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Functional Properties of Flours and Protein Isolates from *Phaseolus lunatus* and *Canavalia ensiformis* Seeds

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The functional properties of flours and protein isolates from the legumes *Phaseolus lunatus* and *Canavalia ensiformis* were evaluated to determine their potential practical applications. The respective protein isolates were obtained from the flours by using isoelectric precipitation, with a protein content of 71.13% for the *P. lunatus* isolate (PPI) and 73.75% for the *C. ensiformis* isolate (CPI). Nitrogen solubility was good in both acid and alkaline pHs for isolates and *Canavalia* flour (CF), with values as high as 80%, but not for the *Phaseolus* flour (PF). The flours and protein isolates had good water-holding capacities, with values between 2.65 and 3.80 g/g sample. Oil-holding capacity was highest in PPI (4.59 g/g sample) and CF (3.15 g/g sample). Under alkaline pH, the PPI foaming capacity (147%) was higher than those for CPI and CF, though the flours produced greater foam. Emulsifying activities for the PF, CF, PPI, and CPI were similar (46.78−53.84%) for pH range 6−10. Emulsion stability (ES) was superior in the CF and the CPI, where values reached 100% at pH 7 and 8. Apparent viscosity was pH-dependent.

KEYWORDS: ***Phaseolus lunatus*; *Canavalia ensiformis*; functional properties; protein isolates**

INTRODUCTION

Animal proteins, such as meat, milk, and eggs, are generally expensive and relatively difficult to acquire, which has led to a worldwide increase in research into vegetable protein sources. Because of their very high protein content, legumes have formed an important part of this search for cheaper, alternative protein sources. With the improvement of the functional properties of legume flours and protein isolates through processing, these vegetable proteins can be used in manufactured foods and texturized products for human consumption (*1*).

Like many tropical regions, the Yucatan Peninsula, Mexico, supports a wide variety of legumes, among them *Phaseolus lunatus* and *Canavalia ensiformis*. Both these legume species have high protein contents (*P. lunatus*, 26% and *C. ensiformis*, 29%) (*2*, *3*). Protein isolates obtained from them through isoelectric precipitation have approximately 72% protein content, which makes them excellent potential protein sources for food industry applications (*4*). This potential usefulness, however, will also depend on their functional properties, which affect the sensory characteristics of food and play an important role in the physical behavior of food or its ingredients during preparation, processing, and storage. Functional properties include emulsification, foam formation, viscosity, improvement of appearance, texture, and water-holding and oil-holding capacities. On the basis of these properties, the specific protein selected to be used in a certain food will depend on its required function in the final product (*5*).

In an effort to understand the potential applications of *P. lunatus* and *C. ensiformis*, two legumes common to the Yucatan Peninsula, a study was done of the physicochemical characteristics and functional properties of flours and protein isolates derived from them.

MATERIALS AND METHODS

Seeds and Chemicals. Baby lima bean (*Phaseolus lunatus)* and jack bean (*Canavalia ensiformis*) seeds were obtained from the February 1998 harvest in the state of Campeche, Mexico. All chemicals used in the experiments were reagent grade, and were purchased from J. T. Baker (Phillipsburg, NJ).

Flours. To produce flours, 10 kg of seeds from each legume species were used. Impurities and damaged seeds were removed from the samples, and the whole, sound seeds were then milled in a Mykros impact mill. The resulting flour was passed through a 20-mesh screen.

Protein Isolates. A modified process (*6*) was used to recover the protein isolates. A flour/water (1:6 w/v) dispersion was prepared, and its pH was adjusted to 11 with NaOH 1 N. After soaking for 1 h, the suspension was milled in a disk mill and passed through 80- and 100-mesh screens to separate the fiber-containing solid fraction from the liquid fraction, which contains the protein and starch. The residual solids

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Table 1. Chemical Composition of *P. lunatus* and *C. ensiformis* Flours and Protein Isolates[a]

component	PF	PPI	CF	CPI
moisture	14.88 ± 0.60	7.87 ± 0.46	11.56 ± 0.12	8.71 ± 0.03
ash	3.40 ± 0.29	2.82 ± 0.10	2.79 ± 0.37	4.16 ± 0.06
protein (N × 6.25)	24.07 ± 0.61	71.13 ± 0.92	26.86 ± 0.1	73.75 ± 0.3
crude fat	3.77 ± 0.36	0.677 ± 0.20	1.67 ± 0.01	5.12 ± 0.01
crude fiber	5.10 ± 0.49	0.20 ± 0.09	13.67 ± 0.55	0.273 ± 0.2
nitrogen-free extract	63.66 ± 0.57	25.12 ± 0.47	55.01 ± 0.36	17.36 ± 0.65

[a] PF, *P. lunatus* flour; PPI, *P. lunatus* protein isolate; CF, *C. ensiformis* flour; CPI, *C. ensiformis* protein isolate; percent dry basis.

were washed 5 times, using a 1:3 solid to distilled water ratio and passed through 150-mesh screen to eliminate the finest fiber; the wash water was then mixed with the supernatants from the initial suspension, and they were allowed to sediment for 30 min to recover the starch and separate the solubilized protein. The pH of the solubilized proteins was adjusted with HCl 1 N to their isoelectric points, which were 4.5 for *P. lunatus* and 4.9 for *C. ensiformis*. The suspension was then centrifuged at 1317*g* for 12 min, using a Mistral 3000i (Curtin Matheson Sci.) centrifuge. The supernatants were discarded, and the precipitates were freeze-dried at −47 °C and 13 × 10^{-3} mbar.

