IJFANS
International Journal of Food And Nutritional Sciences

Volume 4, Issue 4, Jul-Sep 2015, www.ijfans.com e-ISSN: 2320-7876

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

IMPACT FACTOR ~ 1.021

Official Journal of IIFANS

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

e-ISSN 2320 –7876 *www.ijfans.com*
Vol.4, Iss.4, Jul-sep, 2015

Research Paper *Open Access*

PHYSICOCHEMICAL AND STRUCTURAL PROPERTIES OF MAJOR PROTEIN FRACTIONS OF TWO VARIETIES OF NEA-COWPEA (*VIGNA UNGUICULATA L.*): A COMPARATIVE STUDY

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Received on: 16th September, 2015 **Accepted on: 10th November, 2015**

ABSTRACT

In both cowpea varieties, the globulin was the major protein fraction followed by albumins, glutelins and prolamins with 48 ± 2 g/100 g, 33 ± 2 g/100 g, 8 ± 1 g/100 g and 2 ± 0.5 g/100 g of the total seed protein content, respectively. Under non-reducing conditions, the CU and CO globulin fractions, showed four major polypeptides with molecular masses of 80 ± 3, 65 ± 3, 56 ± 2 and 52 ± 2 kDa and some polypeptides with molecular masses ranging 45-25 kDa. Albumin and globulin fractions of CU showed the lowest values of enthalpy (ΔH) suggesting an initial partial denaturation. Globulin fraction showed a higher content of aromatic amino acids than the albumin fraction, displaying a more hydrophobic behaviour. Results provide useful data that will be supplemented with further studies on functional properties of these proteins fractions, followed by the development of legume protein products.

Keywords: albumin, globulin, thermal properties, surface hydrophobicity

INTRODUCTION

Cowpea (*Vigna unguiculata* L. walp) is a legume crop considered of regional importance because of its consumption frequency and its relevance for the agro biodiversity in the NEA. In previous studies, Avanza et al (2013) have found protein contents ranging from 24.3 to 27.1 g/100 g for native flours of Colorado (CO) and Cuarentón (CU) rendering them an attractive source of proteins to replace the more expensive animal proteins. Cowpea protein fractions comprise albumins (Alb), globulins (Glb), prolamins (Pro) and glutelins (Glu) (Vasconcelos IM, et al, 2010). On the basis of their sedimentation coefficient, Glb can be divided into two major groups of proteins: the 11S fraction (legumins) and the 7S fraction (vicilins), which play an important role as storage proteins[3]. Pro are storage proteins found mainly in the seeds of cereal grains with high proline and glutamine content while Glu, have poor lysine content in cowpea (Vasconcelos IM, et al, 2010, Tang CH & Sun X. A, 2011).

Physicochemical and functional properties of protein fractions from seeds are determined by their composition and structure, which is influenced by the environment where the plants are cultivated and physical, chemical and biological treatments. Protein fraction properties from several legumes of different regions have been studied (Vasconcelos IM, *et al*, 2010, Tang CH, Sun X. A, 2011, Lawal O, et al, 2005); however, no information is available about cowpea protein fractions from the NEA.

The aim of this study was to analyze the physicochemical and structural properties of Alb and Glb fractions of two cowpea varieties (CO and CU). The utilization of such proteins could impart specific characteristics to the final product in food formulations.

MATERIALS AND METHODS

MATERIAL

Seeds of two local cowpea varieties (CO and CU) were provided by Estación Experimental "El Sombrero-Corrientes" (Instituto Nacional de Tecnología Agropecuaria-INTA). Healthy seeds were stored at 10 °C until use.

PREPARATION OF FLOURS

Whole seeds were ground in an electric mill (KSM2 Braun, Naucalpan de Juárez, México) and then passed through an 80 ASTM sieve (177 µm pore size). Flours were then defatted by extraction with hexane during 24 h at 4 °C.

PROTEIN FRACTIONATION BASED ON SOLUBILITY CRITERIA

The Alb, Glb, Pro and Glu fractions from flour were extracted according to Rosa et al, 2000. in triplicate. All samples were lyophilized and kept at -25 °C until use. The protein content in cowpea protein fractions was analyzed for nitrogen content according to the Kjeldahl´s method (factor: 6.25) (Lane RH., 1990).

