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Functional and physicochemical properties of a protein isolate from Alu*Prot*-CGNA: A novel protein-rich lupin variety (*Lupinus luteus*)

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ABSTRACT

This study describes the isolation of proteins from the novel lupin variety Alu*Prot*-CGNA (*Lupinus luteus*) and the influence of pH and NaCl on their functional properties. Alu*Prot*-CGNA variety showed to have a great protein content in dehulled seeds (60.60 g protein/100 g, dry matter), which is higher than soybean and other lupin varieties. A lupin protein isolate (97.54 g protein/100 g) from Alu*Prot*-CGNA, LPIA, was prepared from lupin flour by alkali solubilization and isoelectric precipitation. The solubility profile of the LPIA was affected by pH, where the minimal values were observed at pH values close to its isoelectric point range (pH 4–5). The highest values of water absorption capacity (1.71 cm^3 H_2O/g protein), oil absorption capacity (1.43 g trapped oil/g protein), emulsifying capacity (61.94%), emulsion stability (96.43%), foaming capacity (114.29%), foam stability (65.69%) and least gelation concentration (20 g/100 cm^3) were observed at pH values lower and higher than its isoelectric point. In the presence of 100 mM of NaCl, their functional properties were improved. SDS-PAGE showed that LPIA mainly contained high molecular weight proteins (α and β-conglutin). These results are useful for increasing the utilization of this protein isolate as a potential functional ingredient in food industry.

1. Introduction

Proteins are often used as food ingredients for their functional properties and/or provide certain characteristics to the final products. These properties are intrinsic physicochemical characteristics, which affect the behavior of proteins in food systems, manufacturing, storage and preparation (Adebiyi & Aluko, 2011). In the formulation and development of traditional and novel food, emulsification, foaming and gelation are the most important functionalities of proteins. Therefore, it is of great importance to study the effect of environmental conditions, such as pH and ionic strength (NaCl concentration) on functional properties of proteins (Cano-Medina et al., 2011).

Proteins from animal sources are often used in food industry as functional ingredients. However, these proteins may contain large amounts of saturated fat and cholesterol. Therefore, proteins as an industrial ingredient have been turning towards plants as a preferred alternative to animal-based sources, due to increased consumers' concerns for the safety of animal-derived products, as well as their dietary preferences and food choices (Martínez-Villaluenga, Frías, & Vidal-Valverde, 2006). Consequently, vegetable proteins are increasingly being used as food ingredients due to their nutritional composition, satisfactory functional properties and their optimization of consumer's costs.

For example legumes are low-cost and the most common rich vegetable protein source, which constitute a large family of plants, such as bean (*Phaseolus vulgaris*), chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), soybean (*Glycine max*), pea (*Pisum sativum*), or lupin (*Lupinus* sp.). Lupin is an undervalued legume despite its high protein and dietary fiber content and potential health benefits (Lqari, Vioque, Pedroche, & Millán, 2002). The functional properties of some species of lupin have already been studied, such as *Lupinus angustifolius* (Chew, Casey, & Johnson, 2003), *Lupinus albus* (King, Aguirre, & De Pablo, 1985) and *Lupinus luteus* (Lampart-Szczapa et al., 2006). However, a full study of protein isolate functional properties, from *L. luteus* variety, has not yet been made. Major storage proteins in lupin seed are known as globulins, which are classified in four families: α-conglutins (11S globulins), β-conglutins (7S globulins), γ-conglutins (7S basic globulins), and δ-conglutins (2S sulfur-rich albumins) (Foley et al., 2011). γ-conglutins are peptides with reported bioactivity and health and pharmaceutical benefits (Duranti, Consonni, Magni, Sessa, &

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Scarafoni, 2008). Additionally, γ-conglutins are rich in sulfur-amino acids that are scarce in other grain legumes (Mubarak, 2001) and are also able to lower glycaemia (Capraro et al., 2013) and cholesterol (Fontanari, Batistuti, Da Cruz, Nascimento, & Gomes Arêas, 2012) in mammals.

