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Comparative study of functional properties of protein isolates obtained from three *Lupinus* species

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ABSTRACT

Protein isolates from *Lupinus albus*, *Lupinus splendens* Rose and *Lupinus spp.*, seeds, were obtained by isoelectric precipitation of its proteins at pH 4.4, 4.8 and 4.1, respectively. The recovered protein was greater from *L. albus* ($93 \pm 0.77\%$), and the protein content in the protein isolates was greater than 90%. Solubility, emulsion activity index (EAI), emulsion stability index (ESI), foaming capacity (FC) and foam stability index (FSI) of the protein isolates were affected by pH, all of them were minimal at isoelectric pH range (pH 4-5), and increases toward both ends of pH scale. The water absorption average was 2.46 mL/g of protein and no significant difference ($p < 0.05$) between them. *Lupinus spp.*, protein isolate had greater oil absorption (4.69 mL/g protein), whereas the *L. splendens* Rose protein isolate had highest EAI at all pH values, except pH 2, and *L. albus* protein isolate showed the highest ESI at all pH values, except at pH 5 and 8. *L. splendens* Rose protein isolate had greater FC at all pH values, but *L. albus* protein isolate had higher FSI at all values of pH, except pH 10. The gelling minimum concentration was 7.5, 9.0 and 11% w/v for the protein isolates from *L. albus*, *L. splendens* Rose and *Lupinus spp.*, respectively. Thus the protein content as some functional properties of the protein isolates obtained from different *Lupinus* seeds, are similar to those having the soy protein isolates, primary source for obtaining commercial of these additives in the food industry.

Keywords: Protein isolates, Functional properties, *L. albus*, *L. splendens* Rose,

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List of Non-Standard Abbreviations: Emulsion activity index (EAI); Emulsion stability index (ESI); Foaming capacity (FC); Foam stability index (FSI); Gelling minimum concentration (GMC); Water absorption capacity (WAC); Oil absorption capacity (OAC); *Lupinus* protein isolates (LPI)

INTRODUCTION

The rapid global population growth determines the need for new protein sources to supplement existing. Currently interest has been directed towards the use of vegetable protein, where legumes and oilseeds are an important source of these macromolecules [1]. Soybeans, canola, cotton, sunflower and groundnut are the most common sources of vegetable proteins and account for 69, 12.4, 6.9, 5.3 and 2.8% of world production of vegetable proteins, respectively [2]. Soy is the main oilseed used in our country for the production of proteins, in 2010 its demand amounted to 4 million tons of which were produced only 168,000 tons (4.2%), the remaining 3,832,000 tons (95.8%) were imported [3].

Some research indicates that the genus *Lupinus* could be the possible replacement for soy, both in food and feed [4]. Around the world have been reported more than 400 species of the genus *Lupinus* which are distributed in two centers of origin, one is the Mediterranean and another over South America. Only 12 species found in Europe and Africa, but more than 300 species are found in America [5].

Lupinus is a legume whose seeds do not have starch and its biochemical composition is very similar to soybean, especially in its high protein content (35-40%). Additionally *Lupinus* may develop in soils and climates where soy fails to grow [6]. The seeds of this species also contain from 4 to 15% oil, which is

composed of 50-60% oleic acid, 16-23% linoleic acid and 8-9% is α -linolenic acid. His balance of fatty acids Ω -3 and Ω -6 is also similar to soybean oil, which presumably suggests that the health benefits of both types of oils are also similar [7].

Despite the significant nutritional attributes, the low utilization of this legume is due to its high content of anti-nutritional compounds, mainly alkaloids and tannins, so for its use is necessary to reduce the content of these compounds at levels below the limit of toxicity and sensory perceptibility. Among the most common technological processes of removal of these secondary compounds are: its detoxification by heat treatment and soaking in water, its removal by chemical or lactic fermentation and, by obtaining concentrates or protein isolates [8].

The process of obtaining protein isolates involves a series of steps designed to eliminate or reduce non-protein components to obtain a final product with protein content greater than 80%. This process is carried out by two successive operations. In the first, the proteins are solubilized to separate from other non-soluble compounds, mainly insoluble carbohydrates. The second step concerns the concentration of proteins. Today, the procedures are: a) isoelectric precipitation of the proteins and their separation by centrifugation, and b) protein concentration by ultrafiltration [9].

These protein isolates are composed by globulin fractions mainly which determine its technological and functionality properties, which are related to physical and chemical characteristics of proteins such as size, shape, composition, amino acid chain, net charge and charges distribution; ratio hydrophobicity/hydrophilicity, secondary structure, tertiary and quaternary, flexibility, rigidity and the ability to interact with other components [10]. Extrinsic factors such as: nature of the solvent, temperature, pH, ionic strength, divalent cations, denaturants and the presence of other macromolecules also affect technological and functionality properties of these products [10]. Techno-functional properties influence behavior during processing, storage, cooking and consumption of food [2]. The techno-functional properties most important in food processing are: solubility, water absorption, oil absorption, emulsifying capacity, foaming and gelling [1].

The aim of this study was to obtain, evaluate and comparing the techno-functional properties of protein isolates obtained from three *Lupinus* species.

