Functional and some nutritional properties of an isoelectric protein isolate from Mexican cowpea (*Vigna unguiculata*) seeds

LUIS CHEL-GUERRERO – MANUEL MALDONADO-HOIL – ANA BURGOS-PÉREZ – ARTURO CASTELLANOS-RUELAS – DAVID BETANCUR–ANCONA

Summary

Mexican cowpea (*Vigna unguiculata*) is a regionally adapted variety that differs from cowpea varieties in morphology, physiology and possibly some chemical characteristics. Like other varieties, it has potentially good nutritional quality. An isoelectric protein isolate was extracted from seeds harvested in Yucatan State, México, for evaluation of functional and nutritional properties. Protein content was 82.8% and solubility pattern was classical with an isoelectric point (IP) at pH 6. Water-holding capacity (245%) and oil-holding capacity (231%) were balanced. Foam capacity was best (133–190%) at extremely acid and alkaline pH, but low at pH values near neutral or at IP. Foam stability was low at all studied pH values. Emulsifying capacity (45%) was independent of pH value. Emulsion stability was generally low, but improved slightly at alkaline pH. Available lysine content was 64 $g \cdot kg^{-1}$ of protein, although sulfur amino acids were the first limiting factor. True digestibility was good (88.8%), but the protein efficiency ratio was low (1.24) classifying the isolate as having low nutritional value. The protein isolation method applied here clearly improved the functional and nutritional properties of raw Mexican cowpea seeds, but the isolate would require supplementation with other nutrients.

Keywords

Mexican cowpea; *Vigna unguiculata*; protein isolate; functional properties; nutritional properties

Seed legumes, such as cowpea *Vigna unguiculata*, play an integral role in human nutrition due to their protein content (approx. from 17% to 30%), and are consumed in myriad forms, including mature and immature seed, pods and leaves. Pulses are a promising alternative nutritional protein source, especially when blended with cereal proteins [1], and their proteins often have functional properties such as solubility, water- and oil-holding capacity, emulsion stability and foaming that make them vital ingredients in food formulation and processing. Functional properties of the legume proteins have made them useful in the preparation and development of bakery products, soups, extruded products and ready-to-eat snacks [2]. Research also indicates that some legume protein functional properties are comparable to those of other frequently used proteins, such as whey and soybean. Soya protein isolates and concentrates in particular have become ubiquitous as milk substitutes and meat product ingredients [3, 4]. There is currently increasing research interest in legumes focused on complete use of their seeds, in which quality evaluation is done regarding their protein and starch fractions extracted using different processes [5–7].

Studies of cowpea protein concentrates have shown that the processing method used for their preparation may affect their functional properties. For example, minimum nitrogen solubility of a protein concentrate from cowpea grown in Kenya was at pH 4 for the micellar extraction [8] while at an extraction pH of 8.5, minimum solubility was at pH 4.5 [9]. Different methods also lead

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to different functional properties, micellar extraction of cowpea leads to different properties (water absorption capacity 1.24 g of water per 1 g of protein; oil absorption capacity 1.98 ml of oil per 1 g of protein; foaming capacity 50%; foam stability 54%) than isolation done at pH 8.5–12.5 and subsequent precipitation at isoelectric point (water absorption capacity 0.85–1.73 g of water per 1 g of protein; oil absorption capacity 1.27–1.67 ml of oil per 1 g of protein; emulsifying capacity 13% to 48%; emulsion stability 7% to 55%; foaming capacity 15% to 355%; foam stability 65% to 78%) [8].

Legume crops are highly diverse in terms of cultivation methods, uses, environmental adaptation and morphological variability. They can be grown from sea level up to 3000 m above sea level, and are cultivated in monoculture, associations or rotations. The Yucatán Peninsula, México, is home to a wide variety of seed legumes which are well-adapted to regional conditions, including *Phaseolus lunatus, Canavalia ensiformis* and *Vigna unguiculata* [7, 10]. Cowpea (*V. unguiculata)* is known for being a potential protein source in under-developed areas such as Africa and northeast Brazil, where it is widely consumed and can be found in different varieties [9]. Cowpea composition is generally: protein 18–35%, starch 51–67%, fat 17–32% and fibre 32–54%, with minerals and vitamins making up the difference [11], although this can vary between varieties due to genetic manipulation, agricultural practices, post-harvest handling, storage, age and seed treatment for human consumption. For example, the sulfur amino acids content in wild and cultivated varieties is limiting, but selected lines exhibit improved nutritional quality due to lower protease inhibitor and tannin contents. Further research is needed to identify appropriate treatments for removal of non-nutritional lectins, protease inhibitors and other factors [12]. Wet fractionation of legume grains for isoelectric isolate production is a promising option for reducing many antinutritional factor levels [9, 13]. The Mexican cowpea variety very probably differs from other varieties in terms of its morphology, physiology, shape, colour and chemical composition, among other characteristics. For example, Mexican cowpea starch contains approximately 23% of amylose content, which is notably lower than the 50% reported for many other starches from legume seeds [14]. The objective of the present study was to determine technological functional and nutritional characteristics of the Mexican cowpea (*Vigna unguiculata*) variety protein isolate.