Alu*Prot*-CGNA (*L. luteus L.*) is a regional legume crop from La Araucanía Region, Chile, which contains a large amount of protein (~60%) in its dehulled seeds. This high protein-yielding variety is oriented to salmon cluster, eggs farmers, milk and beef producers and human consumption, and it has been developed and registered (resolution number 47/13; -SAG, 2015) by Agriaquaculture Nutritional Genomic Center (CGNA). Consequently, it would be interesting to evaluate the functional properties of the novel lupin protein Alu*Prot*-CGNA. It should be noted that its proteins have not yet been used for this type of study. Therefore, the main purpose of the present study is to obtain a protein isolate from the new high protein content lupin and to evaluate the effect of environmental factors (pH and ionic strength) on functional properties. Preliminary results of this work will provide better understanding of possible applications of this proteins as functional ingredients in food industry.

2. Materials and methods

2.1. Materials

Alu*Prot*-CGNA® is a high protein-yielding variety released by the Agriaquaculture Nutritional Genomic Center (CGNA) in the Araucanía Region, Chile. Dehulled lupin seeds used in this study 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 (USA).

2.2. Preparation of protein isolate

Lupin protein isolate from Alu*Prot*-CGNA (LPIA) was obtained following the method described by Snowden, Sipsas, and St John (2007) with some modifications. First, the lupin flour was mixed with distilled water at 1:10 (w/v). The 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 ×*g* for 10 min. The supernatant containing the extracted proteins was recovered. Then, this supernatant was adjusted to pH 4.5 with HCl (1 M) to precipitate the proteins at the isoelectric point (pI). Then, proteins were separated by centrifugation at 3200 ×*g* for 15 min. The resulting protein was immediately lyophilized after preparation and stored at −20 °C ± 2 until use.

2.3. Chemical composition

Proximate analysis (moisture, ash, protein, total dietary fiber, fat, and nitrogen-free extract contents) was carried out according to the methods described in AOAC (1990).

2.4. ζ-potential measurement

This analysis was performed in order to determine the isoelectric point of LPIA. The determination of ζ-potential was carried out according to Burgos-Díaz et al. (2015) with some modifications. For these analyses, 10 mg/mL solutions of LPIA were prepared in milli-Q water. After wards, a Malvern Zetasizer Nano ZS series HT instrument (Malvern Instruments, U.K.) was used to evaluate ζ-potential as a function of pH (2–10) at 25 °C. It should be noted that pI corresponds to the pH value, in which the net charge of the molecules is zero, and consequently ζ-potential equal to zero.

2.5. Functional properties of protein isolate

2.5.1. Protein solubility profile

The method described by Were, Hettiarachchy, and Kalapathy (1997) was employed for determining the solubility profile of the protein isolate as a function of pH. The protein isolate (125 mg) was dispersed in 25 ml of distilled water and the pH of the solution was adjusted to 2–10, using NaOH (1 M) or HCl (1 M). The slurries were mixed for 1 h at 25 °C using a magnetic stirrer, before centrifuging at 8000 ×*g* for 10 min at 4 °C. Protein content in the supernatant was determined by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951).

2.5.2. Water absorption capacity (WAC)

The WAC was evaluated by the method of Beuchat (1977). One gram of LPIA was vigorously mixed with 10 cm^3 of distilled water for 30 s, and left to stand for 30 min. The slurries were centrifuged at 5000 ×*g* for 30 min. The volume of non-absorbed water was measured using a graduated cylinder. The WAC was expressed as cm^3 of absorbed water per LPIA gram. The effect of different pH (2–10) values and NaCl (100–500 mM) concentrations on the WAC were tested.

2.5.3. Emulsifying capacity (EC) and emulsion stability (ES)

The effect of different pH (2–10) values and NaCl (100–500 mM) concentrations on the EC and ES were tested. For this, EC and ES were determined using the method described by Chau and Cheung (1998) with some modifications. Thus, 5 mL of aqueous solution (containing 10 mg/mL of LPIA in distilled water) and 5 mL of sunflower oil were emulsified for 3 min at 10,000 rpm, using a 400DS homogenizer (PRO Scientific Inc., Oxford, CT, USA). The samples were then centrifuged (1000 ×*g*, 5 min) and the EC was calculated by dividing the height of the emulsion layer by the total height of the emulsion. Emulsion stability (ES) was determined by heating the emulsion at 80 °C for 30 min in a water bath and then centrifuged (1000 ×*g*, 5 min). ES was measured dividing the height of the emulsion layer after heating by the height of the emulsion before heating. Both results were expressed as percentage (%).