MATERIALS AND METHODS

Plant materials

Lupinus albus seeds were donated by the Autonomous University of Guadalajara, *Lupinus splendens* Rose and *Lupinus sp.*, seeds were collected in Hidalgo state, Mexico.

Flours preparation

Dry seeds of *Lupinus albus*, *Lupinus splendens* Rose and *Lupinus sp.*, were pulverized in a mill micropulverizer Micron K-1 (Micropulverizadores Ltd., Mexico) and then were sieved with a 200 mesh. The flours obtained were packed in airtight containers properly identified and stored in a cool dry place for later use.

Chemical proximal analysis of *Lupinus* seeds

Proximal chemical analysis of the different flours was performed according to the methodology proposed in the Official Methods of Analysis [11]. With this methodology were determined the content of moisture, ash, fat, protein (N x 6.25) and crude fiber, while the carbohydrate content was determined by difference.

Obtaining of protein isolates

Previously flours of different varieties of *Lupinus* were detoxified [12] and defatted with hexane reagent grade (98.5%) with continuous agitation (700 rpm) for 8 h at 4°C [9]. Protein isolates from the different flours were obtained by isoelectric precipitation of the proteins [13], which was performed as follows: dispersions of flour in water at 10% (w/v) were made and their pHs were adjusted to 9 by the addition of 0.1N NaOH. The suspensions were shaken at 700 rpm for 30 minutes at room temperature and then were centrifuged in a Beckman model J2-MC centrifuge at 10,000 xg for 15 min. The precipitate was removed and supernatant was filtered through Wattman No.1 paper. Later the isoelectric point of the proteins contained in the different supernatant were determined, understood this as the pH at which the maximum occurs protein precipitation after maintaining the suspension at 4°C overnight. For this, in 11 Erlenmeyer flasks were placed 25 mL of supernatant whose pH were adjusted to 4.0, 4.1, 4.2, ... 5.0 respectively, by addition of 0.1N HCl. The flasks were manually shaken to homogenize the contents and kept to stand at 4 ° C overnight to allow the precipitation of proteins. The protein precipitate was filtered through Wattman No.1 paper and the precipitates were dried in oven at 70°C to constant weight.

Once known the isoelectric point of the proteins contained in the different flours, we proceeded to obtaining of protein isolates from three *Lupinus* species studied for which, applying the above procedure to the point where the supernatants are filtered through Wattman No.1 paper, the pH of the supernatants was adjusted with 0.1 N HCl to its respective isoelectric point determined previously and, after shaking

manually and maintain stand at 4°C overnight so that the precipitation of the proteins is carried out, the suspensions were centrifuged at 10,000 × g at 4°C for 10 min, after which, the protein precipitate was resuspended in water and freeze dried in a lyophilizer Freezone model 18 (Labconco, USA). Finally protein isolates obtained were stored in airtight containers properly identified and stored at -20°C until further use. The determination of the recovered protein was performed using the following equation [14].

$$P_R = \frac{(P_1)(E_P)}{(P_2)(P_{HD})} \times 100$$

Where P_R is the recovered protein (%), P_1 is the protein content in the lyophilized pellet (%), E_P is lyophilized pellet weight (g), P_2 is the protein content in the defatted flour (%) and P_{HD} is the weight of the samples to obtain the defatted protein isolates (g).

Functional properties

Solubility

Solubility of the different *Lupinus* protein isolates (LPI) was determined in the pH range of 2.0-10.0. For this, dispersions 10 mL (0.1% w/v) of the different LPI obtained were prepared, using triple distilled water at room temperature as a dispersing agent. The dispersion pH was adjusted to 2, 3, 4, ... 10, by addition of 0.1N HCl or 0.1N NaOH, keeping the dispersion under continuous stirring for 30 minutes. Subsequently the samples were centrifuged at 10,000 rpm for 10 minutes [15]. The protein content in the supernatant was determined by the method of Bradford using a standard curve of bovine serum albumin as standard and a Perkin Elmer spectrophotometer model Lambda XLS (USA), to determine the samples absorbance at 595 nm. The equation of type curve fit was: $y = 0.0201 x + 0.0354$ with $R^2 = 0.993$. The percent solubility of the protein isolated was determined with the following equation [16].

$$\text{Solubility} = \frac{\text{Protein in the supernatant (g)}}{\text{Protein in the sample (g)}} \times 100$$

Water and oil absorption capacity

The water absorption capacity (WAC) and the oil absorption capacity (OAC) of different protein isolates obtained, was determined as follows. Centrifuge tubes for 50 mL weighed 0.5 g of each protein isolates obtained and mixed with 5 mL of triple distilled water or canola oil respectively. The mixtures were stable for 30 min at 25 ° C. Then the protein dispersions were centrifuged at 1600 xg for 25 min [12]. Subsequently the supernatant liquid volume was measured and the retained liquid was expressed as the milliliters of water or oil absorbed per gram of protein isolates [15].

