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Composition and Functional Properties of *Lupinus campestris* Protein Isolates[1]

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Abstract. Protein isolates from *L. campestris* and soybean seeds were prepared using isoelectric precipitation (PI) and micellization (MI) procedures. The amount of protein recovered was considerably higher with the isoelectric precipitation than with the micellization procedure (60% and 30%, respectively). Protein contents were higher than 90% in protein isolates. Antinutritional factors content (alkaloids, lectins, and tannins) were reduced to innocuous levels after protein isolate preparation. Minimum protein solubility for the precipitated lupin protein isolate (LPI) was at pH 4.0, and between pH 4 and 6 for the micellized lupin protein isolate (LMI), increasing at both extremes of the pH scale. Water absorption for the LMI was 1.3 ml/g of protein and its oil absorption 2.2 ml/g of protein. The LPI had 1.7 ml/g of protein in both water and oil absorption. Foaming capacity and stability was pH-dependent. Foaming capacity was higher at pH 2 and lower near the protein isoelectric points. Minimum protein concentration for gelation in LMI was 8% w/v at pH 4, while for LPI was 6% at pH 4 and 6. Amino acid composition in *L. campestris* flour and protein isolates was high in lysine and low in methionine. Most of the essential amino acids in lupin protein isolates were at acceptable levels compared to a reference pattern for infants and adults. The electrophoretic pattern of both protein isolates showed three bands with different mobilities, suggesting that the protein fractions belong to α-conglutin (11S-like protein), β-conglutin (7S-like protein) and γ-conglutin. It is proven that some of the functional properties of *L. campestris* protein isolates are similar to those soybean protein isolates recovered under equal conditions.

Key words: Functional properties, *L. campestris*, Protein isolates, Soybean, Vegetable proteins

Introduction

Proteins are commonly used as food ingredients as they are of fundamental importance in the human diet. They also contribute to foods sensory properties and provide suitable functionality [1]. The nutritional quality and functionality of protein ingredients depend on their molecular composition and structure, the isolation method used and their interactions with other ingredients in the food product [2].

Due to the high cost of animal proteins, there is an active search for other protein sources to satisfy human nutrition requirements. Excellent alternative sources are legume protein isolates. Soybeans currently constitute the most significant vegetable protein source in food formulations. However, lupins, and particularly *L. albus* from the Mediterranean and *L. mutabilis* from South America, are very promising legumes that can be grown in poor soils [3]. Other species include *L. campestris* which, along with approximately 110 other wild lipin species, has been reported growing throughout México [4].

Lupin seeds have 40% protein content and 20% fat content, which is similar to soybeans but higher than other legumes. Globulins (α-conglutin or 11S-like protein, β-conglutin or 7S-like protein and γ-conglutin) are the main storage proteins (80–90%), in lupins, and have values similar to those reported in most legume seeds [3]. The alkaloid content of lupin seed appears to be the only significant antinutritional component, as hemagglutinin, phytate, α-galactoside, tannin, and trypsin inhibitor levels are lower than those found in other legumes and comparable to those of cereals [5].

Lupins utilize quinolizidine alkaloids as a defense against predators, but this is a limiting factor for human consumption. Elevated concentrations produce a bitter taste, and they do have some reported pharmacological effects [6]. However, alkaloids have been proven non-toxic at low concentrations [7]. Any potential effect from the alkaloids in lupins is eliminated during preparation of protein isolates since the alkaloids are water-soluble and are removed during processing [3].

Protein isolates have become increasingly important in the food industry because of their high protein contents, up to 90% in some legume protein isolates. They represent an alternative protein source for preparation of traditional foods and in development of new foods [2]. Successful use of plant protein isolates depends on the versatility of their functional properties, which are influenced by intrinsic factors (protein composition and conformation), environmental factors (food or model system composition), and isolation methods and conditions [8].

The most widely used procedure to prepare seed protein isolates is isoelectric precipitation. After alkaline solubilization of the proteins (pH 8–10) and removal of the insoluble material by centrifuging, proteins are precipitated by adding acid (pH 4–6) until reaching the isoelectric point [2, 8, 9]. Another process for isolating seed proteins is micellization, which involves precipitation from a neutral salt

[1]The authors dedicate this paper to honor the memory of our dear professors: Dr. Luz María del Castillo and Dr. Manuel Castañeda Agulló.

extract by dilution in cold water. The protein produced in this way has a micellar structure before being dried; hydrophobic interactions may play a major role in the stabilization of such isolates [10].

This study was aimed at recovering *L. campestris* protein isolates using both the micellization and isoelectric procedures, characterizing some of these isolates' antinutritional factors and functional properties, as well as determining their their electrophoretic behavior.