GEL ELECTROPHORESIS (PAGE)

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the Laemmli´s method (Laemmli UK, 1970) (1 h at a constant voltage of 200 V). Alb and Glb samples (1 g/100 mL) were dissolved in 0.125 M Tris-HCl, pH 6.8, 0.02 mL/100 mL glycerol, 0.1 g/100 mL SDS, 0.05 g/100 mL bromophenol blue, and centrifuged at 15800 *g* for 5 min at 4 ºC. Supernatants were loaded onto the gel (30-40 □ g of protein per lane). Samples, boiled [1 min, in 5 mL/100 mL 2-mercaptoethanol (2-ME)] and then centrifuged, were run under reducing conditions (R-SDS-PAGE). The following molecular weight standards were used to estimate the molecular masses of polypeptides (Pharmacia Hepar Inc, Franklin, OH, U.S.A.). Non-denaturing electrophoresis (ND- PAGE) was performed with the same buffer used for denaturing conditions without the addition of SDS and using continuous gels (7%).

GEL FILTRATION CHROMATOGRAPHY

Cowpea Glb were suspended in 0.05 M Tris-HCl pH 7.5 and analyzed by gel filtration chromatography at room temperature, employing a Superose 6B HR 10/30 column linked to a Pharmacia LKB, FPLC System (Uppsala, Sweden). Glb samples (4 mg Glb in 0.2 mL buffer) were eluted with the same buffer in which they were prepared and the optical density at 280 nm was recorded. Column calibration was performed with HMW and LMW gel filtration calibration kits (GE-Healthcare, Buckinghamshire, UK). Data were processed and evaluated by the Pharmacia AB, FPLC director. Protein peaks were pooled and analysed by SDS-PAGE.

PROTEIN DIGESTIBILITY

Protein digestibility was conducted as described by Rudloff and Lönnerdal (Rudloff S, Lönnerdal ,1992) with slight modifications. Alb and Glb samples of CU and CO were dispersed in distilled water (10 mg/mL), adjusted to pH 4.5 (HCl 1 M) and kept in the dark on a shaking water bath (37 °C, 5 min). A pepsin solution (10 mg/ mg sample; the enzyme/substrate ratio: 1/15 to 1/20) was added to the sample and these samples were kept in the dark and on a shaking water bath (37 °C, 30 min). Then, the pH of the samples were gradually (within 10 min) increased to 7.0 ($NaHCO_3$ 0.5 M) and 2.5 mL of a pancreatin solution (0.4 g/100 mL $NaHCO_3$ 0.1 M) was added to each sample to be incubated (1 h at 37 °C). Then, the enzymes were inactivated during 4 min in a boiling water bath. Aliquots were spun (10000 g, 45 min, 4 °C) to separate fat, soluble fraction, and pellet (insoluble proteins). An equal volume of 24% trichloroacetic acid (TCA) was added to the supernatant and then centrifuged at (10000 g, 30 min, 4 °C). The digestibility of each sample was calculated taking into account the initial protein content in supernatant of samples and enzyme blanks. Protein content of samples was determined by Kjeldhal procedure using 6.25 as conversion factor. All analyses were run in duplicate.

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The equipment (Q100 V9.8 Build 296 calorimeter; TA Instrument, New Castle, Del., USA) calibration conditions were: a heating rate of 10 °C/min, using standards [indium, lauric acid and stearic acid (pro-analysis)]. Alb and Glb samples (12-15 mg) were suspended in water (30 g/100mL) and placed in hermetically sealed aluminium pans; a double empty pan was used as reference. Samples were scanned at 10 °C/min from 25 to 130 °C. After each run, the pans were punctured and their dry matter content was determined at 105 °C. The denaturation temperature, Td (°C), and the enthalpy of transition, ΔH (J/g dry solids flour), were obtained from thermograms with Analysis V4.2E (TA Instruments, New Castle, Del., USA).

FLUORESCENCE SPECTROSCOPY

The intrinsic fluorescence was determined at the following conditions: excitation wavelength: 280 nm (slit width, 5 nm), emission wavelength: 300-450 nm (slit width, 5 nm), scanning speed: 500 nm/min (Perkin-Elmer, Watham-Massachusetts, USA). The Alb and Glb fluorescence spectra of both varieties was determined at 25 °C in water and 0.05 M Tris-HCl buffer, pH 7.5, respectively. The protein concentrations of samples ranged between 0.01 and 0.02 g/L (Lowry OH, et al, 1951).