Emulsion activity index and emulsion stability index

The emulsion activity index (EAI) was determined using the method described by Zheng *et al.*, (2008) with some modifications. 10 mg of each one of the protein isolates obtained were mixed with 10 mL of tridistilled water plus 3.33 mL of canola oil. The dispersion pH was adjusted to 2, 3, 4, ... 10, by addition of 0.1N HCl or 0.1N NaOH. Thereafter mixtures were homogenized in a mixer Thermolyne model M16715 (Thermolyne Corporation, Mexico) at maximum speed for one minute. An aliquot of 50 μL of each one of the emulsions formed was mixed with 5 mL of sodium dodecyl sulfate at 0.1%. The absorbances of the samples were measured at 500 nm on a spectrophotometer Perkin Elmer Lambda XLS model. The EAI was obtained by the following equation.

$$EAI \left(\frac{m^2}{g} \right) = \frac{2 \times 2.303 \times A_0 \times DF}{c \times \phi \times 1 - \theta}$$

Where A_0 is the sample absorbance at zero time, DF is the dilution factor, c is the initial protein concentration, ϕ optical path (0.01 m), θ oil fraction used for form the emulsion.

After reading at zero time the absorbances of the respective emulsions at 500 nm, the emulsions were left in rest at room temperature for 10 minutes, after which again was obtained absorbance of emulsions and the emulsion stability index (IEE) was calculated with the following equation, in which A_0 is the absorbance at time zero and A_{10} is the sample absorbance after 10 minutes [17].

$$EEI(\text{min}) = \frac{A_0}{A_0 - A_{10}} \times 10$$

Foaming Capacity and Foam Stability

The foaming capacity (FC) and the foam stability (FE) were determined in the following manner: Initially were prepared 50 mL of solutions 1% w/v of each of the protein isolates, using tridistilled water as dispersant. The pH of these solutions was adjusted to 2, 4, 6, 8 and 10, by addition of 0.1N HCl or 0.1N NaOH as required. Subsequently the dispersions were homogenized by 1 min in an Osterizer mixer and in

this moment, the foam volume formed was registered. The FC was obtained as the percentage increase in volume of the dispersion [18]. Then the dispersion was kept standing at room temperature for 30 minutes after which, the foam volume remaining was measured. With this information was determined the stability of the foam [19].

Gelling Minimum Concentration

The gelling minimum concentration (GMC) of the protein isolates was determined as follows. With phosphate buffer at pH 7.6 as dispersant agent, were prepared 5 mL of dispersions with 5.0, 7.5, 9.0, 10.0, 11.0 and 12.5% w/v of each one of the protein isolates. Tubes containing dispersions were kept one hour in a boiling water bath and then they were cooled and kept for two hours under refrigeration at 4°C. Gelling Minimum Concentration corresponded with that concentration where the sample did not slide down across the tube wall when this was inverted [12].

Statistical Analysis

All determinations were done in triplicate, and data shown represent the average value of the three determinations +/- the series standard deviation. An analysis of variance (ANOVA) was conducted also, and the Duncan's test was used to find statistically significant differences between the different protein isolates obtained at a confidence level of 95%.

RESULTS AND DISCUSSION

Chemical proximal analysis of *Lupinus* seeds

The proximal chemical characterization of the different *Lupinus* flours studied (Table 1) highlights its high protein content, which is similar to soy seeds (38.6-40%), and is twice as high as that found in other seeds of legumes such as chickpea (*Cicer arietinum*) (18.4-29.0%), lentil (*Lens culinaris*) (20.6-28.6%) [20], the pea (*Pisum sativum*) (19.39-34.7%) and beans (16.89-26.40 %) [1, 21, 22].

Lipids are an important component of legume seeds, those containing high amounts of lipids are distinguished from containing high concentrations of starch as energy storage components. The lipid content in *Lupinus* flours studied (Table 1), is higher than that found in seeds of chickpea (6.69%), lentil (2.15%), peas (2.34%) and beans (2.15%) [22], but lower than soybeans contain (19%) [23].

The ash content in flour of various species of *Lupinus* studied is significantly different ($p < 0.05$), was greater in flour *Lupinus spp.* The ash content in flours *L. albus* and *L. splendens* Rose was similar to containing *L. luteus* (4.6 ± 2 g/100 g) and *L. angustifolius* (3.7 ± 2 g/100g) [24]. The variability in ash content in seeds of these plants depends on the type of soil where the plant develops [25]. Meanwhile, the presence of Mn, Fe, Zn and K in the ashes of some *Lupinus* species has been determined [26].

Fiber content in flours of different *Lupinus* seeds (Table 1) is similar to those of other legumes such as peas, beans and lentils (13.6-28.9%) [22, 27, 28]. Fiber content in *L. splendens* Rose flour was higher than that found in flours of *L. albus* and *Lupinus spp.*, which is due that the seeds of *L. splendens* Rose are small and hard coated grains. No statistically significant difference ($p < 0.05$) in the fiber content flours *L. albus* and *Lupinus spp.*

The carbohydrate content in flours of the different *Lupinus* seeds (Table 1) was lower than that found in other legumes such as peas, beans and lentils, which carbohydrate content fluctuates around 50% [22]. The specie with the highest carbohydrate content was *L. albus*, followed by *L. splendens* Rose and *Lupinus spp.*

Obtaining protein isolated

The pH at which had the highest amount of protein precipitate was 4.4 for *L. albus*, 4.8 for *L. splendens* Rose and 4.1 for *Lupinus spp.* These values are within the range of pH reported by Agboola, (2009) [29], who state that vegetable proteins have minimum solubility in the pH range 4 to 5, known this pH as the protein isoelectric point. The final protein concentration in the protein isolates obtained was of 93.55 ± 1.40 for *L. albus*, 92.95 ± 1.91 for *L. splendens* Rose and 91.84 ± 1.11 for *Lupinus spp.* Regardless of the initial content of protein in the starting flour, the final concentration of proteins in the different protein isolates obtained was similar ($p < 0.05$) and in all cases was over 90%.

