Emulsifying and Foaming Properties of Different Protein Fractions Obtained from a Novel Lupin Variety AluProt-CGNA®(Lupinus luteus)

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Abstract: The use of vegetable proteins as food ingredient is becoming increasingly important due to their high versatility and environmental acceptability. This work describes a chemical characterization and techno-functional properties (emulsifying and foaming properties) of 3 protein fractions obtained from a protein-rich novel lupin variety, Alu*Prot*-CGNA®. This nongenetically modified variety have a great protein content in dehulled seeds (60.6 g protein/100 g, dry matter), which is higher than soybean and other lupin varieties. A simple procedure was utilized to obtain 3 different fractions by using alkali solubilization and isoelectric precipitation. Fractions 1 and 3 were mainly composed of protein and polysaccharides (NNE), whereas fraction 2 was mainly composed by protein (97%, w/w). Fraction 3 presented interesting and potential foaming properties in comparison to the other fractions evaluated in the study. Besides, its solubility, foaming and emulsifying capacity were practically not affected by pH variations. The 3 fractions also presented good emulsion stability, reaching values above a 95%. SDS-PAGE showed that fractions 1 and 2 contained mainly conglutin α, β, and δ, but in different ratios, whereas fraction 3 contained mainly conglutin γ and albumins. The results of this work will provide better understanding for the utilization of each protein fractions as potential ingredients in food industry.

Keywords: Alu*Prot*-CGNA, emulsifying property, foaming property, *Lupinus luteus,* protein fractions

Practical Application: In recent years, there is great interest in the food industry in the use of vegetable proteins as natural emulsifier to create novel emulsion and foam systems with improved stability. Alu*Prot*-CGNA have great potential as a source of functional ingredients for food applications. In this work, the utilization of protein fractions from Alu*Prot*-CGNA® as emulsifiers to form stable emulsions and foams were evaluated. The fractions presented good emulsion stability (ES) at acidic pH found in many food products. Moreover, pH stability exhibited by fraction 3 could also be an important characteristic for its use as emulsifying and foaming in food products.

Introduction

In general, proteins are essential ingredients in food industry, not only due to their nutritive value, but also because of their potential functional properties (Fligner and Mangino 1991). Many vegetable proteins are surface-active molecules that can be used as emulsifiers because of their ability to facilitate the formation, improve the stability, and produce desirable physicochemical properties in O/W emulsions and foams (McClemnets 2004). As a result of their amphiphilic nature, proteins are also involved in functional aspects of foods, such as the formation of emulsions and foams (Damodaran 2005). An amphiphile compound can be adsorbed at oil–water and air–water interfaces decreasing surface tension values, thus facilitating the formation of emulsions and foams. Several studies have shown that proteins from animal and vegetable sources can be used to prepare emulsion and foam based-products (Damodaran 2005). Nevertheless, there is still a relatively poor understanding of the influence of specific proteins on emulsifying and foaming properties.

In the past few years, proteins from vegetables are increasingly being used as food ingredients due to their nutritional composition, satisfactory functional properties, and their optimization of consumer's cost. In food application, for example, vegetable proteins are known to be less allergic compared to animal derived proteins. Among vegetable proteins used as functional ingredient, we can find mainly soy protein isolate, pea protein isolate, broad beans, and cereal proteins (Hu and others 2003; Makri and others 2005). Legumes are low-cost and the most common rich vegetable protein source, which constitute a large family of plants, including soy, lupin, and peanut among many others (Gepts and others 2005). For example, lupin is an undervalued legume despite its high protein and dietary fiber content and potential health benefits (Piornos and others 2015). There are 4 species of sweet lupin considered edible for human and animals (low alkaloids): *Lupinus albus, Lupinus luteus, Lupinus mutabilis,* and *Lupinus angustifolius* (Jappe and Vieths 2010). Lupin crop has been recognized as a nongenetically modified organism (non-GMO) with low production costs, low levels of potentially hazardous phytoestrogenic compounds, high dietary fiber, high antioxidants, and protein-rich seeds (Hall and others 2005; Erbaş and others 2005; Sirtori and others 2012). According to Wong and others (2013), lupin proteins consist of albumins (approximately 13% of total protein) and globulins (approximately 87% of total protein). Albumins are

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a diverse group of proteins having a wide range of biochemical functions within plant cells. Globulins are high-molecular-weight storage proteins, the majority of them are α and β conglutins, with lower levels of γ and δ conglutins (Duranti and others 2008). The different protein types have been associated to distinct biochemical properties. The fraction that is rich in α and β conglutins has been reported to display a high emulsifying capacity (EC; Piornos and others 2015). The γ conglutin is a protein with higher nutritional quality than α and β conglutins, which are more abundant, and a potential glucose-lowering bioactivity (Lovati and others 2012). The lupin seeds used in the present study belong to a novel variety that was developed and registered by Agriaquaculture Nutritional Genomic Center (CGNA), Alu*Prot*-CGNA® (Piornos and others 2015). This nongenetically modified variety has an especially high protein yield, corresponding to around 60% of the dehulled seeds content. In our previous study, we prepared a protein isolate from Alu*Prot*-CGNA® through the alkali solubilization and isoelectric precipitation method (Piornos and others 2015). During this process, we additionally obtained 2 fractions that corresponded to an aqueous alkaline extract and an acid-soluble protein, which presented potential functional properties due to their high protein content, polysaccharides. Consequently, it has been interesting to evaluate influence these byproduct on the stability of emulsions and foams. It should be noted that the emulsifying and foaming properties of lupin proteins have already been studied. However, a full study on surface properties of its products and byproducts and a chemical characterization of proteins obtained from this a novel lupin variety (*Lupinus luteus*) has not yet been made. Therefore, the main purpose of this study was to characterize and evaluate the functional properties, such as foaming and emulsifying activities, of the different protein fractions obtained from Alu*Prot*-CGNA. The results of this work will provide better understanding for increasing the utilization of this protein isolate as a potential functional ingredient in food industry.