UV SPECTROPHOTOMETRY

Direct absorption spectra of Alb and Glb were obtained using a UV-visible spectrophotometer (Jasco V 630 Bio-spectrophotometer, England). Alb and Glb samples (1 mg/mL) were dispersed in distilled water and adjusted to pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 9.0 and 10.0 with either 0.1 or 1.0 M NaOH or HCl. The dispersions were shaken for 30 min at room temperature, and centrifuged (10000 g for 30 min at 25 °C).

Alb and Glb samples were evaluated at different ionic strengths (0 M, 0.1 M, 0.5 M and 2 M NaCl). Alb and Glb fractions (1 mg/mL) were dispersed in NaCl-H_2O and 0.05 M Tris-HCl-NaCl buffer, pH 7.5, respectively. All dispersions were stirred during 30 min at room

temperature and then centrifuged (10000 g for 30 min at 25 °C).

SURFACE HYDROPHOBICITY (H_0)

The surface hydrophobicity (H_0) was determined as described by Cardamone and Puri (Cardamone M, Puri NK, 1992), by using the 1,8-aniline-naphthalene-sulfonate (ANS) fluorescent probe (Aldrich Chemical Co., Milwakee-Winsconsin, USA). Alb samples (0.033 mg/mL distilled water) and Glb samples (0.020-0.030 mg/mL, 0.05 M Tris-HCl buffer, pH 7.5) were equilibrated with different concentrations of ANS (from 0.0 to 100 µM). Then, the emission spectra (370-600 nm) of samples were recorded and corrected taking into account the corresponding blanks to obtain, the increase in fluorescence as a result of ANS binding (*ΔFI)*. The *ΔFI* at 470 nm (the maximum emission of an ANS-protein complex) was plotted against the ANS concentration (*Δ*M) and the data adjusted with the following equation:

$$\Delta FI = \frac{A \times ANS}{B + ANS} \quad (1)$$

Where B = 1/Ka and $A = \Delta FI_{max}$. (A = ΔFI_{max} is the fluorescence intensity at saturation).

The H_0 is proportional to ΔFI_{max} per mg protein and therefore estimated from Equation 1 by dividing A by the protein concentration of each sample determined by the Lowry's method (Lowry OH, et al, 1951).

ALBUMIN AND GLOBULIN SOLUBILITY

Protein solubility at different pH was determined by the modified method described by Bera and Murkherjee (Bera MB, Murkherjee RK, 1989). Briefly, Alb and Glb samples (1 mg/mL) were dispersed in distilled water and adjusted to pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 9.0, 10.0 with either 0.1 M NaOH or HCl. Solubility at different ionic forces was evaluated (0 M, 0.05 M, 0.1 M, 0.5 M, 1 M and 2 M NaCl). Alb and Glb fractions (1 mg/mL) were dispersed in NaCl-H_2O and 0.05 M Tris-HCl-NaCl buffer, pH 7.5, respectively.

All dispersions were stirred during 30 min at room temperature and then centrifuged (10000 *g*, 30 min, 25 °C). In both cases, the protein content of the supernatants was determined according to the Lowry's method (Lowry OH, et al, 1951) using a UV-visible spectrophotometer (Jasco V 630 Bio-spectrophotometer, England). Protein solubility was calculated considering the soluble protein determined by Lowry's method (Lowry OH, et al, 1951) and the initial protein content determined by the Kjeldhal's method. Bovine serum albumin was used as standard protein.

STATISTICAL ANALYSIS

Protein fractionations were performed in triplicate. All experimental analyses were carried out in triplicate except the protein digestibility. The means values were compared using the least significant difference (LSD) test (α=0.05) and the analysis of variance (ANOVA) was performed. The statistical analysis was done using the Infostat software (Di Rienzo JA, et al, 2008).

RESULTS AND DISCUSSION

PROTEIN FRACTIONS

In both varieties, Glb was the major seed protein fraction with 48 ± 2 g/100 g of the total seed protein content, followed by Alb with 33 ± 2 g/100 g. The Glu content was 8 ± 1 g/100 g and Pro was 2 ± 0.5 g/100 g. Our results are consistent with those reported by Vasconcelos et al. (2010). Mohan VR, Janardhanan K., 1993 have found only 9 g/100 g of Glu in *Vigna sinensis*, while Vasconcelos et al. (2010) have informed higher values for three different cowpea varieties (21-24 g/100 g). The very low values for the Pro fraction are typical of *Vigna* species (Mohan VR, Janardhanan K. , 1993). The differences between values reported by several authors could be attributed to species variances and/or the protein extraction methods used.