Materials and Methods

Plant Materials

Wild *L. campestris* seeds were collected in the State of Morelos, Mexico. The mature seeds were separated of the pod and exposed to the sun to eliminate excess moisture. Soybeans (*Glycine max*) were supplied by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Celaya, Mexico. Seeds were stored in close containers at 4 °C until use.

Flour Preparation

Seeds were ground in Cyclotec mill (model 1093) and screened using 8XX mesh. After drying at room temperature, the resulting flour was defatted in a Soxhlet for 5 h, and stored in closed containers at 4 °C until use.

Protein Isolate Recovery

Isoelectric Precipitation. Isoelectric precipitation isolates were prepared as described by Betschart [9]. For protein extraction, the meal was suspended in water (10% w/v) and the pH adjusted to 9.0 adding 1 N NaOH, the suspension was stirred for 30 min at room temperature and then centrifuged during 30 min at $10.000 \times g$. Supernatant was adjusted with 0.1 N HCl to pH 4.5 for protein precipitation and then the suspension was left at 4 °C overnight to allow proteins to precipitate; after that, centrifugation at $10000 \times g$ for 10 min at 4 °C followed. Protein precipitate was resuspended in water and freeze dried in a Freezone 4.5 l Freeze Dry System (Labconco, model 18), and then was stored at −20 °C until further study. All reported values are the average of triplicate experiments.

Micellization

Flour samples were extracted in a 10% (w/v) suspension of sodium chloride (0.5 M) at pH 7, and mixed on a stirring plate for 30 min at room temperature. The extract was centrifuged in a Beckman model J-25 centrifuge at $5000 \times g$ for 10 min and the supernatant was diluted with deionized water (1:4 v/v). After standing for 30 min at room temperature, the protein was recovered by centrifugation at $10.000 \times g$ for 10 min and then freeze-dried (Labconco, model 18) [10]. All values reported are the average of triplicate treatments.

Proximal Analyses

Protein content ($N \times 6.25$), crude fiber, fat, moisture and ash contents were determined according to standard methods [11]. Carbohydrates were determined by difference.

Amino Acid Analyses

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 (HPLC) using D,L-α-aminobutyric acid as an internal standard [12]. 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 done using the Maxima 820 3.3 version computer software (Waters). Separations were done 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 m*M* sodium acetate pH 6.0 and acetonitrile. The column was maintained at 18 °C by a temperature controller (Julabo F 10). Amino acid composition was expressed as g of amino acid per 16 g of nitrogen.

Antinutritional Factors

Alkaloid Analyses. Total quinolizidine alkaloids (TQA) determination was done using a colorimetric method described by Baer et al. [13] and modified by Luccisano et al. [14]. Briefly, lupin flour or lupine protein isolate (0.5 g) was extracted with 3 ml of 15% KOH, mixing on a stirring plate for 5 min. After adding 9.0 g of aluminum oxide and 25 ml of chloroform, the extract was filtered and washed with 25 ml of chloroform. It was then dried in a rotator evaporator (Buchi model R-200), and the resulting powder dissolved in 5 ml chloroform. Alkaloid concentration was determined with acid-base titrimetry with p-toluensulphonic acid. This acid was evaluated with sparteine as a standard. Soybean was used as a negative control.

Tannin Analyses

A quantitative analysis of tannin was carried out using a spectrophotometric method (Singleton and Roos [15]), using Folin-Dennis reagent. Extraction was done with methanol/water. Tannic acid was used to prepare the standard curve.

Lectin Analyses

Lectin activity was determined in according to Jaffé et al. [16]. In a round-bottomed wells of microtitre plates (Greiner, Labortechnik, Solingen, and Germany) using human red blood cells (2%) which were added to a serial dilution of an extract which was prepared by shaking 0.5 g of meal or protein isolates samples with 10 ml of NaCl 0.9% for 2 h at 25 °C. For the haemagglutination assay, plates were left at room temperature for 1–2 h and read. A positive pattern which indicated agglutination was a uniform coating of the bottom of the well by erythrocytes while a negative pattern (indicating no agglutination) was a circular clump of erythrocytes surrounded by a concentric, clear zone of equal size to the blank. Lectin activity was expressed as reciprocal of the minimum quantity (in mg) of meal per ml of the assay which produced agglutination.