Materials and Methods

Materials

Seeds of the lupin variety Alu*Prot*-CGNA® were used in this study. Dehulled seeds were ground in an electric mill so as to obtain fine flour (particle size of 625 μm) and kept at 25 °C until usage. Sunflower oil was purchased at a local market and all other chemicals of analytical degree were obtained from Sigma-Aldrich Corp. (St. Louis, MO, U.S.A.).

Preparation of protein fractions

Lupin protein fractions from Alu*Prot*-CGNA® were obtained following the method described by Snowden and others (2007) with some modifications. A schematic flowchart for the preparation of the protein fractions is shown in Figure 1. First, the lupin flour was mixed with distilled water at 1:10 (w/v). Previous oil extraction was not carried out due to low oil content in the seeds (4.95% wt). pH of the suspension was adjusted to 9.0 using NaOH (1 M) followed by stirring for 90 min at 25 °C. After that, the suspension was centrifuged at 3200 $\times$ *g* for 10 min. The pellet obtained was tagged as "lupin cake," and then discarded. The supernatant (fraction 1) containing the alkaline soluble proteins was recovered. Then, pH was adjusted to 4.6 (isoelectric precipitation point) using HCl (1 M), the sample was incubated for 15 min and then centrifuged (3200 $\times$ *g*, 15 min). The so obtained pellet corresponded to the protein isolate (fraction 2) and the separated supernatant (fraction 3), which was also subjected to further analysis. The supernatants (fractions 1 and 3) and the protein isolate (fraction 2) were immediately submitted to freeze-drying and stored at -20 °C until use.

Chemical analysis

The protein concentration in each freeze-dried fraction was determined by a TruSpec®N (LECO Corporation St. Joseph, Michigan 49085, U.S.A.) instrument following manufacturer's instructions. Other compositional parameters (moisture, ash, ether extract, crude fiber, and non-nitrogen extract contents) were determined according to the AOAC (1990) procedures.

Electrophoresis (SDS-PAGE)

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a 10 or 15% gel in an SE 260 (Equipment model code) mini-vertical gel electrophoresis unit (GE Healthcare Bio-Sciences, Pittsburgh, Pa., U.S.A.) as described by Laemmli (1970). Protein bands on the gel were visualized by colloidal blue Coomassie (CBB) staining, and the gel was digitalized using LAS 3000 (FujifilmCo. Ltd., Tokyo, Japan). All the samples were prepared under reducing conditions by addition to the sample buffer of DTT (1,4-Dithiothreitol) and EDTA (Ethylenediaminetetraacetic acid) at a final concentration of 50 and 20 mM, respectively. According to the estimated distribution of protein in each fraction, 10 μg protein of fraction 1, 8.9 μg protein of fraction 2, and 1.1 μg protein of fraction 3, were loaded on the gel.

For bidimensional gel electrophoresis (2DE), a protein sample was treated with 2-D Clean-Up Kit (GE Healthcare Bio-Sciences Pittsburgh, PA 15264-3065, U.S.A.) as described in the manufacturer's instruction manual. This treated protein was dissolved in 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 0.5% (v/v) IPG Buffer (3-11 NL; GE Healthcare Bio-Sciences, Pittsburgh, PA 15264-3065, U.S.A.), and 15mg/mL DeStreak Reagent (GE Healthcare Bio-Sciences, Pittsburgh, PA 15264-3065, U.S.A.). For isoelectric focusing (IEF), an IPG strip (Immobiline DryStrip pH 3 to 11NL, 7 cm; GE Healthcare Bio-Sciences) was rehydrated with the protein solution in a Protean IEF Cell (Bio-Rad Laboratories, Hercules, Calif., U.S.A.) by the active rehydration method described in the manufacturer's instruction manual. IEF was performed in the IEF cell at 20 °C. After solubilization and carboxyamidomethylation of the proteins, SDS-PAGE was performed as described earlier. Three independently prepared samples per fraction were subjected to 2DE. Only spots observed in all 3 gels per fraction were considered for further analysis. The molecular masses and isoelectric point (p*I*) values of spots were manually determined by comparison with the mobilities of standard molecular markers (Mark 12 Unstained Standard; Life Technologies, Grand Island, N.Y., U.S.A.) and their distances from the acidic end of the IPG strip. Conglutin α, β, and γ were partially purified as described by Bush and Tai (1994) with slight modifications, and used as standards in this study.