MATERIALS AND METHODS

Seeds and flour

Mexican cowpea (*Vigna unguiculata)* pods were obtained from a harvest in southern Yucatán state, México. The pods were dried at 60 °C for 24 h, manually threshed, and all impurities and damaged seeds were discarded. The remaining sound seeds were course-ground in a mill (Tecator, Hoganäs, Sweden), the hulls removed with an air blower and the dehulled seed fragments milled in a Cyclotec Mill (Tecator) using a 20 mesh screen to produce a flour.

Protein isoelectric isolate

The flour was processed using the wet-fractionation method reported by Betancur-Ancona et al. [5]. Briefly, three batches (250 g each) of flour were processed by preparing a flour/water (1:6 by mass per volume) dispersion, and adjusting its pH to a value of 11 with 1 mol·l^{-1} NaOH. After soaking for 1 h, the suspension was milled in a disk mill and passed through 80- and 100-mesh screens to separate the fibre-containing solid fraction from the liquid fraction, which contains the protein and starch. Residual solids were washed 5 times with distilled water at a 1:3 (by volume/volume) ratio and then passed through a 60-mesh screen to eliminate the finest fibre. Wash water was mixed with the supernatants from the initial suspension and this solution was allowed to sediment for 30 min to recover the starch and separate the solubilized protein. Solubilized protein pH was adjusted to the isoelectric point (4.3) with 1 mol·l^{-1} HCl. The suspension was then centrifuged at 1425 $\times g$ for 12 min, the supernatants discarded and the precipitates freeze-dried. Percentage of recovered protein (*RP*) was calculated using this equation:

$$RP = \frac{PFw \times Pp}{Fw} \times 100 \quad (1)$$

where *PFw* is protein fraction weight in percent of dry base (d.b.), *Pp* – protein purity (% d.b.) and *Fw* – flour weight (% d.b.)

Chemical analysis

Following AOAC procedures [15], nitrogen (method 954.01), fat (method 920.39), ash (method 923.03), crude fibre (method 962.09) and moisture (method 925.09) contents were determined for the flour and protein isolate (PI). Nitrogen was determined with a Kjeltec System (Tecator) and protein calculated as nitrogen ×6.25. Fat content was quantified with a 4-hour hexane extraction. Ash content was calculated based on re-

maining sample weight after heating to 550 °C for 2 h, while moisture content was measured based on sample weight loss after oven-drying at 110 °C for 2 h.

Functional properties

The following functional properties were studied to evaluate the possible improvement of protein isolate properties with more technological application in the food industry.

Nitrogen solubility

According to the method of WERE et al. [16], samples of PI (125 mg) were dispersed in 25 ml distilled water and dispersion pH was adjusted to 2, 3, 4, 5, 6, 7, 8, 9 or 10 with either 0.1 mol·l^{-1} or 1 mol·l^{-1} NaOH or HCl. The dispersions were shaken for 30 min at room temperature, and centrifuged at 4 320 ×*g* for 30 min. Supernatant nitrogen content (N_S) was measured using the Kjeldahl method (method 954.01) [15], and soluble protein percentage (*S*) calculated as follows:

$$S = \frac{N_S}{N_{PI}} \times 100 \quad (2)$$

where N_S is nitrogen content in supernatant and N_{PI} is nitrogen content in sample of PI.

Water- and oil-holding capacity

These capacities were quantified by weighing 1 g d.b. sample and stirring into 10 ml distilled water or corn oil (Mazola, CPI International, México DF, México) for 1 min. The protein suspensions were then centrifuged at 2 200 ×*g* for 30 min and the supernatant volume measured. Water-holding capacity (*WHC*) and oil-holding capacity (*OHC*) were calculated with equations 3 and 4. Oil density was 920 mg·ml^{-1} [5].

$$WHC = \frac{Wh}{PS} \quad (3)$$

$$OHC = \frac{Oh}{PS} \quad (4)$$

where *Wh* is water held (g), *PS* – protein sample (g) and *Oh* – oil held (g).