Making a matter balance, the quantity of recovered protein with isoelectric precipitation technique of proteins, it was considerably greater from *L. albus* seeds ($93 \pm 0.77\%$). It has been observed that both purity as the quantity of recovered protein are affected by the characteristics of the starting seeds, extraction time and temperature, flour-solvent ratio and pH at which is performed the proteins precipitation [30].

Functional Properties

Solubility

The solubility of protein isolates was influenced by the medium pH; it was low at pH values near the proteins isoelectric point (pH of 4 to 5) and increases toward both ends of the pH scale (Figure 1). The

solubility profile of the different protein isolates obtained was very similar, their solubility were greater both as acidic pH values (pH 2) as alkaline pH (pH 10).

The explanation given for this phenomenon is that in the isoelectric pH, the net charge of the protein is zero, which makes the intermolecular electrostatic repulsion and their ionic hydration at this point is minimal, causing thereby precipitating the protein. The low solubility of the legume proteins at pH 4 to 5, is due to the formation of aggregates at a pH near of the proteins isoelectric point[30], but this property can be improved by adding sodium caseinate conjugates with maltodextrin, when so required[31].The proteins solubility is mainly related with its balance of hydrophilic/hydrophobic aminoacids, however, the solubility characteristics of the protein must be related to the balance hydrophilic/hydrophobic aminoacids that form the surface protein, and not necessarily in overall amino acid composition [32].

The solubility of protein isolates provides important information about of their possible technological applications [10].The protein isolate more soluble was the obtained from *Lupinus spp*, since at both ends pH, its solubility was 100%, while the solubility of *L. albus* protein isolated was 84.18% and 96.19% at pH 2 and 10, respectively. The least soluble of the protein isolates was the obtained from *L.splendens* Rose, whose solubility maxim was 60.68% and 72.15% at pH 2 and 10, respectively. The solubility profile of the obtained protein isolates maintains "U"shape, similar to presented by protein isolates obtained from rice [33], pea [1], peanut [34], canola [35],*Lupinus angustifolius*[18], *Lupinus albus*[36], and soybean [17].

The solubility of the different protein isolates at pH near the isoelectric point of their proteins (pH of 4-5), it is significantly different ($p < 0.05$) from each other (Table 2). This was higher (2.32% w/v) in the protein isolate of *L. splendens* Rose, and was similar to that reported for other protein isolates obtained from sunflower, peanut and green lentil [32].

Water and Oil Absorption Capacities

There were not statistically significant differences ($p \geq 0.05$) in the water absorption capacity (WAC) of the different protein isolates obtained (Table 2). The WAC of the protein isolates obtained were similar (average of 2.46 mL/g) to those presented by the soy commercial protein isolate (Supro 670) and pea, but higher than the presented by the protein isolates of sesame *L. campestris*, sunflower and peanut.The carbohydrate content affects the WAC, property that is related to theproteins hydration capacity and affect the foods texture and flavor [15]. The WAC of the different protein isolates has been asociated to presence of polar amino acids at the interaction sites protein-water, and can be affected by conformational changes in the protein molecules, which can result in exposure of the amino acid side chains previously hidden, by increasing or decreasing its interaction with the water, depending on the polar nature of the amino acids [37].

Moreover, the protein isolate of *Lupinus spp* had the highest oil absorption capacity (OAC), which was 60.76% higher than *L. albus* (Table 2), and 58, 51, 45 and 37% higher than the OAC presented for the protein isolates of *L. angustifolius*, soybean (670), sunflower and sesame respectively. High values of OAC are convenient in the protein isolates that are used as ingredients in the cold meat industry, particularly for sausages, where the WAC and OAC are determinants properties to develop a food of acceptable quality [15].The OAC is an important functional property because it improves mouthfeel and flavor retention [12]. Conversely to what happens in the WAC, the OAC is influenced by the presence of nonpolar amino acids at the interaction sites of protein-oil that may be affected by conformational changes in the protein molecules.

Emulsion Activity Index (EAI) and Emulsion Stability Index (ESI)

Due to the importance of emulsions in food systems such as mayonnaise and dressings, it is necessary to know the behavior of these properties as a pH function. Both theemulsion activity index (EAI) as emulsion stability index (EEI) of the different protein isolates obtained was dependent on medium pH (Figures 2 and 3, respectively). Both properties were minimal in the isoelectric pH range (pH 4 and 5) and as solubility; both properties also increase towards both ends of the pH scale. *Lupinus albus* protein isolated showed a maximum EAI at pH 2, it decreases to a minimum at pH 4, 5 and 6, with no significant differences ($p < 0.05$) between them, and again reaches a maximum value at pH 10. Similar behavior is seen in the ESI of this protein isolate, but in this case, the ESI was greater at pH 10 than at pH 2.The protein isolate of *Lupinus splendens* Rose developed an EAI maxim to pH 10, which was 2.57 and 1.87 times higher than that obtained at pH 4 and 2, respectively. There was not statistically significant differences ($p \geq 0.05$) between the EAI developed by the protein isolate of *Lupinus spp* at pH 2 than at pH 10, although in this case the emulsion was more stable at pH 2 (ESI = 48.85 min) than at pH 10 (ESI = 37.64 min). The profile of the emulsion properties compared with the dispersions pH of the different protein isolates studied was similar to that presented by protein isolates of Indian walnut, soybean [15] and *L. campestris*[12].