2.5.4. Foaming capacity (FC) and foam stability (FS)

The effect of different pH (2–10) values and NaCl (100–500 mM) concentrations on the FC and FS were tested. FC was determined following the method described by Chew et al. (2003). Foam formation was carried out using a 400DS homogenizer (PRO Scientific, Inc., Oxford, CT, USA). Thus, 20 mL of protein solution, containing 20 mg/mL of LPIA, were homogenized at 10,000 rpm for 1 min. FC was calculated as follows:

$$FC(\%) = \frac{V_1\text{-}V_2}{V_2} \times 100 \quad (1)$$

where V_1 is the volume after foam formation and V_2 is the initial volume. FS was evaluated measuring the total volume 2 h after foam formation (V_3) and calculated using the equation proposed by Yuliana, Truong, Huynh, Ho, and Ju (2014) (Eq. (2)).

$$FS(\%) = \frac{V_3\text{-}V_2}{V_1\text{-}V_2} \times 100 \quad (2)$$

2.5.5. Gelation properties

Gelation properties of protein isolates were determined according to Lawal (2004). LPIA suspensions (2 − 20 g/100 cm^3) were prepared in distilled water. Thus, 10 cm^3 of protein solution was transferred into a test tube and heated for 1 h in a dry bath at 100 °C, followed by rapid cooling in cold water bath. Test tubes were further cooled at 4 °C for 2 h. The least gelation concentration (LGC) was considered as the lower concentration when the sample did not fall or slip from the inverted tube.

2.5.6. Oil absorption capacity (OAC)

The OAC was determined following the method described by Chavan, McKenzie, and Shahidi (2001). LPIA (0.5 g) was mixed with sunflower oil (5 cm^3) for 1 h at 30 °C. The slurry was centrifuged (1500 ×*g*, 30 min) and the non-retained oil was decanted. The LPIA with trapped oil was then weighed. The OAC was expressed as grams of trapped oil per gram of LPIA.

2.6. Bulk density

Bulk density of the lyophilized LPIA was determined using the method described by Wang and Kinsella (1976) with modifications. LPIA powder (10 g) was placed in a graduated cylinder and tapped until volume was constant. Bulk density was calculated as the weight of LPIA divided by the final volume of the sample.

2.7. SDS-PAGE

Protein composition of the LPIA was determined following SDS-PAGE method described by Laemmli (1970). For this analysis, the protein sample was prepared by dissolving a piece of LPIA in distilled water, containing 50 mM DTT and 20 mM EDTA at pH 9. Then, proteins were separated by SDS-PAGE using a 10% (w/v) T, 2.6% (w/v) C polyacrylamide gel on a SE 260 mini-vertical gel electrophoresis unit (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA). Protein bands on the gel were visualized by colloidal CBB staining, and the gel was digitalized using LAS 3000 (Fujifilm Co. Ltd., Tokyo, Japan). The standards used in this analysis, Osborn's albumin and α, β and γ conglutins, were partially purified following the method described by Bush and Tai (1994), with slight modifications.

2.8. Statistical analysis

Each determination was performed in triplicate and the data subjected to a one-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 2010.

3. Results and discussion

3.1. Lupin dehulled seeds and protein isolate chemical composition

Table 1 shows the estimated composition of seed flour and protein isolate from the lupin variety (Alu*Prot*-CGNA). The results showed that the main components of the dehulled seeds were protein (60.60 g/100 g) and nitrogen-free extract (22.42 g/100 g). The protein content obtained with Alu*Prot*-CGNA was higher than other lupin varieties (mean average 45.6 g/100 g) and commercial soybean (mean average 49.2 g/100 g) (Bähr et al., 2014). On the other hand, in LPIA the content of protein increased up to 97.54%, and a reduction of other components, such as soluble sugars and fat, was observed with respect to the flour. Additionally, the aqueous extraction process used to obtain the isolate protein in lupins could also reduce the content of antinutritional constituents, such as α-galactosides (Martínez-Villaluenga et al., 2006).