POLYPEPTIDE COMPOSITION OF ALBUMINS AND GLOBULINS

Alb and Glb fractions were separated by PAGE under non-denaturing conditions (ND- PAGE) (Figure 1a). Electrophoresis profiles showed three Glb fractions (α, β and γ -vignin) similarly to those reported by Freitas et al. (Freitas RL, et al, 2004).

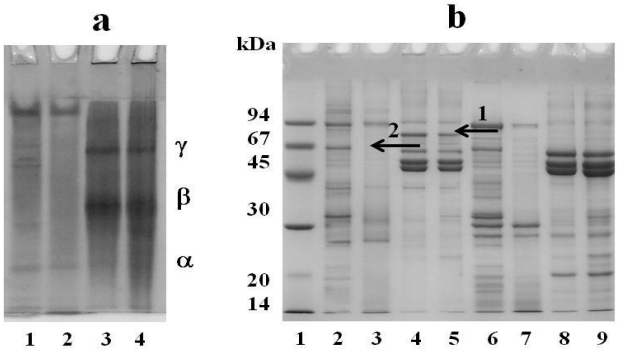

Figure 1: (a) ND-PAGE of Alb and Glb fractions of Cuarenton (CU) and (Colorado (CO) varieties without 2-ME. Lane 1: Alb CO; lane 2: Alb CU; lane 3: Glb CO; lane 4: Glb CU. (b) SDS-PAGE of albumin (Alb) and globulin (Glb) fractions of CU and CO varieties without 2-ME (NR-SDS PAGE) (lane 2, 3, 4, 5) and with 2-ME (R-SDS PAGE) (lane 6, 7, 8, 9). Lane 1: molecular weight standard proteins; lanes 2 and 6: Alb CO; lanes 3 and 7: Alb CU; lanes 4 and 8: Glb CO; lane 5 and 9: Glb CU.

Moreover, Alb and Glb fractions were separated by SDS-PAGE with and without 2-mercaptoethanol (2-ME) as shown in Figure 1b. Under non-reducing conditions (NR-SDS-PAGE), the CU and CO Glb fractions showed four major polypeptides with molecular masses of about 80 ± 3, 65 ± 3, 56 ± 2 and 52 ± 2 kDa

and some polypeptides with molecular masses distributed over a range of 45-25 kDa (Figure 1b lanes 4 and 5). Under reducing conditions (R-SDS PAGE) the 80 kDa polypeptide was not present (Figure 1b arrow 1) and an increase in the intensity of 30 and 20 kDa bands was observed, in both Glb (Figure 1b lanes 8 and 9).

The major CO Alb polypeptides at NR-SDS PAGE were those of molecular masses close to 99, 94, 67, and 32 kDa, with a lower proportion of polypeptides with molecular masses between of 60-45 kDa (Figure 1b lane 2). Alb from CU had almost the same polypeptide composition, except for the 67 kDa (Figure 1b arrow 2). The R-SDS PAGE revealed the presence of polypeptides with molecular masses of around 30 kDa in both varieties (Figure 1b lane 6 and 7). Alb from CO presented a high proportion of polypeptides with molecular masses about 20 kDa (Figure 1b lane 6); whereas in CU, these polypeptides were scant (Figure 1b lane 7). The electrophoresis profiles of the Alb fraction obtained from both varieties (Figure 1b R-SDS PAGE) showed several polypeptides that were not bound by disulfide bonds. Alb electrophoresis profiles showed similar bands as those reported by Vasconcelos et al. (2010), with a prevalence of three polypeptides with molecular masses in the range of 81-93, 27-30, and 16-19 kD. Polypeptide bands of 95, 63, and 32 kDa were found for Alb fractions of other cowpea cultivars (Pedalino M, et al, 1990).

Gel filtration chromatography of crude Glb was performed from CU and CO cowpea cotyledons (Figure 2). Chromatographic profiles on both varieties, showed one major peak (Figure 2a and 2b-peak 2, 300 kDa, Ve=12.5 mL), which could correspond to 11S globulin as it was suggested by Barba de la Rosa et al. (1992) for the globulin fraction obtained from *Amaranthus hypochondriacus*. Major protein peaks showed on Figure 2 were subsequently analyzed by NR-SDS PAGE. The minor peaks (Figure 2a and 2b- 3,4 and 5) would correspond to a molecular association of 7S trimers, since cowpea is a legume whose content in 7S Glb is higher than that of 11S Glb with estimated molecular masses between 150 kDa - 67 kDa (peaks 3 and 4- Figure 2a and 2b).