Chemical Analysis. The nitrogen (method 954.01), fat (method 920.39), ash (method 923.03), crude fiber (method 962.09), and moisture (method 925.09) contents of the flours and protein isolates were determined according to official AOAC procedures (*7*). Nitrogen was determined with a Kjeltec System (Tecator, Sweden). Protein was calculated as nitrogen × 6.25. Fat was obtained from a 4-h hexane extraction. Ash was calculated from the weight remaining after heating the sample at 550 °C for 2 h. Moisture measurement was determined on the basis of sample weight loss after oven drying at 110 °C for 4 h.

Functional Properties. The functional properties of the flours and protein isolates were evaluated under the same conditions according to the following methods.

Nitrogen Solubility. This was determined using the method of Were et al. (*8*). Samples (125 mg) were dispersed in 25 mL of distilled water, and the solution pH was adjusted to 2, 3, 4, 5, 6, 7, 8, 9, and 10 using either 0.1 N NaOH or HCl. The dispersions were agitated for 30 min at room temperature, and then centrifuged at 4320*g* for 30 min. Nitrogen content in the supernatant was determined by the Kjeldahl method (AOAC method 954.01; *7*). The percentage of soluble protein was calculated as follows:

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

Water-Holding and Oil-Holding Capacity. To determine these holding capacities, 1 g of sample was weighed and then stirred into 10 mL of distilled water or corn oil (Mazola, CPI International) for one minute. These protein suspensions were then centrifuged at 2200*g* for 30 min, and the volume of supernatants was measured. Water-holding capacity was expressed as g of water held per g of protein sample. Oil-holding capacity was expressed as g of oil held per g of protein sample. The density of the corn oil was 0.92 g/mL (*9*).

Foaming Capacity and Foam Stability. These properties were evaluated over a pH range of 2 to 10. A 100-mL sample of 1.5% (w/v) suspension was blended at low speed in a Waring blender (Osterizer 10S-E) for 5 min, and the foam volume was recorded after 30 s. Foaming capacity was expressed as the percentage increase in foam volume measured at 30 s. Foam stability was determined according to residual foam volume at 5, 30, and 120 min after blending. Both properties were determined as a function of pH (*9*).

Emulsifying Activity (EA) and Emulsion Stability (ES). Samples of 100 mL of 2% (w/v) suspension adjusted to pHs ranging from 2 to 10 were homogenized using a Caframo RZ-1 homogenizer at 2000 rpm for 2 min. Then, 100 mL of corn oil (Mazola, CPI International) was added to each sample, and the mixture was homogenized for 1 min. The emulsions were centrifuged in 15-mL graduated centrifuge tubes at 1200*g* for 5 min, and the emulsion volume was measured. Emulsifying activity was expressed as percentage of the emulsified layer volume of the entire layer in the centrifuge tube. To determine the emulsion stability, the prepared emulsions were heated at 80 °C for 30 min, cooled at room temperature, and centrifuged at 1200*g* for 5 min. Emulsion stability was expressed as percentage of the remaining emulsified layer volume of the original emulsion volume (*9*).

Viscosity. Suspensions (10%, w/v) with pHs ranging from 2 to 10 were stirred for 30 min at 25 °C. Viscosity was measured using a Brookfield LV viscosimeter (Brookfield Engineering Lab., Stoughton) at 100 rpm, adapting spindle number 21, and the results expressed in cP. The protein samples were then heated to 60 °C, at a rate of 1.5 °C/min, and the viscosity was measured. Samples were then cooled to 25 °C and the viscosity was measured again as mentioned above (*10*).

Statistical Analysis. All determinations were done in triplicate, and data were analyzed using a one-way variance analysis and Duncan's multiple range test (*11*).

RESULTS AND DISCUSSION

Chemical Composition. The moisture, ash, fat, protein, fiber, and carbohydrates contents are shown in Table 1. Protein content was 24.07% for *Phaseolus lunatus* flour (PF) and 26.86% for *Canavalia ensiformis* flour (CF). Protein content was similar for the two legumes, being above 70% in their protein isolates, and moisture content was lower than 10% in both isolates. The higher fat content of the *C. ensiformis* protein isolate (CPI) versus its flour (CF) may be due to lipids saponification, which would have solubilized in the aqueous phase and been carried into the protein precipitate. This saponification, however, is probably dependent on fatty acids composition since other legumes, such as *Vigna unguiculata*, exhibit the same behavior in concentrate form, with 7% fat content, which is higher than the corresponding flour (*12*). In contrast, fat content diminishes in the *P. lunatus* protein isolate (PPI).