Functional Properties

Protein Solubility. Solubility determinations were made within a pH range of 2.0–10.0. Five milliliter of a 1% protein isolate sample were dissolved in distilled water at room temperature, and the solution adjusted to each pH level by adding 0.1 N HCl or 0.1 NaOH and stirring 30 min. Each sample was later centrifuged at 10.000 × *g* for 10 min. Nitrogen content in the supernatant was determined by the Kjeldahl method, N × 6.25 was used to convert nitrogen to protein [17]. The percentage of soluble protein was calculated as follows:

$$\text{Solubility}\,(\%) = \frac{\text{amount of nitrogen in the supernatant}}{\text{amount of nitrogen in the sample}} \times 100$$

Water and Oil Absorption Capacity

To determine these absorption capacities, 0.5 g of protein isolate sample was weighed and then stirred into 5 ml of deionized water or corn oil (Mazola, CPI International) for 1 min in a graduated glass cylinder standing 30 min at 25 °C. These protein suspensions were then centrifuged at 1600 × *g* for 25 min. The volume of free liquid was measured and the remaining liquid was expressed as milliliter of water or oil absorbed per gram of protein [17].

Foaming Capacity and Foam Stability

These properties were evaluated over a pH range from 2 to 10. Fifty millileter of protein isolate solution at 1% were blended at low speed for 1 min in a Waring blender (Osterizer 10S-E) at 25 °C and the foam volume was recorded after 30 s. The volume of foam present above the surface of the liquid contained in a graduated glass cylinder was measured. Foam expansion was expressed as percent volume increase due to whipping. After standing 30 min at room temperature the volume of the remaining foam was recorded and foam stability calculated [17].

Gelation Capacity

Protein isolate sample suspensions of 6, 8, and 10% were prepared in 5 ml of distilled water at room temperature. Test tubes containing these suspensions were heated for 1 h in a boiling water bath (Lab-line, model imperial III) followed by rapid cooling under running cold tap water. The tubes were then cooled for 2 h at 4 °C. The lowest gelation concentration was determined by the sample which did not fall out of or slip from the test tube when inverted [18].

Gel Electrophoresis

Non-denaturing polyacrylamide gel electrophoresis (native-PAGE) was used to examine the principal protein fractions in the protein isolate samples. This was performed using the following continuous buffer system: 0.125 M Tris-HCl pH 6.8 for the stacking gel: 0.375 M Tris-HCl pH 8.8 for the separating gel, and 0.025 M Tris-HCl/0.92 M glycine pH 8.3 for the running buffer in 6% (w/v) polyacrylamide gel (Laemmli [19]). Protein samples of 1 mg were dissolved in 1 ml of 0.75 M Tris-HCl pH 8.8, 20% (v/v) glycerol/0.05% (w/v) bromophenol blue and centrifuged at 1000 × *g* for 5 min. The supernatant was separated and used to load the gel (15 μl/lane).

The gel obtained by electrophoretic technique was fixed and stained with 0.1% R-250 Coomasie Brilliant Blue in water/methanol/acetic acid (5:5:20) for 12 h and destained later with 25% (w/v) methanol and 10% (w/v) acetic acid.

Statistical Analysis

All determinations were done in triplicate, and data were analyzed using a one-way variance analysis and Duncan's multiple way range test [20].

Results and Discussion

Composition of Protein Isolates

Chemical composition of *L. campestris* and soybean flour and protein isolates is presented in Table 1. Among the common legume seeds, those containing high amounts of lipids can be distinguished from those having starch as energy storage components. The former are mostly found in *Lupinae* and *Glycinae* subfamilies, to which lupin and soybean belong. As non-starch leguminous seeds lupins, have a biochemical composition closer to soybean, and are

Table 1. Chemical composition of flour and protein isolates of *Lupinus campestris* and soybean[a]

Components (g/100 g)	LF	LMI	LPI	SF	SMI	SPI
Protein (N × 6.25)	55.3b ± 3.16	95.7a ± 1.25	93.2a ± 1.61	52.4b ± 2.45	93.8a ± 1.15	92.3a ± 3.16
Fat	0.2b ± 0.04	–	–	0.5a ± 0.06	–	–
Crude fiber	5.8a ± 0.36	1.0c ± 0.26	0.5cd 0.10	3.8b ± 0.28	0.5cd 0.09	0.2d ± 0.04
Ash	3.8b ± 0.20	1.7c ± 0.10	2.4c ± 0.15	5.4a ± 0.30	3.8b ± 0.60	4.5ab ± 0.20
Carbohydrate[b]	34.9b ± 1.01	1.6d ± 0.10	3.9c ± 0.09	37.9a ± 1.09	1.9cd ± 0.02	3.0cd ± 1.01
Quinolizidinic alkaloids	0.5a ± 0.02	0.005b ± 0.00	0.005b ± 0.00	–	–	–
Tannins	0.075a ± 0.02	0.017b ± 0.01	0.02b ± 0.02	–	–	–
Lectins[c]	8.0b ± 0.0	4.0c ± 0.0	4.0c ± 0.0	512.0a ± 0.0	–	–