Foam capacity and foam stability

Foaming properties were evaluated by blending 100 ml (V_1) of a 15 mg·ml^{-1} (by mass per volume) suspension blended at low speed in a Warning blender (Osterizer 10S-E, Oster, México DF, México) for 5 min, and recording foam volume at 30 s. Foam capacity (*FC*) was expressed as a percent increase in foam volume measured at 30 s (V_2). Foam stability (*FS*) was determined according to residual foam volume (V_3) after 5, 30 and 120 min after blending, using equations 5 and 6, respectively. Both properties were determined at pH 2–10 [6].

$$FC = \frac{V_2 - V_1}{V_1} \quad (5)$$

$$FS = \frac{V_3 - V_2}{V_1} \quad (6)$$

Emulsifying activity and emulsion stability

Emulsion properties were measured by homogenizing 100 ml of a 20 mg·ml^{-1} solution (by mass per volume) in a Caframo RZ-1 homogenizer (Caframo RZ-1, Heidolph Schwabach, Germany) at 33 Hz for 2 min, adding 100 ml corn oil (Mazola) and homogenizing for 1 min. The resulting emulsions were then centrifuged in 15 ml graduated centrifuge tubes at 1 200 ×*g* for 5 min and emulsion volume measured. Emulsifying activity (*EA*) was expressed as the percentage of the entire emulsion volume (V_t) represented by the emulsified layer volume (V_l). Emulsion stability (*ES*) was measured by heating previously prepared emulsions to 80 °C for 30 min, cooling to room temperature and centrifuging at 1 200 ×*g* for 5 min. Emulsion stability was expressed as the percentage of the original emulsion volume (V_t) represented by the remaining emulsified layer volume (V_r) , using equations 7 and 8, respectively. Both properties were determined as a function of pH in a 2 to 10 range [6].

$$EA = \frac{V_l}{V_t} \times 100 \quad (7)$$

$$ES = \frac{V_r}{V_t} \times 100 \quad (8)$$

Protein profiles of protein isolates

Amino acid analysis

Amino acid profiles were obtained by a method of ALAIZ et al. [17], using pre-column derivatization with diethyl ethoxymethylenemalonate and reversed-phase high-performance liquid chromatography (HPLC) with spectrophotometric detection at 280 nm. The HPLC system consisted of a Waters 600E multi-solvent delivery system (Waters, Milford, Massachusetts, USA), a Wisp Model 712 automatic injector (Waters) and a model 484 UV-VIS detector (Waters). Samples containing D,L-γ-aminobutyric acid as an internal standard were dissolved in 6 mol·l^{-1} hydrochloric acid. These solutions were flushed with nitrogen,

sealed in hydrolysis tubes under nitrogen and incubated in an oven at 110 °C for 24 h. Formation of *N*-[2,2-bis(ethoxycarbonyl)vinyl] derivatives from the sample hydrolysates was done by adding 0.8 μl diethyl ethoxymethylenemalonate to a dried isolate sample (200 μg) in 1 mol·l^{-1} sodium borate buffer (pH 9; 1 ml) containing 0.2 g·ml^{-1} sodium azide. The reaction was run at 50 °C for 50 min under vigorous shaking. Amino acid derivative separation was done using a binary gradient system. Solvents used were (A) 25 mmol·l^{-1} sodium acetate containing 0.2 mg·ml^{-1} sodium azide (pH 6) and (B) acetonitrile. Solvents were applied to the column at a 0.9 ml·min^{-1} flow rate, as follows: time 0.0–3.0 min, linear gradient from A–B (91 : 9) to A–B (86 : 14); 3.0–13.0 min, elution with A–B (86 : 14); 13.0-30.0 min, linear gradient from A–B (86 : 14) to A–B (69 : 31); 30.0–35.0 min, elution with A–B (69 : 31).

Tryptophan

Tryptophan content was determined by HPLC with spectrophotometric detection at 280 nm [18] using instrumentation described above. Samples (10 mg) were dissolved in 3 ml of 4 mol·l^{-1} sodium hydroxide, sealed in hydrolysis tubes under nitrogen and incubated in an oven at 100 °C for 4 h. The hydrolysates were cooled on ice, neutralized to pH 7 using 12 mol·l^{-1} HCl, and diluted to 25 ml with 1 mol·l^{-1} sodium borate buffer (pH 9). Aliquots of these solutions were then filtered through 0.45 µm Millex filter (Millipore, Billerica, Massachusetts, USA) prior to injection. Standard tryptophan solutions were prepared by dilution of a stock solution (0.51 mg·ml^{-1} of 4 mol·l^{-1} NaOH) to 3 ml with 4 mol·l^{-1} sodium hydroxide, followed by incubation. Samples (20 μl) were injected into the column. An isocratic elution system was used consisting of 25 mmol·l^{-1} sodium acetate and 0.2 mg·ml^{-1} sodium azide (pH 6)/acetonitrile (91 : 9), delivered at 0.9 ml·min^{-1}.