Table 1
Chemical composition of dehulled yellow lupin flour, variety Alu*Prot*-CGNA (dry weight basis).

Component quantity, g/100 g	Lupin flour	Lupin protein isolate, LPIA
Protein	60.60 ± 2.40	97.54 ± 1.64
Fat	4.95 ± 1.63	0.55 ± 0.01
Total dietary fiber	7.14 ± 1.35	0.18 ± 0.00
Ash	4.90 ± 0.42	1.42 ± 0.05
Nitrogen free extract	22.41	0.31

These results showed the mean of three determinations.

SDS-PAGE analysis was performed in order to investigate the molecular weights of Alu*Prot*-CGNA protein isolate components. Osborn's albumin fraction and partially purified α, β, and γ-conglutins were used as standards. The results showed that major bands in LPIA were observed between 39 kDa and 69 kDa (Fig. 1). According to these results and after the comparison with standards used in SDS-PAGE analysis, LPIA was composed mainly of high molecular weight proteins α and β-conglutins. It should be noted that α, β and γ-conglutins are major proteins in lupin seed (Duranti et al., 2008). Additionally, these results are in accordance with those reported by Wäsche et al. (2001), which determined that high molecular weight proteins, such as α and β-conglutins in white lupin (*L. albus*) can be separated using alkaline extraction and isoelectric precipitation procedures.

3.2. Protein solubility profile

Fig. 2a shows the results of pH influence on LPIA solubility. Protein solubility was lower at pH values between pH 4 and pH 5 (~8%), which can be attributed to its pI. However, with the subsequent increase or decrease of pH values (pH < 4 or pH > 5) protein solubility values increased progressively. The highest protein solubility value for LPIA was observed at pH 10 (89%). Proteins are considered as biological amphoteric molecules because the net charge of the molecule is affected by pH, thereby in an acidic medium, protein carries positive charges at the amino group ($-NH_3^+$), and it carries negative charges at the carboxylic group ($-COO^-$) in an alkaline medium. On the contrary, the net charge of the protein at pI is zero, and the repulsive forces between proteins decrease hence promoting protein aggregation and even precipitation. LPIA solubility pattern, as a function of pH, was similar to that observed for protein isolates with other legumes, such as chickpea (Sánchez-Vioque, Clemente, Vioque, Bautista, & Millán, 1999), soybeans (Achouri, Zhang, & Shiying, 1999), cowpea (Prinyawiwatkul, McWatters, Beuchat, & Phillips, 1997), peanut (Beuchat, 1977) and lentil (Bora, 2002).

Fig. 2b shows the influence of pH on ζ-potential (electric charge) of the protein. It can be seen that at pH 4.6 the electric charge of protein was equal to zero, which was attributed to proteins' pI (Dukhin &