The level of remaining nitrogen free extract (NFE) for the PPI was higher (25.12%) than for the CPI (17.36%), whereas for the flours it ranged from 55% for CF to near 64% for PF. This parameter is quite variable in both legume species, with reported ranges from 46% (*13*) to 60% (*4*, *14*) in *C. ensiformis*, and from 55% (*15*) to 64% (*16*) in *P. lunatus*. These differences are probably due to cultivation conditions, maturity of the grain, and the species variety. These carbohydrates mainly consisted of starch, with only small quantities of other soluble carbohydrates, totaling about 3% for whole *C. ensiformis* (*17*). The protein isolates maintained a certain amount of NFE because some of the starch remains trapped in the protein matrix, a result of the difficulty of separation by the procedures used in this study (*18*).

Nitrogen Solubility. Nitrogen solubility for the CF, PPI, and CPI was pH-dependent (Figure 1), whereas for PF it remained within a range between 5 and 20%. Minimum solubility in the isolates was around pH 5, a level similar to that reported for minimum solubility in the protein isolates of *Phaseolus calcaratus* (5%), *Dolichos lablab* (5.08%), and *Glycine max* (5.26%) (*9*). Under neutral conditions, the PPI exhibited higher solubility (37.05%) than did the CPI (28.51%). Except for the

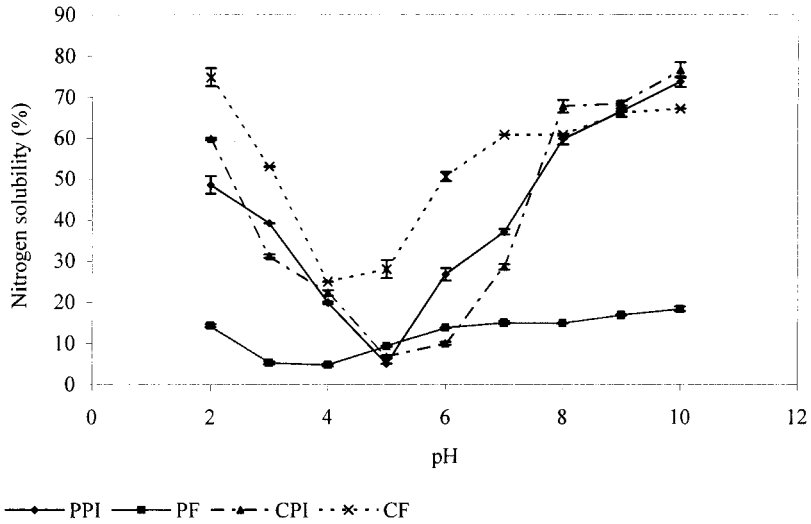

Figure 1. Effect of pH on the nitrogen solubility of *P. lunatus* and *C. ensiformis* flours and isolates.

Table 2. Water- and Oil-Holding Capacities of *P. lunatus*, *C. ensiformis*, and Soybean Flour and Protein Isolates

component	water-holding (g/g sample)	oil-holding (g/g sample)
P. lunatus flour	2.65 ± 0.1	1.83 ± 0.3
P. lunatus protein isolate	3.50 ± 0.0	4.59 ± 0.1
C. ensiformis flour	3.80 ± 01	3.15 ± 0.05
C. ensiformis protein isolate	2.50 ± 0.0	2.70 ± 0.0
soybean flour[a]	1.75	0.56
soybean protein isolate[a]	3.46	3.06

[a] Ref 9.

PF, all the preparations had good nitrogen solubility at both extremes of the pH range (acid and alkaline). A similar behavior has been reported (*19*) for Adzuki bean and soybean protein isolates, with different treatments (enzymatic and alkaline), in which nitrogen solubility was 100% at pH 2 (*8*), and an equivalent value at pH 6 to 10 for Adzuki bean native protein recovered using dialysis was obtained (*19*). This makes the CF, PPI, and CPI potentially useful in applications where high solubility profiles are required to impart certain characteristics in food formulation (*10*). Possible uses include baby food, baked products, carbonated drinks, diet drinks, and desserts (*20*).

Water-Holding and Oil-Holding Capacities. Water-holding capacity was 2.65 g/g for PF, and 3.80 g/g for CF (Table 2). Similar values have been reported (*21*) for the flours of the common beans: red (3.0 g/g), black (2.9 g/g), and white (2.9 g/g), as well as Mung bean (2.1 g/g). Thus, carbohydrate content was also a factor influencing the water-holding capacity of the flours (*22*).

Water-holding capacity was 3.5 g/g for PPI and 2.50 g/g for CPI (Table 2). These values are lower than those reported by Chau et al. (*9*) for protein isolates from *P. calcaratus* (5.28 g/g), *D. lablab* (5.08 g/g), and *P. angularis* (5.05 g/g). The differences in water-holding capacity between the PPI and CPI can be attributed to their different protein fractions. The CPI contains seven times more albumins than the PPI, with a corresponding increase in the globulins fraction (*23*), which allows variations in the number and nature of the water-binding sites in protein molecules (*9*). Additionally, external factors (e.g., stirring velocity, pH, and protein concentration) that can be changed during recovery or measurement can also influence this property.