LF = *L. campestris* defatted flour; LMI = *L. campestris* micelle protein isolate; LPI = *L. campestris* isoelectric protein isolate; SF = Soybean defatted flour; SMI, Soybean micelle protein isolate; SPI = Soybean isoelectric protein isolate.
[a]Results represent the average of three determinations ±SD, values in the same row with different letters are significantly different ($p < 0.05$).
[b]Obtained by difference.
[c]Expressed as reciprocal of the minimum quantity (in mg) of meal ml^{-1} of the assay which produced agglutination.

characterized by high protein content (55.3% in lupin and 52.4% in soybean). Of the lupins, *L. mutabilis* seeds have the highest protein level (40–50%), followed by *L. albus* with 34%, and *L. angustifolius* with 36% [7]. These protein concentrations are higher than in other legumes such as lentils and beans, which range from 6–25% [3]. There are differences in protein and fat compositions among the lupins, due to interspecific genetic differences.

Crude fiber content in the present study (5.8%) was lower than that obtained by Jiménez-Martínez et al. [5] for *L. campestris* (14.7%) and that reported for *L. angustifolius* and *L. luteus* (13–19%). This difference is due to the fact that the lupin seed in the present study was dehulled before processing.

Carbohydrate content for the defatted flour of *L. campestris* (34.9%) was similar to that reported by Jiménez-Martínez et al. [5] for *L. campestris*. The carbohydrate level decreased in the isolates because these compounds were solubilized during the protein isolate recovery treatments.

Protein concentration (55.3%) in the lupin flour increased to 95.7% for LMI and 93.2% for LPI, the protein content values in soybean protein isolates were 93.8% for SMI and 92.8% for SPI. Protein content in both isolates obtained by micellization and isoelectric precipitation was similar ($p < 0.05$).

The amount of protein recovered was considerably higher with isolectric precipitation (60%) than with micellization (30%). These results are better than those obtained by Paredes-López and Ordorica-Falomir [21], for commercially-prepared safflower protein isolate (46% for isoelectric precipitation and 17% for micellization), but lower than they reported for laboratory-prepared safflower protein isolate (78% for MI and 44% for PI)**;** Arntfield et al. [10] recovered 44% protein from faba beans isolates using micellization. The higher levels of protein recovery when using the isoelectric precipitation procedure may result from its more complete protein extraction including solubilization of more proteins from the flour. Micellization is selective for globulin fractions.

Amino Acid Composition

Amino acid profiles of *L. campestris* flour and protein isolates (LMI and LPI) were characterized by low methionine and high lysine contents (Table 2), as in other legumes and their protein isolates. There were slight variations in the

Table 2. Amino acid composition in *L. campestris* flour and protein isolates

				FAO/WHO[c]		
Amino acid (g aa/16 g N)	LF	LPI	LMI	1	2	3
Ile	4.5	4.9	4.8	4.6	2.8	1.3
Leu	7.5	8.2	7.6	9.3	6.6	1.9
Phe	4.0	4.4	4.2	6.6	6.3[b]	1.6
Thr	4.2	3.7	3.3	4.2	3.4	1.7
Val	3.6	3.1	3.1	7.2	3.5	1.9
Lys	6.4	5.9	5.4	4.3	5.8	0.9
Met	0.7	0.4	0.1	5.5	2.5[a]	1.3
Cys	3.1	3.3	3.4			
Asp/Asn	10.9	11.0	11.2			
Glu/Gln	28.9	29.7	32.4			
Ser	7.2	7.5	7.5			
His	3.3	3.3	3.0			
Gly	4.6	3.7	3.6			
Arg	14.8	15.0	15.5			
Ala	2.9	2.6	2.1			
Pro	4.1	4.3	4.2			
Tyr	4.8	4.5	4.1			

LF = *L. campestris* defatted flour; LPI = *L. campestris* isoelectric protein isolate; LMI = *L. campestris* micelle protein isolate. Data are the average of two determinations on separate hydrolyses.
[a]Met + Cys.
[b]Phen + Tyr.
[c]Recommendations from Ref. [25]; 1, children < 2 years; 2,children 2–5 years; 3, adults.

amino acid composition of the two isolates. Both samples had high amounts of arginine, aspartic, and glutamic acids; the total amount of these three amino acids ranged from 55 to 59 g/16 g N.

The amino acid composition for the *L. campestris* isolates from micellization had lower values in six essential amino acids than the isolates recovered by isoelectric precipitation. This difference is similar to that reported by Paredes-López et al. [22] for chickpea protein isolates recovered by micellization and isoelectric precipitation. The yield obtained for *L. campestris* micelle protein isolate was only of 30%, compared with 60% for *L. campestris* isoelectric protein isolate, a difference that can be explained by the differences in the protein isolation techniques. In micellization, the ionic strength of the NaCl used to recover the micelle preferentially attracted one of three globulin fractions, leaving the others proteins in solution. The different protein fractions have distinct amino acid patterns that influence directly the quality and functional properties [3, 23]. These phenomena may be the cause of the lower yield and lower essential amino acids content produced with micellization method.