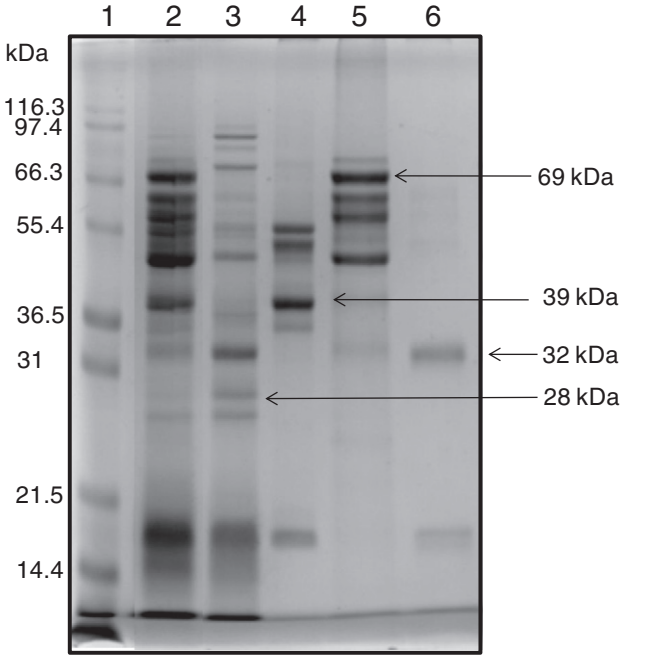

Fig. 1. Coomasie-stained SDS-PAGE profile of *AluProt*-CGNA lupin protein isolate. **1**: molecular weight marker; **2**: lupin protein isolate; **3**: Osborn's albumin fraction, **4**: partially purified α-conglutin; **5**: partially purified β-conglutin; **6**: partially purified γ-conglutin.

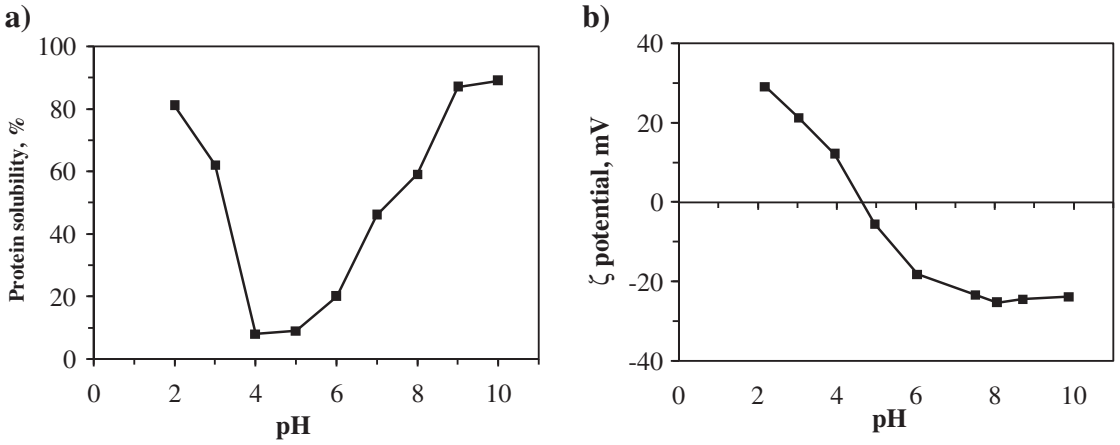

Fig. 2. Influence of pH on protein solubility (a) and ζ-potential (b). These results showed the mean of three determinations. The SD values range from 0 to less than 5%, and thus are regarded as insignificant.

Parlia, 2014). This result is in agreement with the solubility profile obtained in this study, where lower ζ-potential values were found at pH values between 4 and 5. On the other hand, at pH values lower and higher than its pI, the ζ-potential values increased until values close to +30 or −30 mV, respectively. According to Bouyer, Mekhloufi, Rosilio, Grossiord, and Agnely (2012), when all particles present a large positive or negative ζ-potential (greater or lower than +30 mV and −30 mV, respectively), they will repel each other and the dispersion tends to be stable. On the contrary, when particles show low absolute ζ-potential values, there will be no sufficient force to prevent the particles from aggregating.

3.3. Influence of pH on functional properties of the proteins

Table 2 shows the effect of pH on functional properties, such as water absorption capacity (WAC), emulsifying capacity (EC), emulsion stability (ES) and foaming capacity (FC) of the isolated protein. LPIA exhibited WAC pH-dependence, with a minimal value (0.90 cm^3 H_2O/g protein) at pH 5, which may be attributed to the fact that at pI proteins tend to aggregate, decreasing the interaction with water and thus decreasing WAC. However, at extreme acidic and alkaline pH values WAC increased markedly due to protein unfolding, hence their charged functional groups show a higher exposition to hydration (Wani, Sogi, Shivhare, & Gill, 2014). The highest WAC values were found at pH 2 (3.01 cm^3 H_2O/g protein) and pH 10 (1.71 cm^3 H_2O/g protein). At neutral pH, WAC was equal to 1.68 cm^3 H_2O/g protein, higher than that of *L. angustifolius* protein isolates (0.24 to 0.45 cm^3 H_2O/g protein) (Lqari et al., 2002) and lower than that of protein isolated from defatted cashew nut shell (2.56 cm^3 H_2O/g protein) (Yuliana et al., 2014). WAC is an index of the ability of proteins to absorb and retain water, which in turn influences the texture and mouth feel characteristics of foods and food products, like comminuted meats, extenders or analogues, and baked dough (Adebowale, Adeyemi, & Oshodi, 2005).