Oil-holding capacity for PPI (4.59 g/g) was higher than that for CPI (2.70 g/g), and similar to those reported for *P. calcaratus* (4.71 g/g), *D. lablab* (4.77 g/g), and *P. angularis* (4.38 g/g) (*9*). This high oil-holding capacity can be attributed to the high levels of nonpolar residues in the PPI protein molecules, which have been found to have a slightly lower polar amino acids content (61.76 g/100 g) than CPI protein molecules (67.22 g/100 g). Its degree of denaturation was also higher, as demonstrated by the ΔH values: 3.34 J/g for PPI and 5.12 J/g for CPI (unpublished data).

The CF had the highest oil-holding capacity (3.15 g/g), and PF had the lowest (1.83 g/g). These are similar to values reported for common bean flour (2.1 g/g) and green Mung bean flour (2.2 g/g) (*21*), and well above those reported for cowpea (*Vigna unguiculata*) flours (0.69 to 0.93 g/g) (*24*). These oil-holding capacity values, especially the high value of the CF, make these flours potentially useful in structural interactions in food, especially in flavor retention, improvement of palatability, and extension of shelf life in meat products through reduction of moisture and fat loss.

Fiber content in these flours is relatively small, and it does not significantly affect their water- and oil-holding capacities. The fiber of PF had a value of 0.24 g of water/g and the fiber of CF had a value of 0.4 g of water/g, and both had values of 0.2 g of oil/g (*25*). Starch also does not affect these functional properties, because it is a neutral carbohydrate, and gelatinization temperature is not reached (*26*, *27*).

Foaming Capacity and Foam Stability. Foaming capacity for PPI and CPI was pH-dependent (Figure 2), with the lowest values for PPI at pH 3 (22.5%) and 4 (34.5%), and for CPI at pH 5 (24%) and 6 (17.5%). This behavior was similar in both protein isolates with an increase in foam formation at pH 2 and at alkaline pH values (8−10) once the isoelectric point (pI) of the proteins had been passed (pH 4−5). The PPI had significantly higher ($p < 0.05$) foaming characteristics than the other products, with values of 59% at acid pH (2) and 147% at alkaline pH (10). This may be because the protein at isoelectric point has a net charge close to zero and does not allow development of the functional properties. Therefore, better results were obtained when conditions were directed toward either of the two extremes of the pH range. Other legume protein isolates generally react in the same way: for example, soybean protein isolate has a capacity of 102% at pH 10, and *P. angularis*, *P. calcaratus*, and *D. lablab* have values between 65% and 144%, along a pH gradient from 2 to 10. The high foaming capacities at alkaline pHs may be due to an increase in the net charge of the protein which weakens hydrophobic

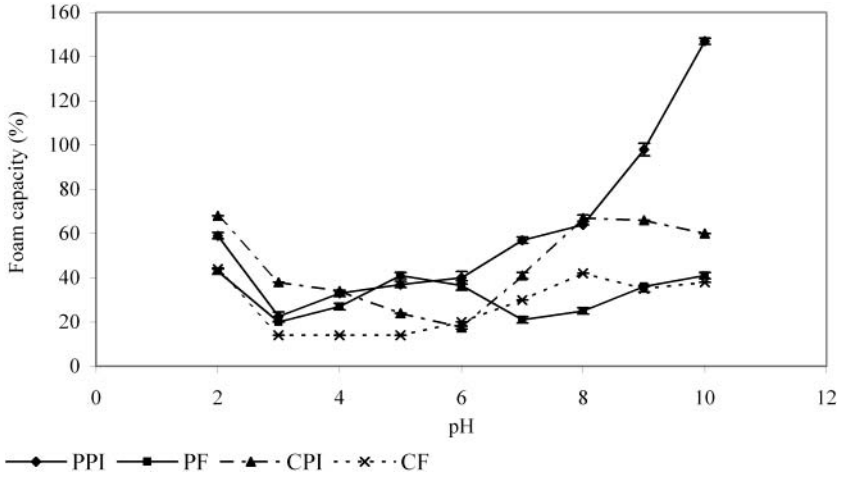

Figure 2. Effect of pH on the foaming capacity of *P. lunatus* and *C. ensiformis* flours and protein isolates.

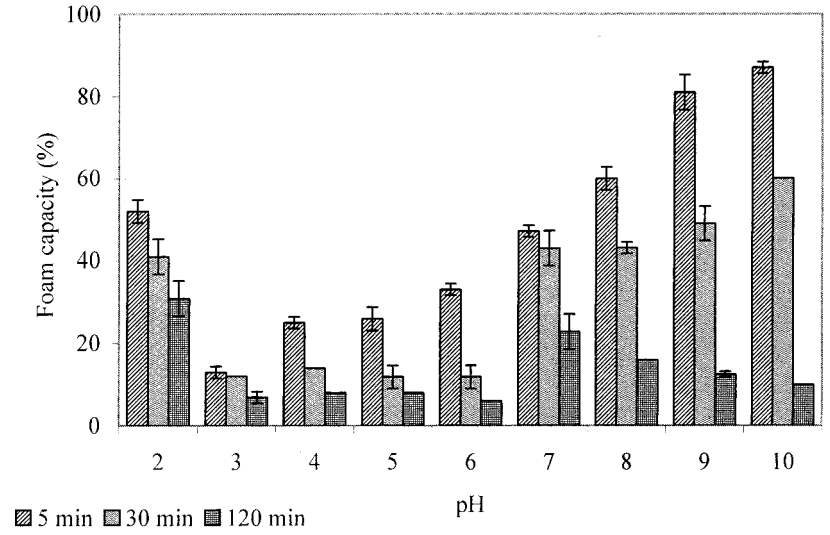

Figure 3. Effect of pH on foam stability (%) of PPI at different times.

interactions and increases protein flexibility, allowing them to spread to the air−water interface more quickly, encapsulating air particles, and increasing foam formation (*9*).