The emulsion activity index (EAI) was determined at pH in which each one of the different protein isolates was obtained and was statistically different ($p < 0.05$) in each of them. The protein isolate of *L. splendens* Rose had the highest EAI, but its EEI which it was 2.48 minutes less stable than that presented by the protein isolate of *L. albus* (Table 2). The protein isolate of *L. splendens* Rose presented an IAE similar to oat and soy protein isolates, but 2.48 times higher than that presented by the protein isolate of bean although these were more stable than the first.

It has been seen that some functional properties of protein isolates such as emulsification, foaming and gelation, can be related to the solubility of the proteins, which are affected by their hydrophobicity, conformational stability and physicochemical factors such as pH, ionic strength and temperature. The proteins with low solubility also present low emulsifying capacity [38]. Ogunwolu *et al.* (2009) indicate that the minimal activity of emulsion in the pH near the isoelectric point of proteins, is correlated with poor solubility at this pH, because the net charge of the peptides is minimized under this condition and they cannot easily migrate to the interface, so that the activity of emulsion is reduced, however, when the pH of the protein is modified, increase the emulsion activity index [15, 16]. This property increases as the pH moves away from the isoelectric point of the protein, due to changes at the surface of the fat globules, which cause a mutual repulsion and form a hydrated layer around the interfacial material, reducing its energy and slows the collapse of the drops [34].

Foaming capacity (FC) and foam stability index (FSI)

Foaming capacity is important in food applications such as bakery products, cakes, biscuits, drinks, meringues, toppings and ice cream, etc. [1, 19]. The isolated protein of *L. splendens* Rose showed the highest FC, which was three times higher than those developed by *Lupinus albus* and *Lupinus spp.*, protein isolates. It was also higher than the FC developed by safflower [39], beans, soybeans, peas, sesame and canola [40] protein isolates, but lower than the FC reported for *Lupinus campestris* protein isolate, whose FC was 500% at pH 2 [12]. By contrast, the foam developed by *L. albus* protein isolate was the most stable of the three, because after staying for 30 minutes at room temperature, still retained 40% of its initial volume (Table 2). The FSI developed by *L. albus* protein isolate was similar to that reported for soy protein isolate [12].

Effect of pH on the foaming capacity and in foam stability index of Lupinus protein isolates

Both the foaming capacity (FC) as the index of stability of the foam (FSI) is affected by the medium pH (Figures 4 and 5, respectively). The FC was highest to pH 2, less at pH 4-6, corresponding to the isoelectric point, and increasing again at alkaline pH. The *L. splendens* Rose protein isolate showed the highest foaming capacity (FC) at all pH values (Figure 4) and there was no statistically significant difference ($p \geq 0.05$) in this property, at pH values of 2 and 10. Meanwhile, the protein isolates of *Lupinus albus* and *Lupinus spp.*, developed maximum FC at pH 2, and its FC at pH 4 and 6 were very similar. The FC of the *L. albus* protein isolate to pH 2 was similar to developed by *L. splendens* Rose protein isolate.

Comparatively, the foam developed by protein isolate of *Lupinus albus* showed the greatest foam stability index (FSI) at all pH values tested except at pH 10, in which the FSI was lower (Figure 5). No significant difference ($p < 0.05$) in the FSI of this protein isolates at pH 6 and 8. The FSI of the different protein isolates obtained was lower in acidic pH conditions (pH 2 to 4), and higher in near neutrality pH conditions (pH 6 - 8). The FSI of peanut protein isolate was of 35% [34], while the Indian walnut protein isolate showed a FSI of 45-50% at pH 6 [15]. The NaCl plus xanthan gum were used to improve the foaming capacity and foam stability developed by sunflower protein isolates [41].

Both the solubility and foaming and emulsifying properties of different protein isolates obtained, were affected by the pH of the medium (Figure 1-5), similar behavior to that presented with protein isolates of India nut [15], peanut [34] and pea [30]. It has been observed that the presence of soluble proteins is an important factor in developing good foaming properties of protein isolates, due to the amphiphilic character of the proteins; it gives them the property of forming a film the air-water interface and the ability to prevent the collapse of the bubbles [33, 37]. Also has been observed that the molecular properties that proteins must have to develop a good foaming capacity, differ from those needed to achieve a good foam stability, because the foams formation of protein-based involves diffusion of soluble proteins toward air-water interface and the rapid conformational change and the rearrangement of the interface, whereas the stability of the foam requires the formation of a thick film, cohesive viscoelastic around each gas bubble [32].