Functional properties of fractions

Protein solubility. The method described by Were and others (1997) was employed for determining the protein solubility profile of the different fractions as a function of pH. Dried fractions were dispersed into distilled water at a concentration of 10 g/L and the pH of the suspensions was adjusted to 2 to 10, using NaOH (1 M) or Hydrochloric acid (HCl) (1 M). The solutions were stirred for 1 h at 25 °C using a magnetic stirrer, before centrifuging at 8000 $\times$ *g* for 10 min at 4 °C. The supernatants were 10-fold diluted in distilled water, and protein content was determined using

Qubit® Protein Assay Kit by Qubit® 2.0 fluorometer (Invitrogen-Thermo Fisher Scientific, Waltham, MA 02451, U.S.A.).

Emulsifying capacity and emulsion stability. The EC_{24} at different pH values (from 2 to 10) was performed according to the methodology described by Burgos-Díaz and others (2015). Sunflower oil was vortexed for 2 min with an equal volume (3 mL) of the aqueous phase, which consisted of each fraction (fractions 1, 2, or 3) at 10 g/L. The tubes were left to stand for 1 h at room temperature. The height of total and emulsion layers was measured in the test tubes at 0 and 24 h. The EC_{24} was referred as the relation of the height of emulsion layer after 24 h (H_{EL}) and the total height of the liquid sample (H_T), whereas the ES was calculated by dividing the EC_{24} by the EC at the start time (EC_0).

$$EC\ (\%) = \frac{H_{EL}}{H_T} \times 100 \quad (1)$$

$$ES\ (\%) = \frac{EC_{24}}{EC_0} \times 100 \quad (2)$$

Foaming capacity (FC) and foam stability (FS). The effect of different pH values was tested on the FC and FS. FC was determined following the method described by Piornos and others (2015). A 400DS homogenizer (PRO Scientific, Inc., Oxford, Conn., U.S.A.) was employed to form the foam. Thus, 20 mL of solution, containing 20 g/L of each fraction, were homogenized at 10000 rpm for 1 min. FC was calculated as follows (Eq. 3):

$$FC\ (\%) = \frac{V_1 - V_2}{V_2} \times 100 \quad (3)$$

where V_1 is the volume after foam formation and V_2 is the initial volume. FS was evaluated measuring the total volume after 2 h of foam formation (V_3), and calculated using the equation proposed by Yuliana and others 2014; (Eq. 4).

$$FS(\%) = \frac{V_3 - V_2}{V_1 - V_2} \times 100 \quad (4)$$

FC and FS were determined for fractions 1 and 3. Fraction 2 was evaluated following the method presented in our previous study (Piornos and others 2015).

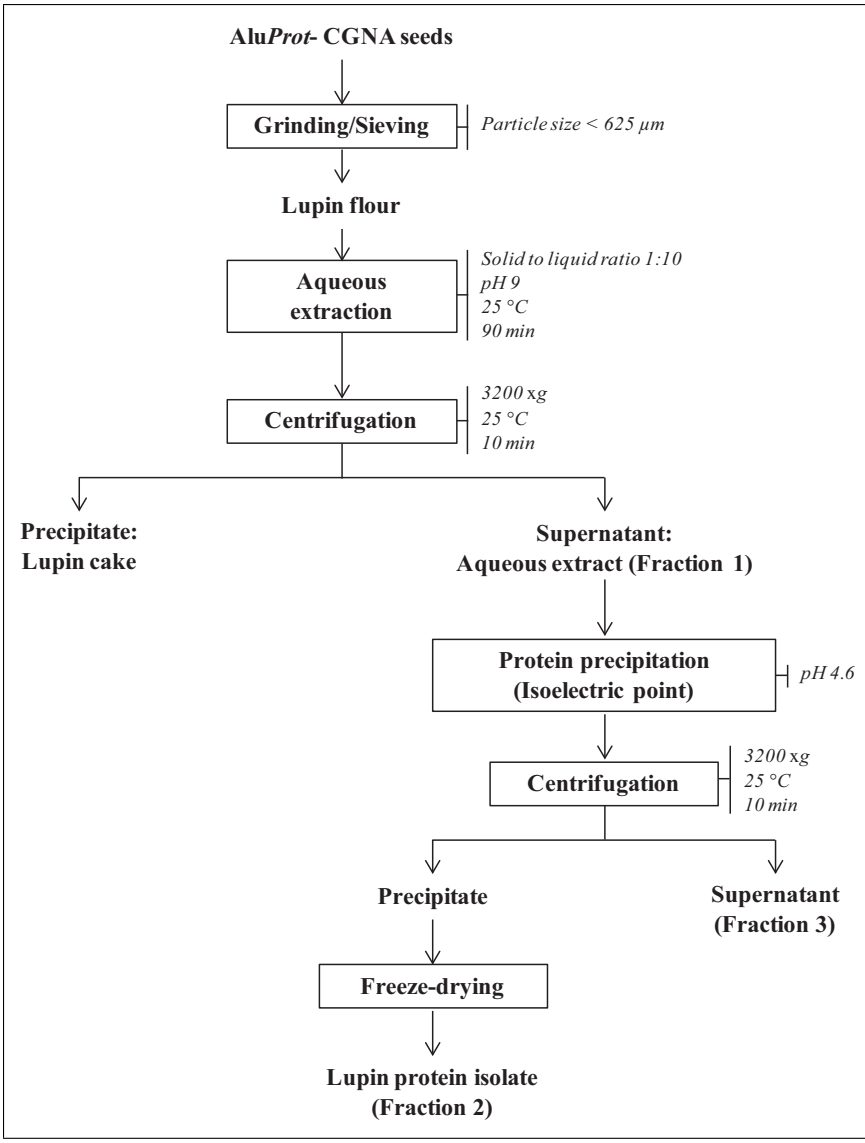

Figure 1–Process and conditions for the extraction of protein fractions from Alu*Prot*-CGNA®. Fraction 1, aqueous alkaline extract fraction; fraction 2, acid protein precipitate fraction; and fraction 3, acid soluble protein fraction.