To explore the emulsifying properties of LPIA, EC and ES were determined. Table 2 shows that the lowest EC value was found at pH 4 (24.10%), whereas the highest EC value (59.94%) was obtained at pH 7. However, in further studies on EC at pH 12, LPIA showed a higher value (61.94%) (Datum not shown in Table 2). The lowest EC observed at pH 4 can be attributed to the pI of the protein (pH 4.6), in which the net charge is equal to zero, and therefore, its colloidal repulsions are weak. According to Lestari, Mulder, and Sanders (2011), EC is closely related to the amount of soluble protein in the solution. In fact, protein unfolding occurs at extreme alkaline or acidic pH values, exposing the most lipophilic functional groups present in proteins. A more soluble protein produces a rapid migration to the oil–water interface, hence favoring the formation and stability of emulsions. Several studies have shown that there is a relationship between EC and protein solubility (Chavan et al., 2001; Lawal, Adebowale, Ogunsanwo, Sonsanwo, & Bankole, 2005; Sánchez-Vioque et al., 1999).

Additionally, Table 2 shows that ES values were between 94.49% and 96.56%, at all pH values tested, except at pH 4 where the lowest ES value (12.56%) was observed. These results are in agreement with pI and solubility of LPIA. Thus, at pI the repulsive forces of proteins are weak, favoring droplets emulsion coalescence and hence a poor emulsifying stability. On the contrary, when the electrical charge around oil droplets is high, repulsion forces help maintaining droplet repulsion and retarding emulsion coalescence (Yuliana et al., 2014). Although the highest EC value for LPIA (61.91%) is lower than that for soy protein isolate (74.7%), as an emulsifier, LPIA formed more thermally-stable emulsions than soy protein isolate (73.2%), except at pI (Franzen & Kinsella, 1976).

Table 2 shows that the lowest FC value was observed at pH 4 (46.43%), which could be attributed to protein pI. On the other hand, FC increased when pH values were lower and higher than its pI, reaching the highest FC (114.29%) at pH 10. Foam capacity is closely related to the concentration of soluble protein, because soluble proteins can reduce surface tension at the interface between air bubbles,

Table 2
Influence of pH on functional properties of lupin protein isolate.

Protein properties	pH								
	2	3	4	5	6	7	8	9	10
WAC, cm^3 H_2O/g protein	3.01^a	1.31^b	1.09^c	0.90^d	1.29^b	1.68^e	1.70^e	1.70^e	1.71^e
EC, %	57.68^a	59.92^a	24.10^b	59.60^a	58.79^a	59.94^a	58.70^a	53.78^c	55.02^c
ES, %	95.12^a	96.43^a	12.56^b	95.16^a	94.49^a	95.76^a	95.05^a	96.08^a	95.91^a
FC, %	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
FS, %	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
LGC, g/100 cm^3	16	14	14	14	18	20	20	20	20

Means within the same row with different superscripts are significantly different ($p < 0.05$). The SD values range from 0 to less than 5%, and thus are regarded as insignificant.

increasing the FC of proteins (Adebowale & Lawal, 2003). However, at pH values close to pI, protein solubility is minimal, resulting in a low FC value. Additionally, the increase in FC with pH could be due to the increased net charge of the proteins, which weakened the hydrophobic interactions and increased protein flexibility. This allows the protein to diffuse more rapidly to the air-water interface to encapsulate air particles, enhancing foaming (Khalid, Babiker, & El Tinay, 2003). It should be noted that, the results obtained for LPIA were higher than commercial soybean concentrate (16.0 to 92.0%) (Cano-Medina et al., 2011).