Available lysine

Following the Booth method [19], 8 ml of a $NaHCO_3$ solution (80 mg·ml^{-1}) and 12.3 ml fluoro-2-4-dinitrobenzene (FDNB) solution (0.3 ml FDNB in 12 ml ethanol) were added to a quantity of sample containing 35 mg nitrogen. The mixture was agitated for 2 h, excess solvent evaporated, 200 ml of 8 mol·l^{-1} HCl added and heated for 16 h. Mixture was then filtered and 2 ml placed in each of two tubes (A and B). Tube B content was extracted with 5 ml sulfuric ether, the residual ether evaporated in a water bath at 80 °C, and phenolphthalein and NaOH 120 mg·ml^{-1} (by mass per volume) added until producing a pink coloration. Then, 2 ml buffer (19.5 g of $NaHCO_3$ + 1 g of Na_2CO_3 in 250 ml of water) and 5 drops methyl chloroformate (MCC) were added, the tube covered and shaken. After shaking, 0.75 ml HCl was added, the solution extracted 4 times with 5 ml sulfuric ether each time, and the tube content transferred to a 10 ml flask. Tube A content was extracted 3 times with ether, the mixture transferred to a 10 ml flask and measured with 1 mol·l^{-1} HCl. Absorbances were read at 435 nm using a spectrophotometer (Thermospectronic Genesis 10UV; Thermo Scientific, Madison, Wisconsin, USA). Lysine (g·kg^{-1} of protein) was determined as dinitrophenyl-Lys (DNF-Lys):

$$Lys = \frac{(L)(250)(0.42)(1000)}{(E)(a)(P)} \tag{9}$$

where L is absorbance, number 250 – volume of sample (ml), number 0.42 – DNF-Lys to lysine conversion factor, E – molar coefficient (1/mol·l^{-1}), a – sample content (g) in 2 ml of solution and P – proteins (in percent of dry matter).

True digestibility and protein efficiency ratio

These properties were measured following methods described by FAO/WHO [20] and TEJADA [21], using one-month-old female Wistar rats from a local source (Centro de Investigaciones Regionales "Dr. Hideyo Noguchi", Mérida, México). Animals were individually housed in cages kept in a temperature-controlled (24 °C) vivarium on a 12 h : 12 h photoperiod, being given free access to feed and water. They received a standard diet containing casein for a one-week acclimation period. This was followed by a 21-day trial in which they were randomly assigned to one of two diets (10 animals per diet): one containing cowpea isolate adjusted to 10% protein, the other containing no protein. Weight gain (*Wg)* was recorded two times a week and feed intake every two days. True digestibility (*TD*) was calculated using these equations:

$$TD = \frac{Ni_{\mathrm{TG}} - (Nf_{\mathrm{TG}} + MNf)}{Ni_{\mathrm{TG}}} \tag{10}$$

$$MNf = \frac{Nf_{\mathrm{FG}} \times Fi_{\mathrm{TG}}}{Fi_{\mathrm{FG}}} \tag{11}$$

where Ni_{TG} is nitrogen in test group (animals feeded with diet containing protein isolate in grams), Nf_{TG} – nitrogen in feces in test group (g), *MNf* – metabolic nitrogen in feces (g), Nf_{FG} – nitrogen in feces in free group (animals feeded with diet containing no protein in grams), Fi_{TG} – food intake in test group (g), Fi_{FG} – food intake in free group (g).

Tab. 1. Proximate composition of Mexican cowpea (*V. unguiculata*) protein isolate compared to that of other legume protein isolates.

Component	Mexican cowpea (*V. unguiculata*)	*Vigna unguiculata* (L.) Walp	*Mucuna pruriens*	*Phaseolus lunatus*	*Canavalia ensiformis*
Moisture [%]	2.9	3.3	8.19	7.9	9.2
Crude protein [%]	82.8	79.9	66.0	71.1	71.3
Crude fibre [%]	0.7	0.4	0.4	0.2	0.2
Crude fat [%]	1.2	6.6	19.3	0.7	0.1
Ash [%]	1.2	2.4	1.8	2.8	3.6
Nitrogen-free extract [%]	14.1	10.8	12.5	25.1	8.3
Reference		TORRES [25]	CORZO et al. [26]	BETANCUR et al. [5]	MOGUEL et al. [23]

Protein efficiency ratio (PER) was calculated as:

$$PER = \frac{Wg_{TG}}{TPi} \quad (12)$$

where Wg_{TG} is test group weight gain (g) and *TPi* – total protein intake (g).

Statistical analysis

All measurments were done in triplicate and the results were analysed using a one-way analysis of variance with a Duncan's test to compare means [22]. The level of significance was 5% and data were processed using Statgraphics Plus version 5.1 software (Statistical Graphics, Warrenton, Virginia, USA).