Table 1–Chemical composition of fractions from Alu*Prot*-CGNA®.

Component quantity, g/100 g	Fraction 1	Fraction 2[a]	Fraction 3
Protein	69.70 ± 0.17	97.54 ± 1.64	35.98 ± 0.12
Fat	0.80 ± 0.00	0.55 ± 0.01	1.59 ± 0.10
Total dietary fiber	0.96 ± 0.40	0.18 ± 0.00	5.05 ± 0.665
Ash	6.11 ± 0.42	1.42 ± 0.05	14.59 ± 3.23
Nitrogen-free extract	22.44	0.31	42.79

These results showed the mean of 3 determinations.
[a]Piornos and others (2015).

Statistical analysis

Each determination was performed in triplicate and the data were subjected to a 1-way analysis of variance (ANOVA) and Duncan's tests to determine significant differences ($P < 0.05$) between mean values within each group, employing MS Excel 2013.

Results and Discussion

Chemical characterization of fractions obtained from lupin seeds

The overall protein content of dehulled seeds of the novel variety Alu*Prot*-CGNA® is around 60%, this value is higher than any reported legume or grain crop (Piornos and others 2015). However, after the preparation of concentrate and protein isolate from Alu*Prot*-CGNA® flour, fraction 1 and fraction 2 respectively, the protein content increased significantly. It should be noted that, a higher protein concentration than seeds was obtained for both fractions, fraction 1:69.70% and fraction 2:97.57%. These values are achieved due to the high protein content present in the dehulled seeds of the new lupin variety (Alu*Prot*-CGNA®). In addition, in the same process, a third fraction was obtained (fraction 3), which exhibited interesting functional properties and correspond to an acid-soluble protein fraction. The compositional analysis (Table 1) showed that these 3 fractions from Alu*Prot*-CGNA® consisted mainly of protein and polysaccharide (non-nitrogen extract, NNE) mixtures. Fractions 1 to 3 contained 69.70%, 97.54%, and 35.98% of protein respectively, and in the case of NNE (w/w), the values were 22.44%, 0.31%, and 43.38%. These results indicated that, by means of this extraction method, it was possible to obtain several fractions with different protein and polysaccharide contents, besides the protein isolate (fraction 2) prepared in our previous study (Piornos and others 2015).

Protein composition of fractions

To investigate the protein composition of each fraction, the samples were applied on a SDS-PAGE and Osborn's albumin fraction, and partially purified conglutin α, β, and γ were used as references (Figure 2). Fraction 1 (alkaline extract) is supposed to contain all conglutins present in lupin seeds. Consistently, this 1st fraction apparently presented the bands of the subunits of conglutin α, β, and γ, determined by comparison with the band profile of the partially purified conglutins (Figure 2A, lanes 6 to 8). Conglutin δ was also contained in this fraction, but it could not be visualized on a 10% SDS-PAGE due to the small size of its subunits (largest subunit 9 kDa and smallest subunit 4.5 kDa). Thus, a sample of the isolated protein was loaded on a 15% SDS-PAGE under nonreducing conditions and a band of approximately 13 kDa was observed, which matched the calculated molecular weight of the combined subunits of conglutin δ, based on the amino acid sequence, 13.5 kDa (Figure 2B). After isoelectric precipitation, clear differences were observed between the band profile of fractions 2 and 3 (Figure 2A, lanes 3 and 4). The bands that were assumed subunits of conglutin α and β were recovered in fraction 2. However, fraction 3 contained the bands related to the large subunit of conglutin γ (32 kDa) and an assumed albumin (28 kDa).

To have a better estimation of the identity of the bands observed in the SDS-PAGE, a 2DE image analysis was performed (Figure 3). The spot pattern between 69 and 39 kDa on the images of fraction 2 (Figure 3B) was identical with the spot pattern of partially purified conglutin α (red circle in Figure 3E) and conglutin β (blue circle in Figure 3F). The spot pattern of 32 kDa protein of Fraction 3 (Figure 3C) was identical with the spot pattern of Osborn's albumin fraction (purple circle in Figure 3D) and the large subunit of partially purified conglutin γ (green circle in Figure 3G). The existence of conglutin γ in fraction 3 was supported by the existence of spots of small subunit of conglutin γ on the image of the supernatant after isoelectric precipitation (19 kDa). Clear spots of conglutin γ were not observed on the image of the fraction 2. The 28 kDa protein was observed on the image of Osborn's albumin fraction (yellow circle on Figure 3D). Based on the results shown it was possible to conclude that the lupin protein isolate (fraction 2) of Alu*Prot*-CGNA® contains