Gelling Minimum Concentration

The main reserve proteins in the seeds of legumes are of globulin type, which can dissociate and associate in different shapes and form gels by heating [32]. These gels are three-dimensional matrices that retain water, lipids, sugars, flavors, and other ingredients, ability which is very useful in the new product development [30, 34]. The gelling minimum concentration was obtained with the isolated protein of *L. albus*, which was 7.5% w/v and this was 20% lower than that obtained with the protein isolated of *L.*

splendens Rose and 46.7% lower than that recorded by the protein isolated of *Lupinus sp.* (Table 2). A gelling minimum concentration greater than 11% w/v has been reported in protein isolates of *L. angustifolius*, soy and India nut [32]. Gelling capacity of the protein concentrates, not only is affected by the protein concentration but also by the presence of non-protein components. Furthermore, depending on their amino acid composition, proteins form two types gels; the clot and the transparent gels [30]. It has been observed that proteins with high presence of nonpolar aminoacids formed clot type gels, whereas proteins with high frequency of hydrophilic aminoacids, formed transparent gels [32].

Table 1. Proximal chemical analysis of *Lupinus albus*, *Lupinus splendens* Rose and *Lupinus spp* flours (g/100 g flour on a dry basis).

Component	<i>Lupinus albus</i>	<i>L. splendens</i> Rose	<i>Lupinus spp.</i>
Protein	37.81±0.77 ^a	34.14±0.75 ^a	48.63±1.22 ^b
Lipids	8.54±0.09 ^a	13.01±0.37 ^b	13.97±0.22 ^b
Ash	3.62±0.12 ^a	4.32±0.41 ^b	5.18±0.02 ^c
Fiber	11.58±0.80 ^a	16.39±1.82 ^b	10.18±0.92 ^a
Carbohydrates	38.46 ^a	32.11 ^b	22.00 ^c

Different letters in rows indicate significant differences (p < 0.05)

Table 2. Functional properties of protein isolates obtained from seed of *Lupinus albus*, *Lupinus splendens* Rose and *Lupinus spp.*

Functional property	<i>Lupinus albus</i>	<i>L. splendens</i> Rose	<i>Lupinus spp</i>
Solubility (%p/v)	0.09±0.01 ^a	2.32±0.79 ^b	0.31±0.03 ^c
WAC (mL/g)	2.57±0.20 ^a	2.60±0.30 ^a	2.21±0.27 ^a
CAO (mL/g)	2.85±0.26 ^a	4.63±0.79 ^a	4.69±0.19 ^a
EAI (m ² /g)	28.65±1.30 ^a	58.81±2.07 ^c	23.40±0.84 ^b
ESI (min)	12.67±0.25 ^a	10.19±1.27 ^b	8.17±0.07 ^b
FC (%)	48.67±3.83 ^a	195.39±2.17 ^b	50.85±7.69 ^a
FSI (%)	40.18±3.78 ^a	20.00±0.00 ^b	25.40±4.49 ^b
GMC (% p/v)	7.5	9	11

Average values of three determinations ± standard deviation of the series. Different letters in the same row indicate significant differences (p < 0.05). WAC: Water Absorption Capacity; OAC: Oil Absorption Capacity; EAI: Emulsion Activity Index; ESI: Emulsion Stability Index, FC: Foaming capacity, FEI: Foam Stability Index; GMC: Gelling Minimum Concentration

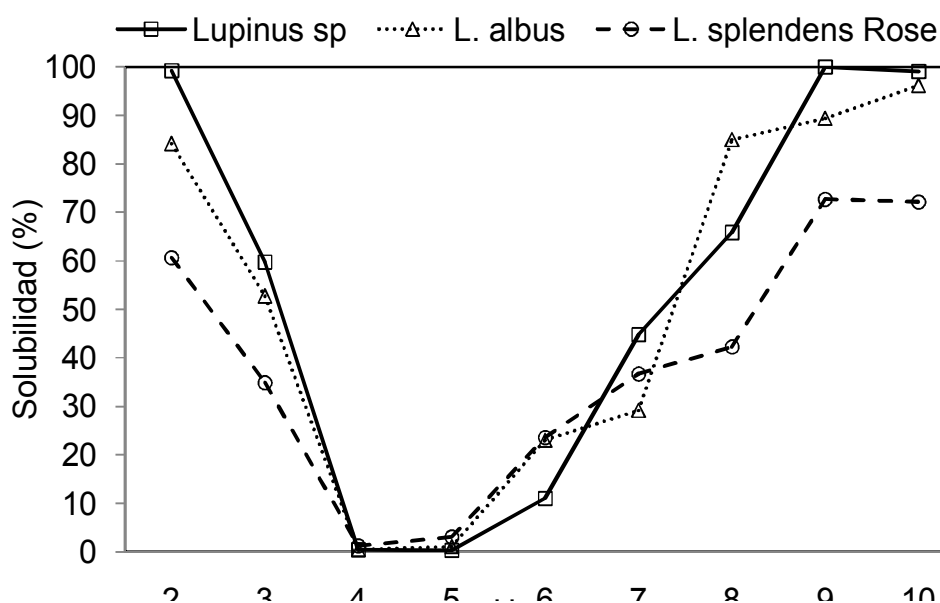


Figure 1: Effect of pH on the solubility of *Lupinus albus*, *Lupinus splendens* Rose and *Lupinus spp* protein isolates