RESULTS AND DISCUSSION

Proteins isolate yield and composition

Protein yield was approximately 55% and the protein content of the isolate was 82.8%, higher than reported for other legumes (Tab. 1). These levels were probably the result of processing conditions such as mesh size and number of washes [23], as well as protein composition, solubilization pH conditions and ionic force. In comparison, MUNE [24] reported an optimal pH of 9.9, and optimal NaCl concentration of 0.15 mol·l^{-1}, with an 87% yield and 84% protein content. In the present study, the cowpea grains were processed with complete use in mind, that is, taking into account the use of starch and fibre fractions. Even so, the protein content attained using the present method was higher than reported for *V. unguiculata* (L.) Walp processed with (76%) and without heat (79.9%) [25]. It was also higher than the value of 78.6% reported in another study of protein extraction with isoelectric precipitation (1 mmol·l^{-1} NaOH, extraction at pH 7) and the value of 80.9% with the Fan-Sosulki method (5 mmol·l^{-1} NaOH, pH 9), but lower than the value of 86.2% produced with salt treatment (1 mol·l^{-1} NaCl, pH 6) [11]. Using the same extraction method as in the present study, CORZO et al. reported a protein content of 65.9% with *Mucuna pruriens* [26].

Functional properties

Nitrogen solubility

Generally, protein solubility in bean proteins follows a typical curve, which for the Mexican cowpea isolate has the same tendency (Fig. 1). Nitrogen solubility of the protein isolate was pH-dependent since this determines protein electrical charge and, in turn, dictates how the protein molecule interacts with water. Minimum solubility (1.5%) was observed at pH 6, which is similar to the value of 1.3% reported for *V. unguiculata* (L.) Walp by TORRES [25], but lower than the values of 5% and 6.6% at pH 5 reported for *P. lunatus* and *C. ensiformis*, respectively [7]. For comparison,

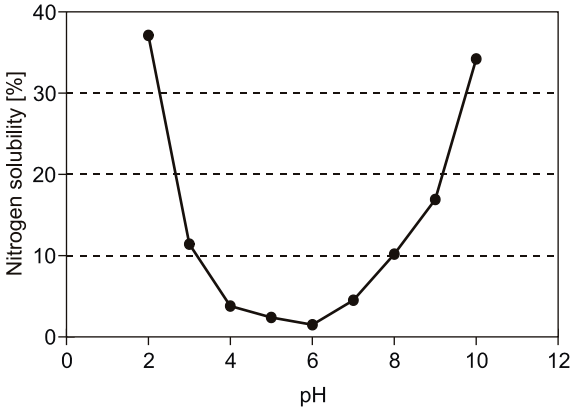

Fig. 1. Effect of pH on nitrogen solubility in Mexican cowpea (*Vigna unguiculata*) protein isolate.

Tab. 2. Water-holding capacity (*WHC*) and oil-holding capacities (*OHC*) of a Mexican cowpea *V. unguiculata* protein isolate compared to those of other legume protein isolates.

Legume protein isolates	*WHC* [%]	*OHC* [%]	Reference
Mexican cowpea	245 ± 0.1	231± 0.1	
Glycine max (Soya)	346	306	Chel-Guerrero et al. [7]
Canavalia ensiformis	250	270	Chel-Guerrero et al. [7]
Vigna unguiculata (L.) Walp	170	280	Torres [25]
Phaseolus lunatus	350	459	Chel-Guerrero et al. [7]

M. pruriens isolate is at pH 4.5 totally insoluble [27]. This variation in isoelectric points is caused by changes in the proportion of charged amino acids versus native proteins due to the concentration process, or by differences in amino acid composition between different raw materials. In both cases, amino acids with ionizable remains are responsible for the electrostatic attraction or repulsion, consequent interaction with water and, finally, protein solubilization.

Water- and oil-holding capacity

Water-holding capacity (*WHC*) is important for product characteristics such as moistness, starch retrogradation, staling, etc. [28]. In the present study, the *V. unguiculata* protein isolate *WHC* was 245% (Tab. 2), similar to the value of 250% reported for a *C. ensiformis* isolate, but lower than the value of 346% reported for *Glycine max* [7]. This legume could be used for comparison purposes due to its extensive use in foods as a functional ingredient [29]. Mwasaru et al. [8] reported *WHC* ranging from 85% to 173%, depending on protein solubilization pH. Bean protein *WHC* may depend on protein type and quantity, and on the presence of non-protein components, principally saccharides, which may increase *WHC*. This need not be true in all cases since the isolate *WHC* observed here is the same as that reported for *C. ensiformis*, although the latter has a minimum of 6% nitrogen-free extract. Bean protein *WHC* may therefore depend on other factors such as pH of the system, because this controls protein electrical charge [28]. This effect is even more pronounced when the protein contains numerous lateral polar chains, which is the present case given the high content of charged amino acids in the isolate (Tab. 3).