Both PF and CF had a generally low foaming capacity across the entire pH range, especially at basic pH, when compared to that of PPI and CPI, which is probably due to their low degree of denaturation.

Foam stability in the PPI diminished through time (5, 30, and 120 min) (Figure 3). It had higher foam volumes but smaller foam stability than either of the flours (Figures 3−6). These values are also lower than those reported for protein isolates from *P. lablal, P. angularis,* and *P. calcaratus* (*9*). Foam stability was lowest at pH 3 to 6 for the established times (5, 30, and 120 min) for both PPI and CPI, but higher at pH 2 and 7 in PPI and at pH 3, 4, 5, and 6 in CPI, when foam formation capacity is lowest. At neutral and alkaline pH (7, 8, and 9), foam stability for the CF was low, diminishing considerably after 2 h, and at pH 10 it disappeared.

Given these results, the relationship of hydrophilic versus hydrophobic properties is a key factor in balancing foam capacity and foam stability (*28*). This is likely a function of protein source as influenced by environmental conditions, as other authors (*9*, *19*, *29*) have reported surface properties for *D. lablal, P. angularis, P. calcaratus, P. vulgaris,* and *V. unguiculata* protein isolates that are better than for the PPI and CPI.

Emulsifying Activity (EA) and Emulsion Stability (ES). The *P. lunatus* and *C. ensiformis* products generally exhibited good EA values (41.78−56.46%) at different pH levels, with values similar to those of soybean protein isolate (54−58%) (*9*). The PPI is the exception in that its insolubility near the pI lowered its EA. Emulsifying activity profiles at different pH (2−10) levels for PPI and CPI (Figure 7) had values ranging from 41.8 to 56%, with no statistical differences ($p > 0.05$) between values for CPI, PF, and CF. Fiber content probably did not influence EA, as the fibrous products from both the legumes have been reported as having very low EAs (0.49 g/g for *P. lunatus* and 0.086 g/g for *C. ensiformis*) (*25*). This makes it unlikely that low fiber content in the isolates influenced EA.

The PPI and CPI exhibited a decrease in EA at pH 4 (44.28% and 50%, respectively) and pH 5 (41.78% and 50.76%, respectively). The CPI had very similar values across the pH scale with levels ranging from 51 to 54%, whereas the PPI had a distinct "V" pattern, the highest value being at pH 2 (56.42%) and the lowest at pH 5 (41.78%), with little variation thereafter (approximately 51% from pH 6 to 10). These values are slightly lower than those reported for other legume protein isolates (*9*), though they do exhibit similar behavior, with minimum values from 53 to 54.7% at pH 4, and higher values at the extremes of the pH range (55.8 to 58.2% at pH 2, and 57.1 to 58.2% at

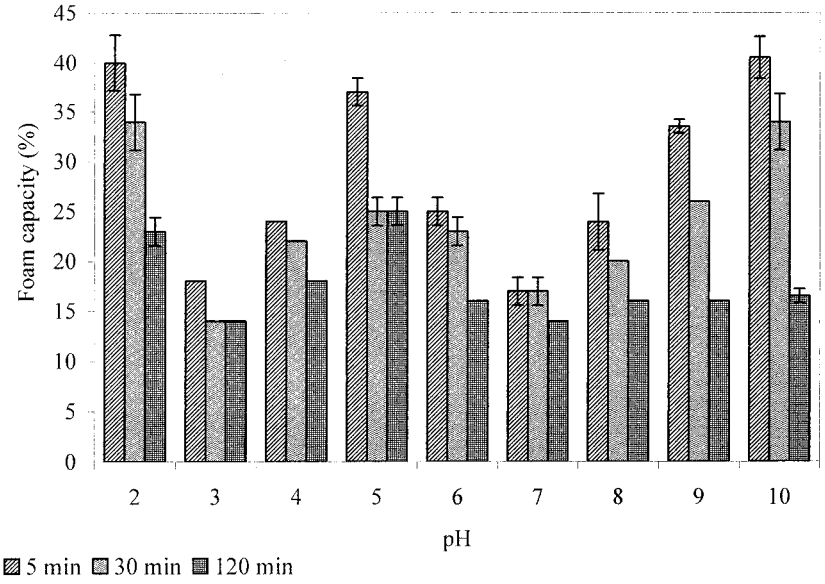

Figure 4. Effect of pH on foam stability (%) of PF at different times.