The essential amino acid content in *L. campestris* flour was higher than that reported for *L. mutabilis* [24], *L. albus* and *L. luteus*, except for isoleucine and leucine [7].

The *L. campestris* flour and protein isolates had amino acid values in agreement with those proposed in the FAO/WHO pattern [25] for infants and adults, with the exception of methionine.

Antinutritional Factors

Alkaloids. Total quinolizidine alkaloids (TQA) concentration in *L. campestris* defatted flour, determined with the tritrimetric method, was 0.5% (Table 1). Jiménez-Martínez et al. [5], found TQA values of 2.74% in *L. campestris* seeds, using quantification by gas chromatography coupled with mass spectrometry. Hatzold et al. [26], found 3.1% in *L. mutabilis*. Lupins are classified into sweet varieties, with low alkaloids content (<1%), and bitter varieties with typical alkaloid contents of 1.0–3.0% and in a few as high as 4.0%. Since the TQA content of the *L. campestris* seed was 2.74%, it can be classified as bitter specie. Quinolizidine alkaloid toxicity varies. Those belonging to the sparteine and lupanine QA types are relatively toxic when injected, but have lesser toxic effects when ingested orally. Lupanine has a moderate toxic effect in vertebrates, while alkaloids such as α-piridone, cytisine, and anagyrine are highly poisonous [27]. Martínez-Herrera et al. [28], did not find these alkaloids in *L. campestris* seeds. The TQA concentration of *L. campestris* was reduced during the protein isolation process from 0.5 in defatted flour to 0.005% in protein isolates ($p < 0.05$). An alkaloid content between 0.02 and 0.04% is through not to have toxic effect [29].

Tannins

Tannin content in the flour was 0.75 mg of tannic acid/g of sample, 0.20 in the LPI and 0.17 in the LMI (Table 1), meaning tannins were reduced by 75% during isolate preparation. Fernández-Quintela et al. [8], observed a reduction of 69% in soybean protein isolates and 95% in faba bean isolates. Tannins decrease protein digestibility [29]; some epidemiological reports suggest a possible relationship between presence of condensed tannins and esophageal cancer [30].

Lectins

Lectin presence (inverse of minimum amount of meal in mg ml^{-1} assay mixture which produced haemagglutination) in the *L. campestris* flour showed a value of 8, lower than those obtained for soybean flour of 512 ($p < 0.05$) (Table 1). The reduction in hemagglutinating activity after protein isolate preparation was probably due to the slight protein mobilization which occurs at alkaline pH values. Similar results were obtained by Fernández-Quintela et al. [8], who did not detect lectins in protein isolates prepared from peas, faba beans and soybeans.

Functional Properties

Protein Solubility. The protein solubility profile (Figure 1) showed that the minimum protein solubility value was at pH 4.0–6.0 near the protein isoelectric points, which is similar to those of other vegetable proteins [8, 9, 18, 21]. *L. campestris* seed globulins comprised 83% of the total protein, this included α-conglutin (33% of globulins) or 11S-like protein with an isoelectric point of pH 4.8, β-conglutin (43% of globulins) or 7S-like protein with an isoelectric point of pH 6.0 and γ-conglutin (10% of globulins) with an isoelectric point of pH 8.0, noted by Martínez-Ayala and Paredes-López [31].

Protein isolate solubility generally increased at both ends of the pH scale. At the acid pH values (pH 2) maximum protein solubility was 91% for LPI and 76% for LMI, while at alkaline pH values (pH 10) it was 99% for LPI and 98% for LMI. These results are better than those of commercially important seeds such as peas, faba beans and soybean protein isolates, which had values lower than 70% for solubility at acid values (pH 2) and similar solubilities at basic values [8]. Safflower protein isolates has a maximum solubility of 89% at pH 3 [32], soybean protein isolate has a maximum solubility of 82.8% at pH 11 [33], and wheat germ protein isolate has a maximum solubility of 78% at pH 6 [34].

Electrostatic repulsion and ionic hydration are minimal at the isoelectric point. However, some proteins, for example, whey proteins (α-lactalbumin, β-lactoglubulin, and bovine serum albumin) are highly soluble at their isoelectric pH's.