Oil-holding capacity (*OHC*) makes products potentially useful in food structural interactions, particularly flavour retention, improved palatability and extended shelf-life in meat products through reduction of moisture and fat loss [7]. In the Mexican cowpea isolate, *OHC* was 231% (Tab. 2), which is lower than the value of 280% reported for *V. unguiculata* (L.) Walp isolates [25], but higher than a 127% to 167% range that depended on protein solubilization pH [7]. It was only slightly lower than the value of 270% reported for a *C. ensiformis* isolate, but notably lower than the values of 306% and 459% reported for soya and *P. lunatus* isolates, respectively [6]. Considering soya a standard ingredient of food [29], other author [28] reports for a soya isolate (89.9% of protein) an *OHC* value of 148%, which indicates that the protein level and composition are important in this functional property. Oil-holding capacity is attributed to non-polar sites on the molecular chain that are normally in its interior [30]. The more denatured they are, the more available they become, until reaching a maximum value that does not make them insoluble. This may be the case with the Mexican cowpea protein isolate since

Tab. 3. Amino acid composition of protein isolates from Mexican cowpea (*V. unguiculata*).

Amino acid	FAO requirement [20]	Mexican cowpea
	[g·kg^{-1}]	
Essential		
Lys	58	73
Trp	11	7
Phe + Tyr	63	95
Met + Cys	25	5
Thr	34	43
Leu	66	92
Ile	28	44
Val	35	54
Non-essential		
Asp		108
Glu		190
Ser		65
His		29
Gly		44
Arg		78
Ala		44

Values are expressed per kilogram of protein.

Tab. 4. True digestibility (*TD*) and protein efficiency ratio (*PER*) values for a Mexican cowpea protein isolate and other legume protein products.

Protein product	*TD* [%]	*PER*	Reference
Mexican cowpea isolate	88.8	1.24	Experimental
Soybean isolate	not reported	1.1–1.7	Berk [42]
Vigna unguiculata isolate	86.9–96.8	not reported	Liu et al.[4], Rangel et al. [36]
Cowpea flour	55–92	0.5-1.4	Sathe [28]
Mung bean isolate	not reported	0.53	Sathe [28]
Mung bean isolate + 0.5% Met	not reported	1.26	Sathe [28]
Chickpea isolate	90.1	NR	Sathe [28]
Mung bean isolate + 0.5% Met	not reported	1.26	Sathe [28]

its relatively high digestibility (Tab. 4) suggests denaturation and consequently the presence of more oil-binding groups.

Foam capacity and stability

The relationship of hydrophilic versus hydrophobic properties is a key factor in balancing *FC* and *FS*, and is probably a function of composition and protein conformation in response to environmental conditions [30]. Foaming capacity in the Mexican cowpea protein isolate was pH-dependent with the highest values ($p < 0.05$) recorded at the two extremes of the pH range (190.3% at pH 2; 133.3% at pH 8; 160% at pH 9; and 173.3% at pH 10; Fig. 2). The lowest *FC* values were observed at pH 3 to pH 5 and were similar ($p > 0.05$), while values at pH 6 and pH 7 were only slightly higher, similar to those of soya isolate at neutral pH. This *FC* pattern was similar to that of the solubility pattern, with increases at extreme pH far from the protein's isoelectric point (IP). At its IP, the protein has a net charge near zero which is probably what prevents development of functional properties such as foaming. The protein content is also very important in a response of *FC*, because soya protein isolate, considered a standard ingredient of food, at 5 mg·ml^{-1} has 98% of this parameter [31]. For a *V. unguiculata* (L.) Walp protein isolate, Torres [25] also reported greater *FC* at pH 2 (157%) and pH 10 (89%), and lower values near IP (34% at pH 4; 46% at pH 5; and 59% at pH 6). For a *P. lunatus* isolate, Chel-Guerrero et al. [7] reported a high *FC* value of 147% at pH 10 and low values near IP (22.5% at pH 3 and 34.5% at pH 4), while for *C. ensiformis* the low values were also near IP (24% at pH 5 and 17.5% at pH 6). Differences in pH-dependent *FC* values probably result from the protein source, since the generally compact, rigid structure of legume proteins is influenced by environmental conditions.