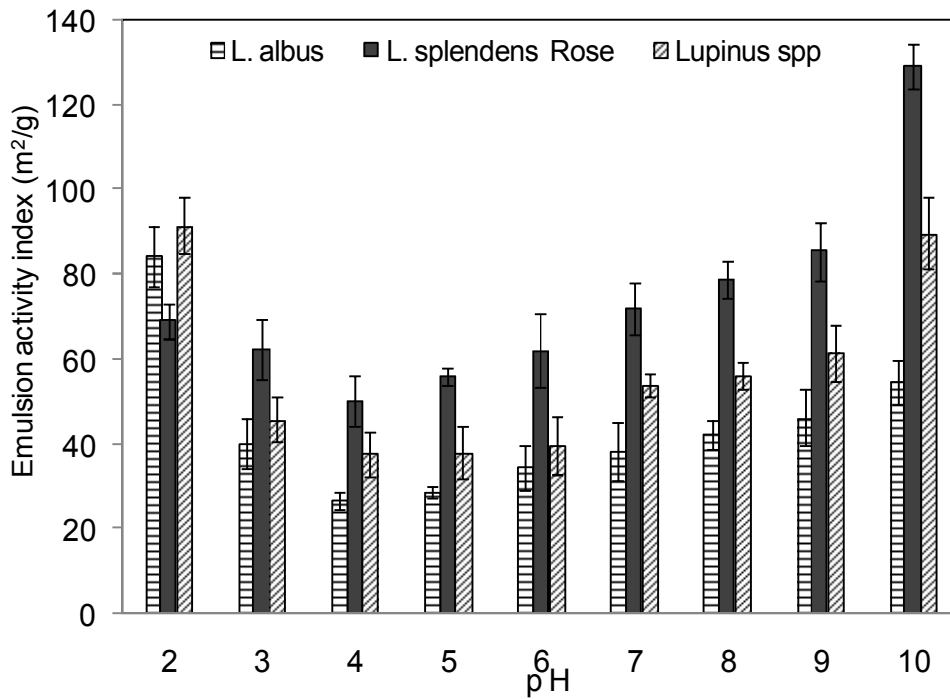


Figure 2: Effect of pH on the emulsion activity index of *Lupinus albus*, *Lupinus splendens* Rose and *Lupinus spp* protein isolates

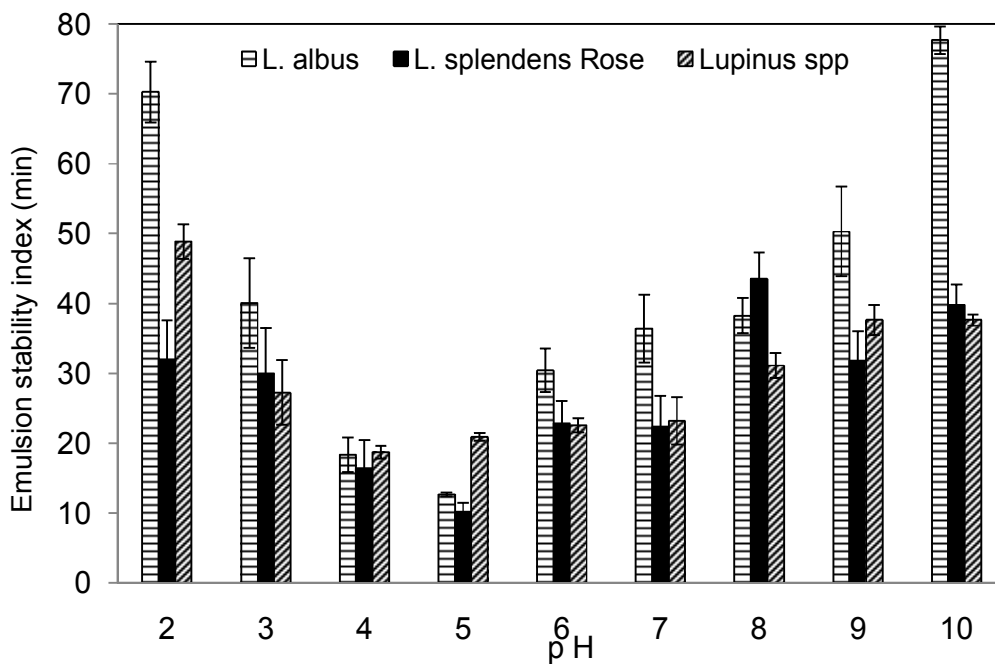


Figure 3. Effect of pH on the emulsion stability index of *Lupinus albus*, *Lupinus splendens* Rose and *Lupinus spp* protein isolates