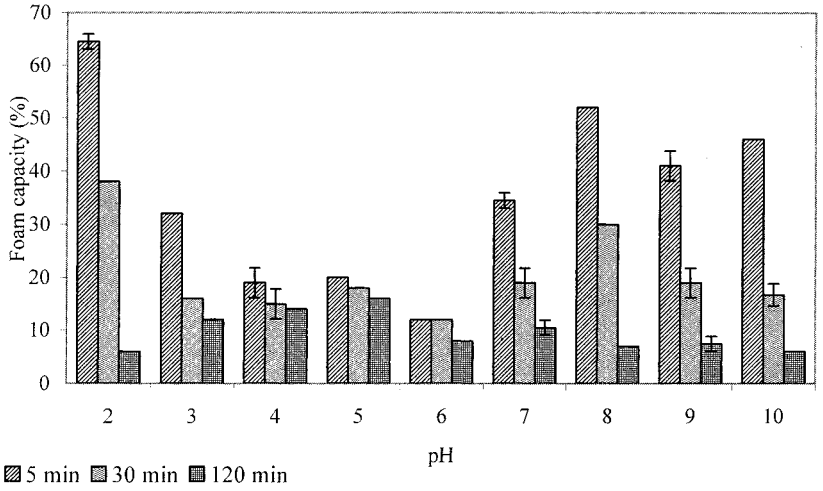

Figure 5. Foam stability of CPI at different times.

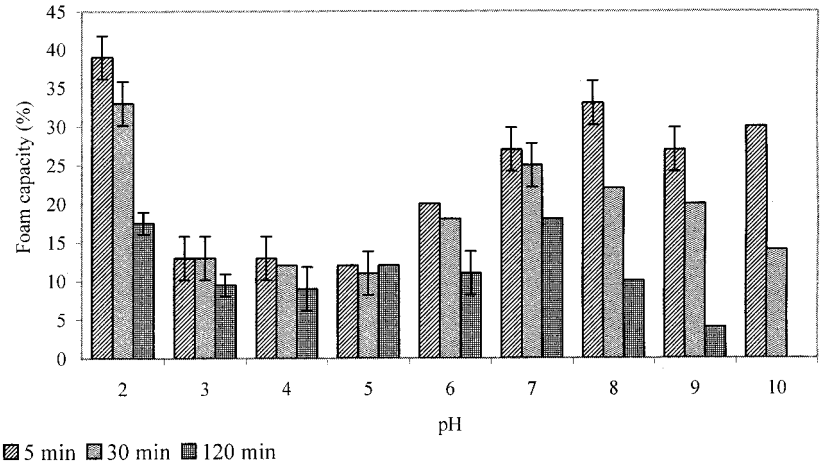

Figure 6. Effect of pH on foam stability of CF at different times.

pH 10). An EA for soybean protein isolate of 87.5% has been reported (5), which is notably higher than those for PPI and CPI in the present study, but the same study also reports an EA of 53% for dry egg albumin, showing that PPI and CPI have values quite similar to this conventional protein.

For the flours, CF had a decrease in EA at pH 4 (48.46%), remaining almost constant at pHs higher than 5 (50−51%). For PF the decrease in EA was seen at pH 6 (46.78%) and 7 (47.85%). Under acid or alkaline conditions the EA values for the CF and PF stayed around 50%, which is notably higher than

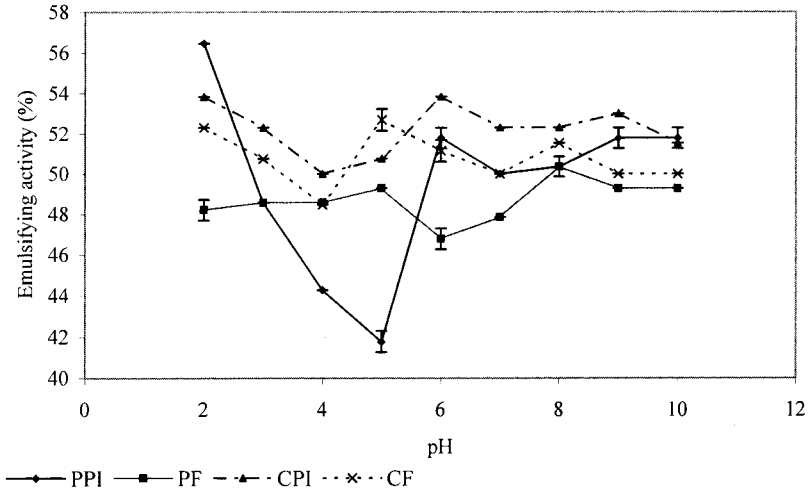

Figure 7. Effect of pH on the emulsifying activity of *P. lunatus* and *C. ensiformis* flours and protein isolates.