The same interaction between hydrophilicity and hydrophobicity produced a level of hydropho-

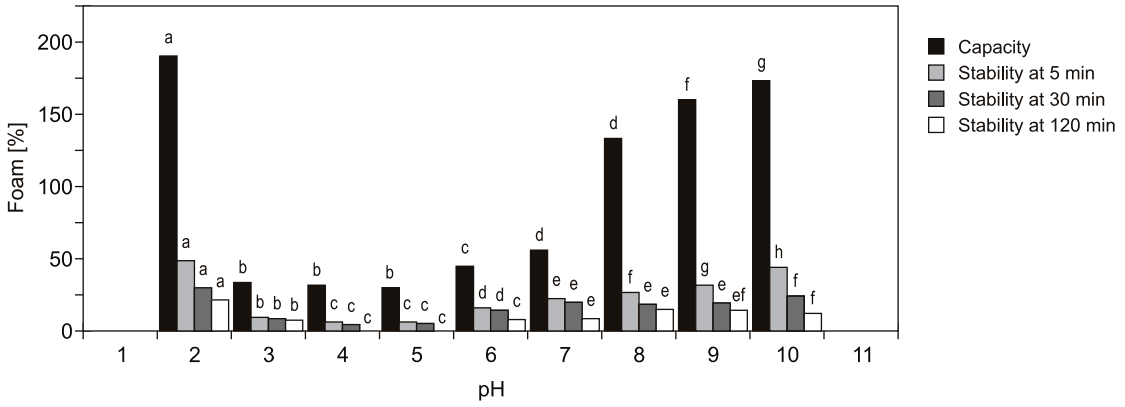

Fig. 2. Effect of pH on foam capacity and foam stability in a Mexican cowpea (*Vigna unguiculata*) protein isolate at different pH values and times.

Different letters on bars of the same colour indicate a statistically significant difference ($p < 0.05$).

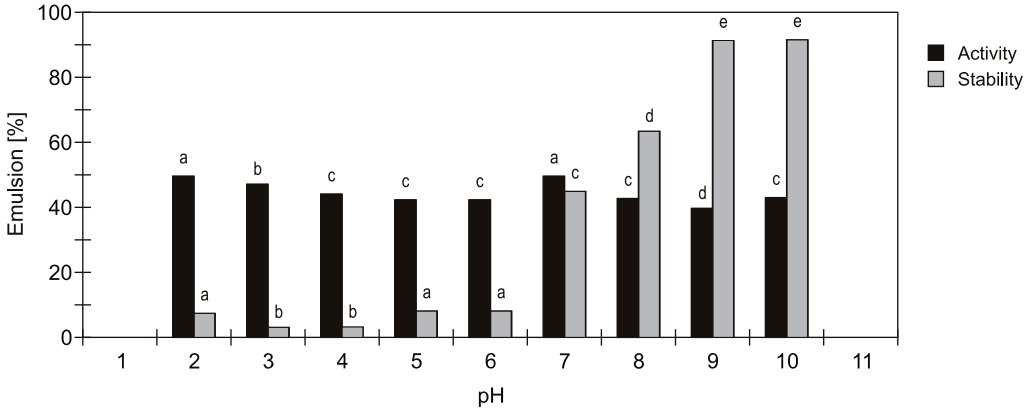

Fig. 3. Effect of pH on emulsifying activity and emulsion stability in a Mexican cowpea (*Vigna unguiculata*) protein isolate.

Different letters on bars of the same colour indicate a statistically significant difference ($p < 0.05$).

bicity insufficient to maintain foam (Fig. 2). Foam stability decreased over time, with notable instability near IP, due to the inability to form the viscoelastic film necessary to resist pressure from bubbles [32].

Emulsifying activity and emulsion stability

The behaviour of surface properties in different bean proteins varies widely, which is shown clearly in the *EA* results. The Mexican cowpea protein isolate exhibited the lowest ($p < 0.05$) *EA* (39.7%) at pH 9, and the highest (49.6%) at pH 2 and pH 7 (Fig. 3). The low *EA* value for Mexican cowpea protein isolate contrasted with low values reported for *V. unguiculata* (L.) Walp protein isolates: 5.6% at pH 4 for [25], but was similar to other isolates: 41.7% at pH 5 for *P. lunatus*; 50% at pH 4 for *C. ensiformis* [6]; and 53% at pH 7 for *M. pruriens* [27]. Similar to *FC*, protein concentration is an important factor in film properties, because in soya products with 66% and 86% of protein content, the *EA* values were 18% and 180%, respectively [33]. Our present results fall in this interval. Emulsion stability (*ES*) was highest ($p < 0.05$) at pH 9 (91.3%) and pH 10 (91.5%), followed by values at pH 7 (44.9%) and pH 8 (63.4%), and finally levels below 10% at pH 2–6 (Fig. 3). Higher *ES* values at neutral to alkaline pH have also been reported for protein isolates from *V. unguiculata* (L.) Walp (92.7% at pH 7; 94.1% at pH 8; and 94.2% at pH 9) [1] and from *C. ensiformis* (near 100% at pH between 7 and 9) [7]. Similar to foaming properties, the highly variable *EA* and *ES* values observed here depend on the degree of protein denaturation and the hydrophobic/hydrophilic balance [30].