On the other hand, a dependence of the FS with respect to pH was also observed, showing a profile similar to protein solubility. The lower FS value was registered at pH 4 (30.68%) because of the protein's pI. However, FS increased at higher and lower pH values, with a maximum FS value at pH 10 (72.56%). FS not only depends on solubility and flexibility of proteins, but also on rheological characteristics of foam (Deng et al., 2011). Thus, higher protein solubility gives a higher viscosity to solution and thereby stiffer foam structure.

Table 2 shows the results of gelation of LPIA at different pH (2–10) and concentrations of protein (2–20 g/100 cm^3). The results were expressed as LGC (least gelation concentration), which refers to the minimal concentration that generated a firm gel. Therefore, a low value of LGC means a better gelling ability of the protein. Lower values of LGC (14 g/100 cm^3) were found at pH range from 3 to 5, while LGC increased to 20 g/100 cm^3 for pH values above 7. At the pI zone, protein aggregation is higher due to the fact that its net surface electrical charge is equal to zero, decreasing intermolecular forces (minimal electrostatic repulsion),and therefore facilitating of gelation of proteins. At pH 7 LGC was 20 g/100 cm^3, higher than protein isolates from *L. angustifolius* (Lqari et al., 2002). After the heating process, partial denaturation of protein allows exposing functional groups, with which proteins interact and generate the three-dimensional gel structure. Thus significant amounts of water are retained into the gel structure, transforming liquid sample to solid. Gelation properties of proteins are attributed to a combined effect of chemical transformations such as denaturation, aggregation and network formation (Kaur & Singh, 2007).

3.4. Influence of NaCl concentration on the functional properties of proteins

NaCl is a common ingredient used in food industry to enhance flavor, preserve food, and improve processing but is a strong modulator of protein functionality. Results showed a dependence of NaCl concentrations on WAC of the lupin protein isolate (Table 3). Adding 100 mM of NaCl WAC increased from 1.55 cm^3 H_2O/g protein to 1.91 cm^3 H_2O/g protein. From 200 mM to 500 mM of NaCl WAC decreased progressively until stabilization (1.75 cm^3 H_2O/g protein). According to Khalid, Babiker, and El Tinay (2003), at low salt concentration, the interactions between protein molecules and NaCl increases protein solubility and hydration. However, when the salt concentration increases, the intermolecular interaction among proteins are strengthened and lead to the dehydration of the protein and a reduction in WAC.

Table 3 shows that in the presence of salt (100 mM NaCl), EC increased from 56.81% to 62.75% (pH 7 and 10 g/L of LPIA). However, when NaCl concentrations were higher, EC decreased. As reported by Lawal (2004), the positive effect of salt ions is due to the increase in protein solubility. The increase of ionic strength, up to 100 mM, enhanced unfolding of proteins and subsequent exposure of functional groups to the environment. Therefore, proteins are able to interact with water and oil phases forming more stable emulsions. However, screening effect exceeds the benefits of unfolding over 100 mM NaCl and EC decreases.

Table 3
Influence of NaCl concentration on functional properties of lupin protein isolate.

Protein properties	[NaCl], mM					
	0	100	200	300	400	500
WAC, cm^3 H_2O/g protein	1.55^{a}	1.91^{b}	1.95^{b}	1.85^{c}	1.75^{d}	1.76^{d}
EC, %	56.81^{a}	62.75^{b}	59.55^{c}	55.88^{a}	55.48^{a}	55.16^{a}
ES, %	$93.76^{a,b}$	98.79^{c}	96.14^{a}	95.43^{a}	92.40^{b}	94.36^{a}
FC, %	90.48^{a}	136.41^{b}	84.95^{c}	78.02^{d}	84.98^{c}	82.60^{c}
FS, %	37.07^{a}	51.85^{b}	40.83^{c}	40.00^{d}	16.67^{e}	14.29^{f}
LGC, g/100 cm^3	20	18	18	20	20	20

Means within the same row with different superscripts are significantly different ($p < 0.05$). The SD values range from 0 to less than 5%, and thus are regarded as insignificant.