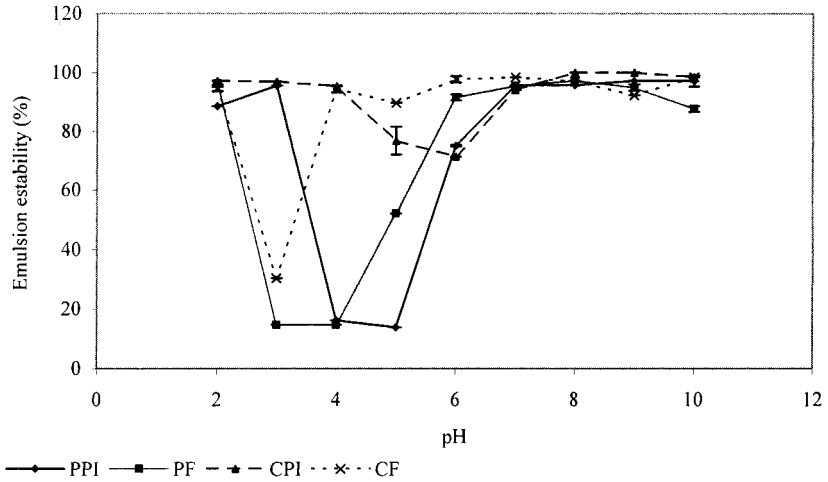

Figure 8. Effect of pH on the emulsion stability of *P. lunatus* and *C. ensiformis* flours and protein isolates.

those reported by Idouraine et al. (*10*) for Tepary bean (32.5% at pH 7). The minimal variation in EA of the CF and PF across the pH scale may be due to interactions of other components of the flours that influence this property (*30, 31*).

Emulsion stability (ES) for PPI and PF was pH-dependent (Figure 8). ES was higher for the CPI than for the PPI, reaching values of near 100% at both acid pH (2, 3, and 4) and alkaline pH (8, 9, and 10). This may partially result from the higher hydrophobic amino acids content of the PPI (*32*), which allows the protein−protein interaction in the interface. This fact, and the presence of smaller molecular weight (<19 Kda) components in the PF and PPI (*23*), may produce instability in the film (32). The CPI had very similar stability values at the extremes of the pH scale used in this study. The lowest value for CF was at pH 3 (30.3%), rising drastically above pH 4 to almost 100%. This "V" pattern is similar to that reported by Chau et al. (*9*), who obtained values for soybean isolate of 96% at pH 2, 60% at pH 4, and 98% at pH 10.

The ES values of PPI (95.71%) and CPI (94.11%) at pH 7 were similar to that reported for chickpea protein isolate at pH 7 obtained through micellization (94.3%), but higher than that for chickpea protein isolate obtained through isoelectric precipitation (85.0%) (*21*). These results indicate that both the flours and the protein isolates from *C. ensiformis* are effective emulsifiers, making them useful in applications such as sausage, mayonnaise, and seasonings manufacture, specially in products that require heating, because the protein−lipid interaction is favored by the temperature increase (greater than 60 °C) causing molecule emulsion before coalescence is present (*33*).

Viscosity. For CPI the viscosity increased after cooling to 25 °C at all pH levels (Table 3). The values for PPI at pH 7 were 11.52 cP at 25 °C before heating, and 13.75 cP at 25 °C after thermal treatment. Similar results have been reported in studies using the same protein concentration (10%) at pH 7 with *P. angularis* (12.3 cP), *P. calcaratus* (10.2 cP), and *D. lablab* (12.5 cP), though they are lower than that reported for soybean protein isolate (23 cP) *(9)*.

The CPI and CF (Table 4) showed marked increases in viscosity at acid and alkaline pHs, with values for CPI of 229 cP at pH 9, 114 cP at pH 10, and 80.12 cP at pH 2. The minimum viscosity values were observed at pH 4 with 4.50 cP for CPI, and 6.50 cP for CF at pH 5. This behavior is similar to that reported for soybean protein isolate (*30*).

Viscosity values recorded in the present study for CPI (i.e., 3.35 cP at 25 °C, 23 cP at 60 °C, and 44.12 cP at 25 °C postheating) are notably different from previously reported values for protein isolate from *C. ensiformis* at pH 7 (i.e., 13.7 cP at 25 °C, 14.2 cP at 60 °C, and 11.2 cP at 25 °C postheating). This is likely a consequence of the different protein conformations of the *C. ensiformis* in each study, a probable result of the different sources and aging of the raw material employed (*10*).

Protein concentration has also been shown to be an important factor in viscosity variation. Values have been reported of up

Table 3. Effect of pH on *P. lunatus* Flour and Protein Isolate Viscosities (cP)[a]

	P. lunatus protein isolate			*P. lunatus* flour		
pH	25 °C[b]	60 °C[b]	25 °C[c]	25 °C[b]	60 °C[b]	25 °C[c]
2	22.0 ± 1.0	19.5 ± 0.0	38.75 ± 0.5	3.50 ± 0.0	1.75 ± 0.5	2.00 ± 0.0
3	8.50 ± 0.5	6.75 ± 0.5	10.75 ± 0.5	2.75 ± 0.5	1.50 ± 0.0	2.25 ± 0.5
4	6.00 ± 0.0	4.50 ± 0.0	7.50 ± 0.0	3.00 ± 0.0	1.50 ± 0.0	2.25 ± 0.5
5	5.25 ± 0.5	3.25 ± 0.0	4.50 ± 0.0	3.75 ± 0.5	1.75 ± 0.5	2.75 ± 0.5
6	7.50 ± 0.0	7.00 ± 0.0	6.00 ± 0.0	4.00 ± 0.0	1.75 ± 0.5	2.75 ± 0.5
7	11.52 ± 0.5	8.75 ± 0.5	13.75 ± 0.5	3.25 ± 0.5	2.50 ± 1.0	2.50 ± 0.0
8	18.75 ± 0.5	8.75 ± 0.5	17.5 ± 1.0	5.00 ± 0.0	2.50 ± 1.0	3.50 ± 1.0
9	12.75 ± 0.5	6.00 ± 0.0	13.75 ± 0.0	4.75 ± 0.5	3.75 ± 1.5	4.00 ± 0.0
10	9.75 ± 2.5	5.00 ± 1.0	9.50 ± 2.0	3.50 ± 1.0	3.50 ± 1.0	4.00 ± 0.0

[a] SPDL 21, 100 rpm. [b] Heating stage. [c] Cooling stage.