Nutritional characteristics

Amioacid profiles of protein isolate

Generally, the Mexican cowpea protein isolate had essential amino acids levels that surpassed FAO-recommended levels for school-age children [20]. Despite a 12% decrease from total lysine levels, the available lysine level (64 g·kg^{-1} of protein) was still above the recommended levels (Tab. 3). The decrease was due to possible reactions of the epsilon-amino group under alkaline conditions, e.g. Maillard reactions [30, 34]. This reduction was less than that of 15–22% reported for soya protein after heating [12]. This level is similar to levels reported for a *P. lunatus* protein isolate, lower than reported for a *M. pruriens* protein isolate (Tab. 3), and very similar to that reported for egg (66 g·kg^{-1} of protein) [35].

Sulfur amino acids are the primary limiting amino acids in cowpea [12]. This held true for the Mexican cowpea protein isolate sulfur amino acids fraction, which contained only 5 g·kg^{-1} of protein, far less than the same fraction in protein isolates from *M. pruriens* and *P. lunatus*. RANGEL et al. [36], in contrast, stated that tryptophan was the primary limiting factor. As is the case with most plant proteins, acidic amino acids accounted for the greatest proportion (298 g·kg^{-1} of protein) of total amino acids content in *V. unguiculata*, a proportion similar to that in *P. lunatus* flour and protein isolate, yet lower than in *M. pruriens* products (Tab. 3). Legume protein isolates contain mostly reserve proteins, specifically globulins (510–720 g·kg^{-1} of protein) [37, 38]. The largest globulin fraction in legumes is the vicilins [28], which have very low sulfur amino acids levels [9]. Protein isolate

preparation conditions may also have an effect. For instance, RANGEL et al. [36] reported that an isolate with 32 g·kg^{-1} of protein sulfur amino acids may be produced at less drastic pH conditions (pH 8.5), although the vicilin fraction contained only 13 g·kg^{-1} of protein.

Digestibility and protein efficiency ratio

True digestibility (*TD*) for the Mexican cowpea protein isolate (88.8%) was within ranges reported for isolates from other cowpea varieties, and slightly lower than reported for a chickpea (*Cicer arietinum*) isolate [28] (Tab. 4). Differences in *TD* values are largely caused by variations in composition and processing conditions that lead to changes in protein structure, a limiting factor in digestibility [39]. Legumes generally have low protein digestibility values (73–90%) [12] because the globulin fraction accounts for the highest proportion of protein in the dry grain (50–75%) [40]. Consequently, when these proteins are in native state, they are not very susceptible to proteolytic enzyme attack [41]. The relatively high *TD* of the Mexican cowpea protein isolate can be attributed to protein denaturation during the treatment of the flour at a highly alkaline pH of 12.

Protein efficiency ratio (*PER*) for the Mexican cowpea protein isolate was 1.24, higher than reported for a Mung bean isoelectric protein isolate and within the range for an equivalent soya product (Tab. 4). Processing influences product amino acid balance, for example, *PER* of soya flour decreases from 2.2 to 2.3 for the protein isolate [42]. According to FRIEDMAN [12], *PER* of the Mexican cowpea protein isolate studied here would classify it as a low-quality protein source. It must be taken into account that *PER* includes the contribution of digestibility and essential amino acids. This suggests that, in the present case, processing made the product more accessible to enzymatic action by denaturation, but lowered sulfur amino acids content and, in turn, compromised the nutritional quality (Tab. 4). Adding the limiting factors to the diet can improve nutritional value quantified as *PER* as shown in a study of Mung bean protein isolate, in which *PER* increased from 0.5 to 1.26 when methionine was added to the diet [28].

CONCLUSIONS

Protein content of Mexican cowpea isoelectric protein isolate was 82%. Solubility pattern was unchanged with respect to the raw material, with high solubility at extremely acid or alkaline pH, and an isoelectric point of pH 6. Based on several functional properties, it may be an attractive functional ingredient for food systems due principally to its water-holding, oil-holding and foam capacities. The protein isolate could be incorporated into food products such as bakery products, seasonings, sausages or ice cream. Like most legume products, the Mexican cowpea protein isolate contained lower amounts of sulfur amino acids and higher amounts of acidic amino acids. Protein denaturation during processing probably positively affected the nutritional quality of the isolate, which was reflected by a higher true digestibility. After processing, lysine content decreased by 12% to 64% of protein, but was still slightly higher than that of eggs, being within levels recommended by FAO. However, its low sulfur amino acids content kept the protein efficiency ratio at 1.24, classifying it as a low-nutritional-value protein, although this *PER* was still better than that of soya protein isolate. The protein isolation method applied in the present study is a viable alternative method for improving the functional and nutritional properties of Mexican cowpea *Vigna unguiculata* seeds.

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