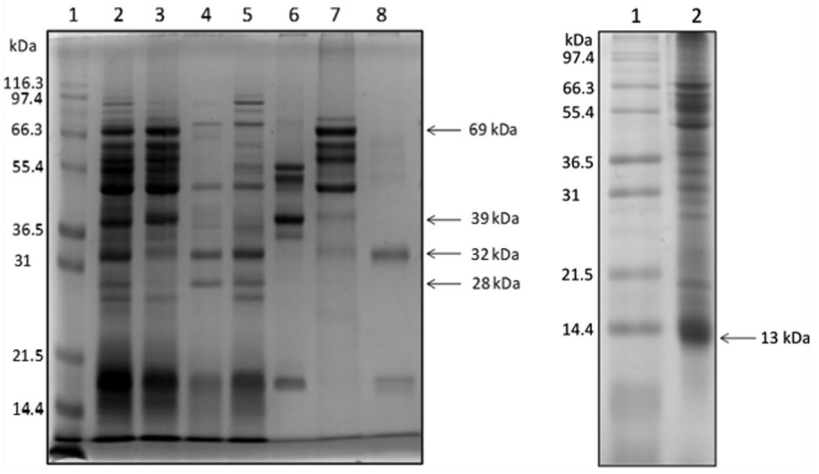

Figure 2–(A) SDS-PAGE (10%) of extracted fractions and partially purified conglutins of Alu*Prot*-CGNA CGNA® seeds stained with colloidal CBB. Lane 1: molecular mass markers; lane 2: alkaline extracts (fraction 1); lane 3: acid precipitated fraction (fraction 2); lane 4: acid supernatant fraction (fraction 3); lane 5: Osborn's albumin fraction; lane 6: partially purified conglutin α; lane 7: partially purified conglutin β; lane 8: partially purified conglutin γ. Partially published in Piornos and others (2015). (B) SDS-PAGE (15%) of extracted fraction 2 stained with colloidal CBB. Lane 1: molecular mass markers; lane 2: acid precipitated fraction (fraction 2).

α and β conglutin as described previously (Piornos and others 2015).

In terms of protein composition, conglutin α, β, γ, and δ are described in literature as the main proteins in lupin seeds (Duranti and others 2008). In this study, it was possible to identify the subunits of α and β conglutins present in fractions 1 and 2. It was observed that the band profile of these 2 conglutin families showed most of the major polypeptides described previously in literature for the proteins of *Lupinus luteus* (Ratajczak and others 1999).

Fraction 2 also contained conglutin δ, as shown in previous studies (Sironi and others 2005), but the complete confirmation of its presence was not possible due to the lack of partially purified reference. Nevertheless, a band of the corresponding size was observed on a 15% SDS-PAGE. Conglutin δ is rich in cysteine and, together with conglutin γ, is responsible for the sulfur-bearing amino acid content in lupin seeds. The yellow lupin has been shown to contain the highest levels of conglutin δ between sweet lupin species at transcript level (Foley and others 2015), and because the Alu*Prot*-CGNA® variety was derived from this species, a similar protein distribution pattern for this improved cultivar was expected. This feature becomes more important in the context of livestock and aquaculture feed, because other lupin species have always been deficient in sulfur-bearing amino acids and lysine. Because of the significant increase of protein content of this novel variety, the aforementioned amino acid deficit has been overcome and the amino acid profile of the seeds matches the requirements of the world animal feed industry.

The protein isolate obtained here as fraction 2 could be also beneficial for human health, because it was observed that comparable isolates from other lupin species had a cholesterol-lowering effect in hamsters, rats, and humans (Parolini and others 2012; Bähr and others 2013), which could give added value to our isolate in case of human comsumption.

Conglutin γ was not present in fraction 2 because it remained in the supernatant after isoelectric precipitation at pH 4.6, and it was a component of fraction 3. The isoelectric point of this