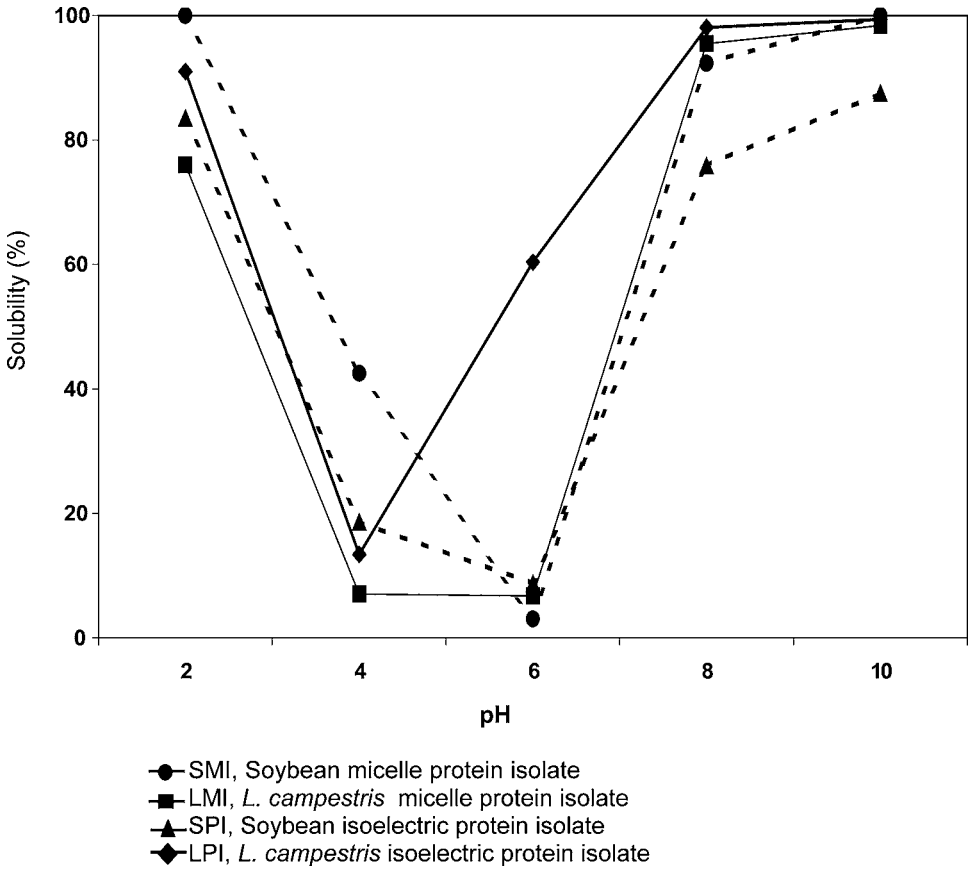

Figure 1. Solubility of *L. campestris* and soybean protein isolates.

This is primarily because the exposed surfaces of these proteins contain a high ratio of hydrophilic to hydrophobic groups [1]. Several functional properties, such as protein foaming, emulsification and gelation may be related to solubility and conformation stabilities. Protein solubility is fundamentally related to the hydrophilicity/hydrophobicity balance [1]. The solubility characteristics of protein, however, should be related to the composition of the protein's surface and not necessarily to its overall amino acid composition.

Water and Oil Absorption Capacities

Protein isolate water absorption capacity was 3.5 ml water/g protein for SMI and 1.3 ml water/g protein for LMI, while the SPI showed 2.2 ml water/g protein and LPI had 1.7 ml water/g protein (Table 3). Results showed influence of the type of protein, soybean protein isolates were higher ($p < 0.05$) than *L. campestris* protein isolates. Sathe et al. [21] reported water absorption capacity values of 1.2 ml water/g protein for *L. mutabilis* seed flour and 1.4 for its protein concentrate. These values are similar to those obtained for protein isolates of peas (1.7 ml water/g protein), faba beans (1.8) and soybeans (1.3) [8], as well as safflower protein isolates (1.3–1.8 ml water/g of protein [32]). Protein isolate water absorption depends on polar amino acids availability on the primary sites for protein/water interactions. Isolates' water absorption capacity may be affected by their conformation and environmental factors. Conformational changes in the protein molecules may expose previously enclosed amino acid side chains, thereby making them available to interact with water. Differences in carbohydrate content may also have affected water absorption capacity [22].

Table 3. Water and oil absorption capacity of *L. campestris* and Soybean protein isolates[a]

ml/g of protein	LPI	LMI	SPI	SMI
Water absorption	1.7c ± 0.10	1.3d ± 0.00	2.2b ± 0.10	3.5a ± 0.20
Oil absorption	1.7a ± 0.10	2.2b ± 0.10	1.5a ± 0.00	2.5c ± 0.17

LPI: *L. campestris* isoelectric protein isolate; LMI: *L. campestris* micelle protein isolate; SPI: Soybean isoelectric protein isolate; SMI: Soybean micelle protein isolate. [a]Results represent the average three determinations ± SD, values in the same row with different letters are significantly different ($p < 0.05$).