A similar trend was observed with ES, where the maximal ES value (98.79%) was observed at 100 mM. The effect of NaCl concentration on ES may be attributed to the formation of a charged ion layer around the emulsion droplets (Kinsella, Damodaran, & German, 1985). This way, the repulsion forces are more intense among fat globules and the emulsion becomes more stable against coalescence. For NaCl concentrations above 100 mM, the salting-out effect resulted in a reduction of ES values.

FC was also evaluated, the maximal FC value (136.41%) was observed at 100 mM of NaCl (Table 3). Above this salt concentration, FC decreased to 82.60% at 500 mM. The addition of salt (up to 100 mM) weakens the hydrophobic binding among protein molecules, enhancing the interaction through hydrophilic functional groups. This better cohesion between proteins improves rheological properties of foam (Yuliana et al., 2014). However, Lawal et al. (2005) reported that at high NaCl concentration, salt ions screen protein interaction because of salting-out effect, even promoting aggregation, precipitation and flocculation.

The highest FS value (51.85%) was observed at 100 mM of NaCl. This may be attributed to high protein solubility, enhancing the rheological properties of the protein solution. A more viscous solution forms stiffer foam, thus increasing the stability of the water-air protein structure (Lawal, 2004).

The effect of NaCl concentration on LGC was studied at pH 7. LGC decreased from 20 to 18 g/100 cm^3 with an addition of NaCl up to 200 mM. The decrease of LGC, by means of low addition of salt ions, is due to the enhancing of unfolding hidden functional groups of proteins. Thus, interactions among protein molecules can be improved by facilitating electrostatic bindings. Above 200 mM NaCl, the activity of the functional groups was reduced because of the salting-out effect. Protein gelation is a particular type of cross-linking that implicates different molecular forces, such as hydrogen (Elridge & Ferry, 1954) and disulfide bonds (Huggins, Tapley, & Jensen, 1951).

3.5. Oil absorption capacity

The oil absorption capacity (OAC) of proteins is an important functional property to prepare products based on emulsions in food industry. The oil absorption capacity of LPIA was 1.43 g trapped oil/g protein. This value was lower than other protein isolates, such as kidney bean protein isolate (5.82–7.99 g/g) (Wani et al., 2014) or Indian chickpea protein isolates (2.08–3.96 g/g) (Kaur & Singh, 2007). Similar OAC values (1.59–2.58 g/g) were reported for adzuki bean protein isolate by Tjahjadi, Lin, and Breene (1988). According to Sathe, Deshpande, and Salunkhe (1982), the OAC can be related to the amino acid composition of proteins, in particular to hydrophobic residues that interact with hydrocarbon chains in fat molecules.

3.6. Bulk density

Bulk density is a very interesting property used to characterize powder products and it is of great importance for economical and functional reasons, for example, for reducing packaging costs. The LPIA bulk density was found to be 0.535 $g \cdot cm^{-3}$. This value was lower than soybean protein isolate (0.678 $g \cdot cm^3$) (Al-Kahtani & Abou-Arab, 1993) and higher than lupin flour (0.39 $\cdot$ g $\cdot$ cm^3) (Cerezal, Urtuvia, Ramírez, & Arcos, 2011). Bulk density depends on the combined effects of interrelated factors, like particle size, number of contact point, and intensity of attractive inter-particle forces (Peleg & Bagley, 1983).

4. Conclusion

A protein isolate was successfully obtained from a novel lupin variety (*L. luteus* cv. Alu*Prot*-CGNA), reaching high protein content (97.54 g protein/100 g). The SDS-PAGE analysis showed that the LPIA was composed mainly by α and β-conglutins, therefore, LPIA functional properties might be attributed to this type of proteins. The highest values of protein solubility, EC, ES, FC, FS and WAC were observed at acidic and alkaline pH values. Adjusting pH and NaCl concentrations the characteristics of this protein product could be improved by affecting its solubility, water absorption capacity, foaming and emulsifying properties. The protein isolate obtained from the novel high yielding protein crop (Alu*Prot*-CGNA) studied in this work, could be employed in the preparation of different products where the functional properties of proteins have an important role in the texture and quality of foods.

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