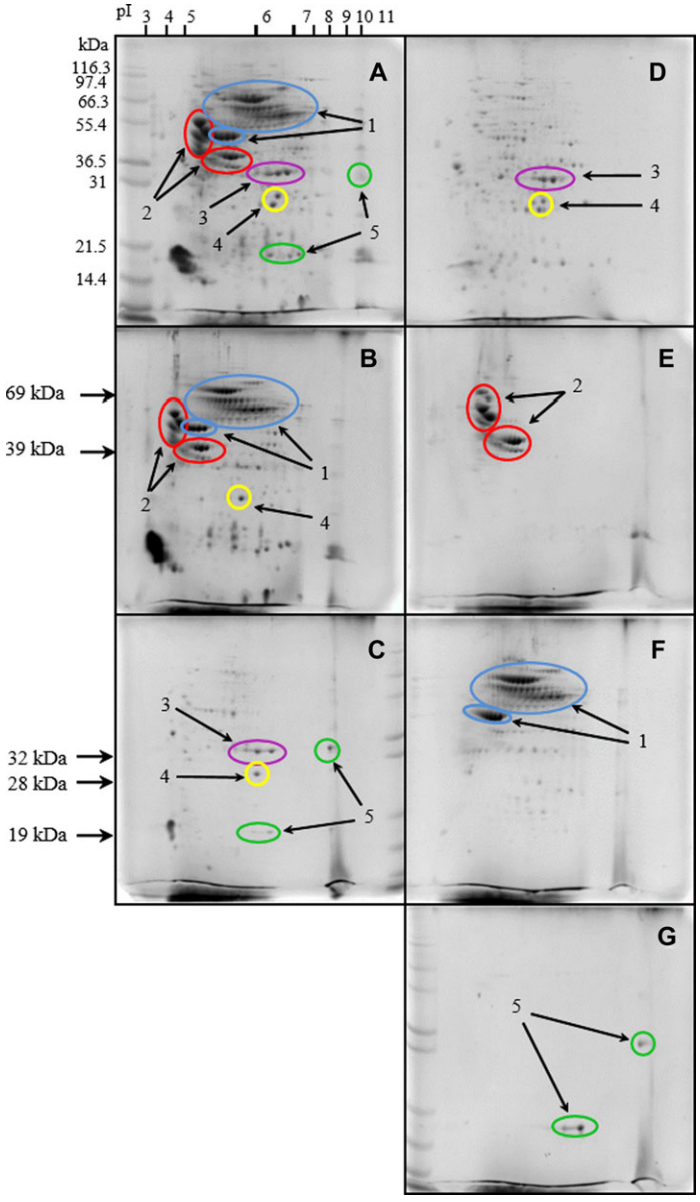

Figure 3–Bidimensional electrophoresis gel (2DE) of extracted fractions and partially purified conglutins of Alu*Prot*-CGNA® seeds. The proteins were visualized by colloidal CBB staining. (A) alkaline extracts (fraction 1); (B) acid precipitated fraction (fraction 2); (C) acid supernatant fraction (Fraction 3); (D) Osborn's albumin fraction; (E) partially purified conglutin α; (F) partially purified conglutin β; and (G) partially purified conglutin γ.

protein is rather basic (around pH 8); therefore, other steps would be required for its isolation as described previously (Sironi and others 2005). Obtaining an enriched product with this protein from Alu*Prot*-CGNA® could be of great interest because it has been associated to reduced glycaemia in rats by interacting with insulin in a similar way as the antidiabetes drug metformin (Lovati and others 2012).

Protein solubility profile of the fractions

The effect of pH on protein solubility was tested on the 3 obtained fractions (Figure 4). As shown in section 3.3, the main components of fractions 1 and 2 are quite alike. Hence, protein solubility patterns on both fractions were also very similar. The lowest solubility values were found at pH 5 (approximately 28% in both cases) and the highest solubility was observed at pH lower than 3 and higher than 6 (from 70% to 80%). Minimal solubility of proteins is related to the p*I*, where net charge of molecules is zero. Net charge of the main constituent proteins for fractions 1 and 2 is null between pH 4 and 5 (isoelectric point), thus the repulsive forces between protein molecules are reduced, promoting the aggregation and precipitation of the sample. At pH higher and lower than p*I*, proteins carry negative, and positive net charges, respectively, which favoured solubility. This behavior, which was observed for lupin's conglutins α, β and δ, was also reported for main proteins in other legumes, such as chickpea (Sánchez-Vioque and others 1999), lentil (Bora 2002), and other lupin species (Sironi and others 2005). Solubility of fraction 2 (protein isolate) was already reported in our previous study, but in that case we employed the Lowry method to determine the concentration of soluble protein (Piornos and others 2015).

Protein solubility of fraction 3 was not widely affected by pH changes, being higher than 65% for all the studied pH values. This aqueous fraction was mainly comprised by carbohydrates (42.79%) and proteins (35.98%), conglutin γ and albumins being their main components. Parekh (1991) proposed that glycosylation of conglutin γ in its natural conformation could be the reason for special characteristics of this glycoprotein. The oligosaccharide chain of lupin conglutin γ may contribute to the solubility of the protein and also to the unusual resistance to proteolysis (Duranti and others 1995). The methods usually employed to precipitate and separate conglutin γ are based on metal quelation using ions, mainly Zn^{2+}, which promote the selective reversible precipitation of this glycoprotein (Duranti and others 2001). Besides, the presence of polysaccharides in a protein solution can inhibit the precipitation of protein at pH values in the normal isoelectric range, and thus, it is possible to use polysaccharides to maintain a protein in solution under conditions that would normally lead to precipitation.

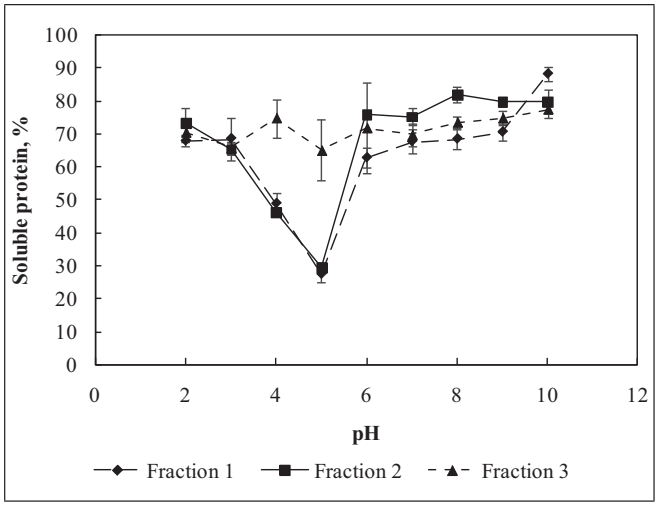

Figure 4–Influence of pH on protein fractions solubility. These results showed the mean of 3 determinations.