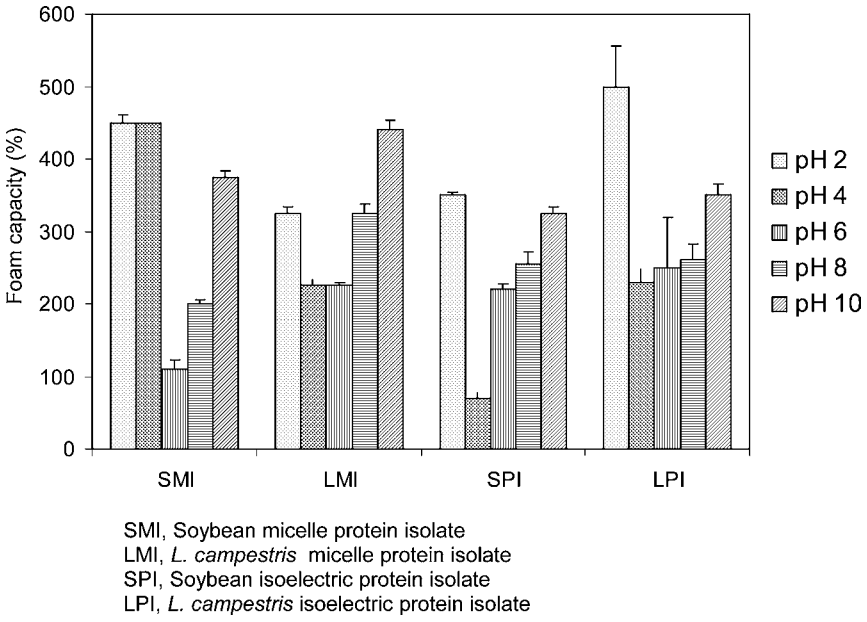

Figure 2. pH effect on foam capacity of *L. campestris* and soybean protein isolates.

Oil absorption in food products is an important functional property because it improves mouthfeel and flavor retention [35]. Oil absorption was 2.5 ml oil/g protein for SMI, 2.2 for LMI, 1.5 for SPI and 1.7 for LPI (Table 3). These values are similar to values for guava protein isolate (1.7 ml oil/g protein) reported by Bernardino et al. [23], but better than those reported by Fernández-Quintela et al. [8] for commercial legume protein isolates (1.2 mg oil/g protein for peas, 1.6 for faba beans, 1.1 for soybeans) and *L. mutabilis* seed protein concentrate (1.3 ml/g [24]). They were lower, however, than safflower protein isolate (2.4 ml oil/g protein) [32]. The oil absorption capacities recorded for *L. campestris* and soybean protein isolates recovered by micellization and isoelectric precipitation, suggest that several non-polar side chains may be binding to the hydrocarbon chains of fats, resulting in a good absorption of oil.

Capacity and Stability Foaming

The foaming properties of the *L. campestris* protein isolates were pH- dependent (Figures 2 and 3). Foaming capacity was highest at pH 2 (500% for LPI) and lowest in the pH 4–6 isoelectric range (220% for LPI and LMI), increasing again in the alkaline region (450% for LMI and 330% for LPI). Similar behavior was observed for foaming stability. These kinds of pH-dependent foaming characteristics were also observed in the soybean isolates. The foaming capacities of both *L. campestris* protein isolates, were better than the capacities reported for safflower protein isolates [32], *L. mutabilis* protein isolates [24] and commercial legumes protein isolates [8].

Foam stability of *L. campestris* and soybean protein isolates was high at pH 2 (95% for SPI, 85% for LPI, 70% for SMI and 65% for LMI). At alkaline pH (8 and 10) foam stability for soybean protein isolates (25% for SPI and 5% for SMI) was lower than for *L. campestris* protein isolates (70% for LPI and 65% for LMI).

It has been shown that molecular properties of proteins required for good foaming capacity and good foaming stability are different. The formation of protein-based foams involves the diffusion of soluble proteins toward the air–water interface and rapid conformational change and rearrangement at the interface, the foaming stability requires

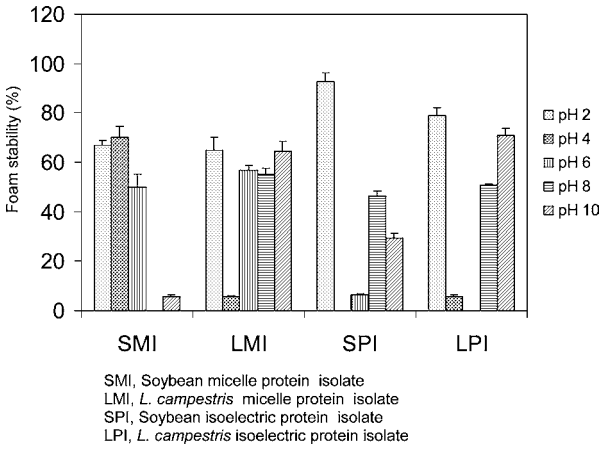

Figure 3. pH effect on foam stability of *L. campestris* and soybean protein isolates.

formation of a thick, cohesive and viscoelastic film around each gas bubble [1].

