysine (Table 4). Also, a loss of the content of sulphur amino acids in isolates is observed, probably due to the elimination of albumins, that are rich in lysine, cysteine and methionine (Sánchez-Vioque et al., 1999).

The earlier results show that it is possible to obtain, from lupin, protein isolates of acceptable nutritional value with a high protein digestibility, and low content of undesirable substances such as lipids, sugars, polyphenols and fibre. Main proteins of protein isolates correspond to globulins. The extraction of lupin seed proteins at different pH values produce protein isolates with different functional properties. This difference may be explained by the different polymeric state of conglutin-α in both isolates. Isolate B with white colour, could be appropriate to lighten the colour in a wide variety of foods and with high fat absorption, could be suitable for the preparation of cheese and meat products. Isolate A could be suitable in foods such as frankfurters or creams.

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