Effect of pH on emulsifying and foaming activity of Alu*Prot*-CGNA fractions

To explore the possible applications of the fractions from Alu*Prot*-CGNA as functional ingredient, the FC, FS, EC_{24}, and ES were evaluated (Table 2). The results showed that fraction 3 presented interesting foaming properties and good stability at all pH values evaluated. For example, FC and FS was mildly affected with pH variations and the behavior in both cases (FC and FS) was similar to the solubility profile curve (Figure 4), resulting on a low variation of the FC and FS values for all pH tested. This behavior could be attributed to the fact that conglutin γ, main protein present in this fraction, is not affected with pH variations (Duranti and others 2001). In addition, high concentration of polysaccharide (42.79%) present in this fraction could help to stabilize the system. According to Ye (2008), polysaccharides are often employed as stabilizers in order to improve the stability of foam and emulsions. Proteins and polysaccharides can form protein–polysaccharide complexes at determinate conditions of pH and ionic strength, being able to increase the viscosity of the dispersion medium and to form gel-like charged and thick adsorbed layers (Ye 2008). Note that the stability of the foam prepared with fraction 3 was higher than fractions 1 and 2, reaching, for example, an FS value of 100% at pH 3. However, fraction 1 showed the lowest FC values of all tested pH in comparison to the other fractions in the study.

Nevertheless, fraction 1 presented an unexpected behavior in the FC profile, increasing its values at pH values between 4 and 5, where its minimal solubility was observed. Normally, protein solutions obtained by solubilization/isoelectric precipitation show a decrease of its functional properties (in this case FS and FC values) at pH values close to p*I*, similar to that was obtained with fraction 2. This type of behavior could be attributed to the fact that fraction 1 is mainly composed by a complex mixture of proteins (conglutins α, β, and δ, and albumins), polysaccharides, and other minor water soluble compounds, which could interact and influence on the increase of FC at pH values between 4 and 5. According to Dee and others (2002), unfolding of a protein at its p*I* is likely to expose more sites (points) for protein-surface contact, resulting in a greater surface activity. Polysaccharides present in this fraction (36%) could interact with the exposed amino acids, and increase functional properties at this pH values. Regarding FS, the highest value was obtained at pH 4 (89.93 %). However, above this pH value, FS decreased progressively reaching a value of 38.43% at pH 9. A similar behavior was reported for extracts prepared directly from defatted cashew nut kernel powder (Ogunwolu and others 2009), presumably having a complex composition similar to fraction 1.

Table 2 also shows results obtained previously in our laboratory with fraction 2 (Piornos and others 2015). This fraction showed better FC values, presumably due to its high protein content, in comparison to fractions 1 and 3 for all pH values evaluated, except at pH values close to p*I* (4 and 5). However, foam formed with fraction 2 presented a poor stability after 2 h, reaching the

Table 2–Effect of pH on emulsifying and foaming activity properties of fractions.

		pH								
Functional property	**Fraction**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
FC (%)	F1	24.72_{bc}	20.68_{ab}	35.32_{e}	29.05_{cd}	19.09_{a}	26.14_{c}	28.43_{cd}	29.55_{cd}	32.57_{de}
	F2[a]	82.14_{a}	89.29_{b}	46.43_{c}	53.57_{d}	75.00_{e}	89.29_{b}	103.57_{f}	96.43_{g}	114.29_{h}
	F3	68.56_{a}	69.73_{a}	72.05_{ab}	68.56_{a}	69.73_{a}	72.97_{ab}	72.57_{ab}	77.62_{b}	70.89_{ab}
FS (%)	F1	86.31_{d}	83.33_{d}	89.93_{d}	75.93_{c}	76.67_{c}	60.71_{b}	56.08_{b}	38.43_{a}	41.48_{a}
	F2[a]	52.27_{a}	51.92_{a}	13.39_{b}	15.48_{b}	28.64_{d}	43.91_{e}	58.57_{f}	62.91_{g}	65.69_{h}
	F3	93.25_{abc}	100.00_{c}	98.41_{c}	88.25_{a}	93.24_{abc}	90.30_{ab}	95.07_{abc}	96.96_{bc}	93.64_{abc}
EC (%)	F1	59.73_{cd}	58.48_{bc}	66.22_{e}	62.16_{d}	56.76_{bc}	55.88_{b}	58.68_{bc}	56.73_{bc}	45.23_{a}
	F2	62.51_{b}	63.60_{b}	50.00_{a}	49.32_{a}	60.25_{b}	59.46_{b}	58.90_{b}	58.86_{b}	58.90_{b}
	F3	61.51_{c}	59.75_{bc}	64.47_{d}	65.33_{d}	59.46_{abc}	58.11_{ab}	56.76_{a}	57.13_{ab}	57.70_{ab}
ES (%)	F1	95.83_{b}	95.28_{b}	99.30_{b}	97.92_{b}	97.30_{b}	98.75_{b}	98.68_{b}	98.94_{b}	80.60_{a}
	F2	98.65_{b}	98.68_{b}	77.66_{a}	75.13_{a}	97.26_{b}	98.65_{b}	96.88_{b}	98.61_{b}	97.70_{b}
	F3	98.68_{a}	98.72_{a}	98.00_{a}	99.30_{a}	97.30_{a}	95.09_{a}	97.30_{a}	94.90_{a}	98.34_{a}

Means within the same row with different subscript are significantly different ($P < 0.05$). The SD values range from 0% to less than 5%, and thus are regarded as insignificant.
[a]Results from Piornos and others (2015).

lowest FC value (13.39% and 15.48%) at pH 4 and 5. Besides, the results obtained with this fraction presented an expected behavior, increasing FC and FS values below and above of its isoelectric point (V shape-profile).