Table 4. Effect of pH on *C. ensiformis* Flour and Protein Isolate Viscosities (cP)

	C. ensiformis protein isolate			*C. ensiformis* flour		
pH	25 °C[b]	60 °C[b]	25 °C[c]	25 °C[b]	60 °C[b]	25 °C[c]
2	26.75 ± 1.5	31.0 ± 2.0	80.12 ± 4.0	4.00 ± 1.5	2.25 ± 0.0	6.37 ± 1.75
3	6.75 ± 0.5	4.50 ± 0.0	10.25 ± 1.5	4.00 ± 0.5	2.75 ± 0.5	9.25 ± 2.5
4	3.75 ± 0.0	3.97 ± 1.45	4.50 ± 0.0	3.75 ± 1.0	3.25 ± 0.75	10.50 ± 0.0
5	5.12 ± 1.25	3.75 ± 1.50	5.37 ± 1.75	8.25 ± 1.0	6.25 ± 1.5	6.50 ± 1.0
6	11.0 ± 1.0	7.00 ± 0.0	12.0 ± 0.0	8.25 ± 0.5	4.50 ± 1.0	7.12 ± 0.25
7	3.35 ± 0.0	23.0 ± 1.0	44.12 ± 0.25	7.25 ± 0.5	3.75 ± 0.0	6.62 ± 0.25
8	28.25 ± 1.0	22.5 ± 1.0	51.0 ± 1.0	6.75 ± 2.0	4.37 ± 0.25	6.37 ± 0.25
9	31.0 ± 0.0	8.5 ± 2.5	229 ± 2.0	8.62 ± 0.75	5.12 ± 0.75	7.50 ± 0.0
10	10.25 ± 1.0	11.75 ± 2	114 ± 2.0	8.75 ± 0.5	4.12 ± 0.75	8.87 ± 1.25

[a] SPDL 21, 100 rpm. [b] Heating stage. [c] Cooling stage.

to 238 cP at 25 °C after heating at a concentration of 15% for soybean protein isolate (90% protein concentration) and of 22 cP for soybean flour (45% protein concentration) under the same conditions (*10*). In the present study, the viscosities obtained from the protein isolates were also higher than those of their respective flours. The difference in protein concentration in relation to viscosity is due to the fact that the flours have a higher starch content. As a result, the flours' viscosity values are influenced by the physical competition of water for starch (*24*) preventing it from reaching gelatinization temperature range (76−83 °C for *C. ensiformis* and 75−87 °C *P. lunatus*) and thus forming the gel that provides viscosity *(26, 27)*.

The high viscosities attained with the CPI, in contrast to those of the PPI, were likely due to the higher globulin fraction of the CPI (22.9%) in comparison to that of the PPI (15.7%). Also, the CPI has a 25% higher protein content type 11S (hexameric nature) when compared to that of the PPI (*23*). The 11S proteins are larger than the 7S proteins present in higher proportions in the PPI, and also have stronger chemical bonds that are more difficult to break. This likely leads to the formation of gels with different structures and physical properties, affecting viscosity (*35*).

All these results demonstrate that *P. lunatus* and *C. ensiformis* protein isolates, and to a lesser degree their flours, can be used in food systems as thickening agents, such as in dry foods and in soup mixes, to obtain a certain viscosity when reconstituted with water (*10*).

CONCLUSIONS

The chemical composition of protein isolates obtained from *P. lunatus* and *C. ensiformis* seeds was similar, with protein levels of 71.13% and 73.75%, respectively. Solubility was highest at acid and alkaline pHs for all products (greater than 60%), save for the *P. lunatus* flour (PF). The *P. lunatus* protein isolate (PPI) and *C. ensiformis* flour (CF) had the highest water-holding capacity with values of 3.50 and 3.80 g/g of sample, respectively, whereas oil-holding capacity was greatest in the *P. lunatus* protein isolate (4.59 g/g sample). Foaming capacity was highest in PPI, reaching values to 147%, and the foam stability was lower in flours and protein isolates at all pH levels evaluated. The CF and CPI had the best emulsifying activity properties across the range of pH values, though PPI had the highest (56.42%) in acid pH. The best emulsion stability was observed in the CF and CPI with values of almost 100% at pH 7. The CPI had better viscosity values at acid (2) and alkaline (9) pHs. Because of these properties the protein isolates of both legumes are very attractive as functional ingredients in food systems. These could be incorporated into products such as bakery products, seasonings, and sausages, among others, but sensory and texture analyses of the products would be necessary.

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