Gelation Capacity

The major reserve proteins in legumes are the 7S and 11S globulins. These proteins may dissociate and associate in different ways and form gels upon heating [1, 3]. The capacity of gels to retain water, lipids, sugars, flavors, and other ingredients is very useful in developing new products. Heat-induced gels were obtained from lupin and soybean protein isolates, at different pH values and protein concentrations. The lowest gelation concentrations for the *L. campestris* isolates were 6% (LPI) and 8% (LMI) (w/v), which are better than the 8% (SPI) and 8% (LMI) recorded for the soybean protein isolates. Soybean protein isolates (SPI) did not show gelation at pH 4 at the three tested concentrations. While gelation capacity of *L. campestris* and soybean protein isolates recovered with MI indicated a minimum protein concentration of 8% for forming a gel at pH 4, no gel was formed at pH's 6 and 8 at the three tested concentrations. Proteins form two types of gels: the coagulum and transparent gels. The type of gel a protein forms is primarily influenced by its amino acid composition. Proteins containing a high frequency of non-polar amino acid residues tend to form coagulum type gels [1], whereas proteins containing a high frequency of hydrophilic amino acids form transparent gels. All the tested proteins formed coagulum type gels under all the tested conditions.

Gel Electrophoresis

The *L. campestris* protein isolates native-PAGE indicated that most components were common to the two isolates, and there were three main bands that exhibited different mobilities (Figure 4). The globulins isolated from *L. campestris* exhibited different mobilities (α-conglutin, legumin or 11S-like protein $>$ β-conglutin, vicilin or 7S-like protein $>$ γ-conglutin) when subjected to native-PAGE [31] as reported by Blagrove and Gillespie for *L. angustifolius* [36]. As shown in Figure 4, only a slight difference in mobility was observed in the three main electrophoretic bands between LPI and LMI. These differences may have been due to changes in protein structure, composition and interactions of proteins with residual salts in the isolates.

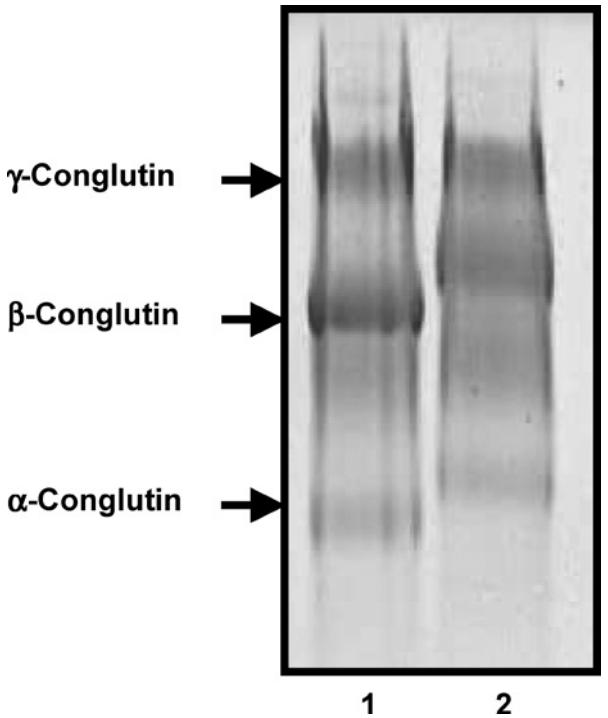

Figure 4. Native-PAGE of *L. campestris* protein isolates; lane 1, isoelectric protein isolate; lane 2, micelle protein isolate.

Conclusions

The processing of *L. campestris* into protein isolates led to an increase in the protein concentration of 49%–90%, which is consistent with the FAO/WHO (1985) amino acid pattern for infants and adults. It also lowered alkaloids and antinutritional factors to safe levels after protein isolate preparation. The efficiency of protein recovery in the isolates was 30% for LMI and twice that for LPI (60%). Similar yield extraction was obtained for soybeans. The smaller yield extraction from LMI, versus the LPI procedure, which uses more extreme conditions, may be due also to the nature of protein–protein interactions, which appear to exclude low molecular weight protein components during the micelle formation.

The *L. campestris* protein isolates native-PAGE exhibited three bands, which likely belong to α, β y γ conglutin.

The high protein content and functional properties of *L. campestris* protein isolates suggest their potential applications in new product formulations and fortification.

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