In addition, Table 2 shows the results of EC_{24} and emulsifying stability (ES) for each fraction. Fractions 1 and 3 presented the same EC_{24} profile, showing an increase, whose highest values (>62%) were observed at pH values between 4 and 5. This behavior could be attributed to the same factors aforementioned in FC with fraction 1. Both fractions are composed mainly by a mixture of proteins and polysaccharides, the latter could help increase the stability and functionality of protein even at pH values close to its isoelectric point. However, fraction 1 showed a reduction of EC_{24} values at pH 10 (45.23 %). This decrease can be attributed to the interactive repulsive forces of polysaccharides and proteins at very alkaline pH (Ogunwolu and others 2009).

The effect of pH on EC_{24} was also evaluated for fraction 2, where a reduction of EC_{24} values were observed at pH values close to the p*I* of this fraction, between pH 4 and 5 (EC_{24}: ≤50%). In a previous study, where the emulsifying properties of fraction 2 were measured (Piornos and others 2015), the EC showed a similar trend when compared with the data obtained in this study. In this work, we evaluated the EC as the creaming index at 24 h after the preparation of emulsions and no significant differences were observed for most pH values with the exception of pH 4 and 5. Thus, the lowest EC_{24} values for fraction 2 are closely related to the protein solubility, which is minimal near the p*I*, suggesting a relation among EC_{24} and protein solubility (Lestari and others 2011).

Furthermore, Table 2 shows the effect of pH on ES for all protein fractions. The values showed that emulsions prepared with the 3 fractions presented good stability, reaching EC values above a 95% of stability with all evaluated samples. These high values of ES might be due to the fact that the emulsions were submitted only to a decantation process (see section "Emulsifying capacity and emulsion capacity") and they were not submitted to centrifugation or temperature.

The lowest ES values were obtained with fractions 1 and 2, whereas fraction 3 was not statically affected by pH variations. All ES values were close to 100%. As mentioned earlier, the polysaccharide/protein ratio of fraction 3 would favor the emulsions stability, which were stable for at least 7 d (data not shown). It is known that emulsions can be stabilized through a combination of proteins and polysaccharides, reducing surface tension between oil and aqueous phases and augmenting viscosity (Ye 2008).

However, the lowest ES value for fraction 2 (75.13%) was observed at pH 4 and 5, being in agreement with the low solubility of these fractions. At p*I*, repulsive forces between protein molecules are minimal, hence favoring fat globules' coalescence and destabilizing the emulsion (Yuliana and others 2014). It should be noted that, fraction 1 is composed mainly by protein (97.54%) and a little amount of polysaccharide (0.31%) in comparison to the others fractions. Therefore, at pH values close to p*I* (pH in which the solubility is minimal), the protein functionality is markedly diminished in the absence of polysaccharide.

Finally, fraction 3 showed to have great functionality as a foaming and emulsifying agent at all pH values tested in this study. Moreover, it was proved that this lupin fraction has potential in food industry because of its good surface activity, adding economical value to this otherwise waste of lupin protein isolation process.

To date, several food prototypes, using protein isolate from Alu*Prot*-CGNA®, have been developed and characterized in our laboratory (Food Technology and Process Unit, CGNA; Temuco-Chile). The potential emulsifying and foaming properties found with this protein isolate (Alu*Prot*-CGNA®) have allowed its use as a functional ingredient in order to prepare different food products to laboratory scale.

Conclusions

When we analyzed the properties of each fraction separately, it was possible to observe that fraction 1 was a mixture of compounds, with high protein content (69.70%; comprising all the globulins and many albumins) and also a significant amount of NNE (22.44%). The proteins of this fraction are responsible for the decrease in solubility at pH close to the p*I* of the main proteins. This fraction had the lowest FC of the 3 (19% to 35% depending on pH), but it seemed to have good EC and stability.

Fraction 2 had the highest protein content (97.5%) and neglectable NNE. The solubility of this fraction is strongly affected by the p*I* of its main proteins (α, β, and δ conglutins), which also influences its foaming and emulsifying capacities. Although fraction 2 was able to form foam and to emulsify, these capacities and the stability of them were reduced at the p*I* of the proteins. Nevertheless, this fraction was best suited for high protein content preparations.

However, fraction 3 had the lowest protein content (35.98 %), highest NNE (42.79 %), and fiber (5.05 %). This combination of elements conferred the fraction good foaming and emulsifying capacities, which were also very stable. This fraction was not affected by the p*I* of the constituent proteins. This overall stability made fraction 3 the best candidate for surfactant preparations. In addition, the main protein of this fraction was conglutin γ, which had been linked to glucose reduction in a similar way as antidiabetes drugs.

In this study, we could also see that several fractions isolated from lupin seed flour had good techno-functional properties with a great potential for usage in food industry.

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