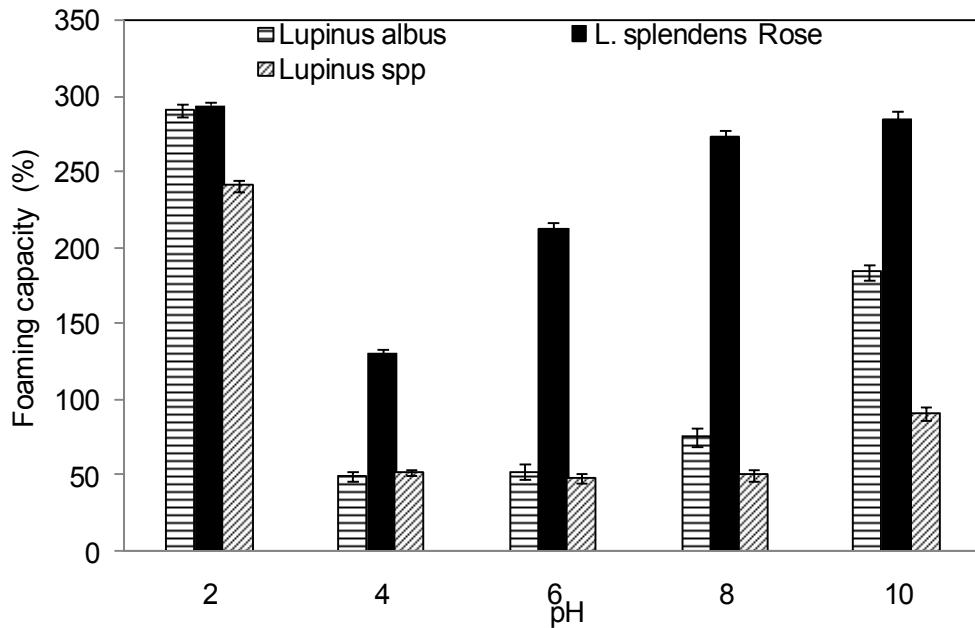


Figure 4. Effect of pH on the foaming capacity of *Lupinus albus*, *Lupinus splendens* Rose and *Lupinus spp* protein isolates

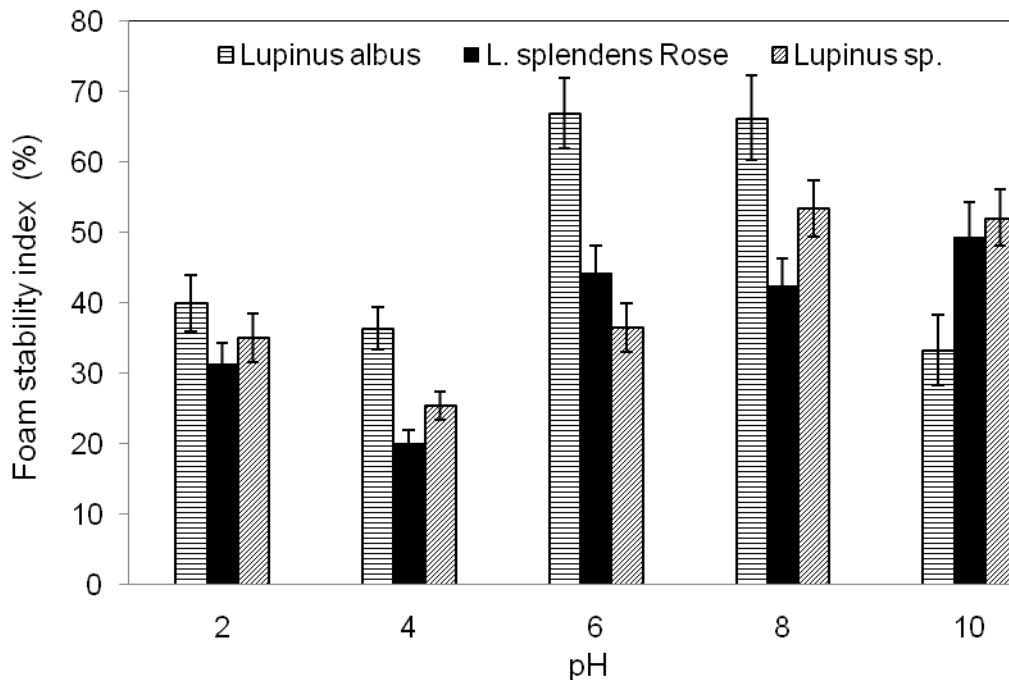


Figure 5. Effect of pH on foam stability index of *Lupinus albus*, *Lupinus splendens* Rose and *Lupinus spp* protein isolates

CONCLUSIONS

The *Lupinus sp* seeds presented a higher protein content, oil and ash than seeds of *L. splendens* Rose and *L. albus*, but the fiber content was highest in *L. splendens* Rose seeds. The functional properties such as solubility, emulsifying and foaming capacities of the different protein isolates obtained were influenced by the pH of the medium, in general, all of them were minimal at pHs near the isoelectric point of the proteins (pH 4-5) and they reached maximum values at both acidic pH (pH 2) as at alkaline pH (pH 10). *Lupinus splendens* Rose protein isolated was more soluble than *Lupinus albus* and *Lupinus spp* protein isolates. Water absorption capacity of the different proteins isolates obtained was similar but the highest oil absorption capacity was obtained with the protein isolates from *L. splendens* Rose and *Lupinus spp*,

without there being a significant difference ($p < 0.05$) between both. The protein isolate obtained from *Lupinus splendens* Rose seeds, had higher emulsifying and foaming capacities, but its stability index was lower in both properties. Given its high protein content, *Lupinus* seeds represent an important source altern for the production of protein isolates. *Lupinus* protein isolates could be used as alternative protein source in the preparation of foods and diets that require high protein content.

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