Both Glb cowpea varieties (CO and CU) presented different structural features. CO Glb presented high molecular mass protein aggregates (AAMM) (Figure 2a- peak 1> 600 kDa, Ve= 7.5 mL) which can also be seen on the electrophoretic profile seed point (Figure 2a- inset), whereas the CU Glb fraction did not present such aggregates. On the other hand, CU Glb exhibits a prevalence of low molecular mass protein molecules. (Figure 2b- peaks 4 and 5).

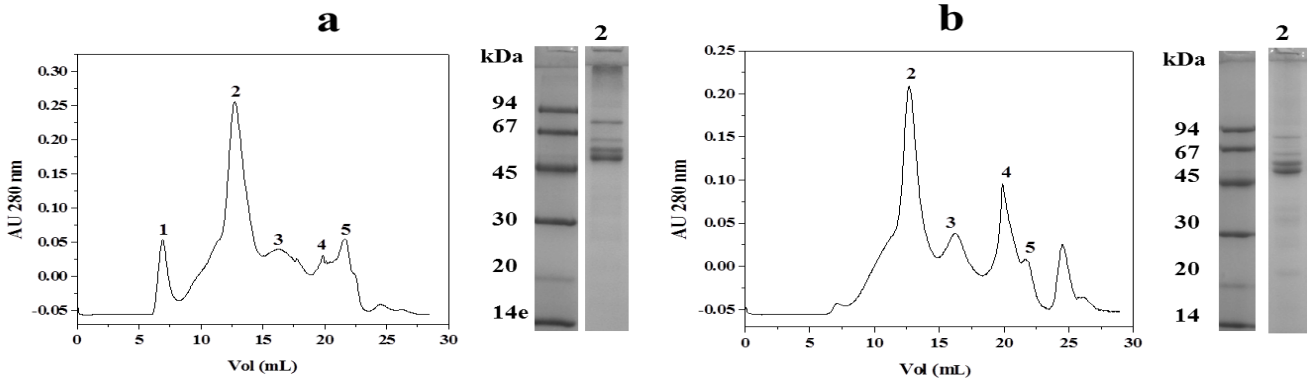

Figure 2: Fractionation of the globulin fraction from cowpea by FPLC gel filtration chromatography and electrophoresis profiles NR SDS-PAGE (a) CO and (b) CU.

PROTEIN FRACTIONS DIGESTIBILITY (%PD)

Protein quality could be considered as a function of amino acid composition and protein digestibility. Cowpea major protein fractions obtained from both varieties showed good *in vitro* protein digestibility that varied according to the protein fraction (Alb or Glb) and cowpea variety analysed. The protein fractions of CO did not show significant differences in digestibility values (Alb CO: 78.02±1.80% and Glb CO: 78±1.67%), but the protein fractions of CU exhibited significant differences (Alb CU: 66.95±2.01% and Glb CU 90.95±2.64%). The protein digestibility of Glb protein fraction from CU was higher than Glb from CO. The difference observed could be due to the presence of high molecular mass protein aggregates (AAMM > 600 kDa) in Glb fraction from CO which were mentioned in section 3.2.

CONFORMATIONAL CHARACTERISTICS

DIFFERENTIAL SCANNING CALORIMETRY

Alb and Glb protein fractions were analysed using a differential scanning calorimeter (DSC) to investigate their thermal stability. The Alb and Glb fractions of CU and CO showed only one major endotherm. The denaturation temperature (Td) and denaturation enthalpy (ΔH) of the proteins are shown in Table 1. The Td values of CO Alb and CO Glb are similar to those of CU fractions ($p>0.05$). These Td values are in agreement with those reported by Horax et al. (2004) for isolates from different cowpea varieties.

The ΔH represents the proportion of undenatured protein in a sample and it is correlated with the degree of order in the protein structure (Meng GT, Ma CY, 2004). The ΔH values of CU protein fractions were lower than

those of CO and both were lower to those reported for cowpea isolates and protein fractions of other legumes (Tang CH & Sun X. A, 2011, Horax, et al, 2004). The minor values of ΔH obtained for major protein fractions of CU, as compared to CO, suggest that the first ones are partially denatured. This finding could also be explained considering that the protein extraction protocol used in this work might promote protein denaturation, being this the cause of the low ΔH values.

Table 1: Differential scanning calorimetry (DSC) thermogram parameters of cowpea major protein fractions

Protein fraction	Td (°C)	ΔH (J g^{-1})
Alb CU	91.17 ± 3.11a	0.46 ± 0.01d
Glb CU	89.38 ± 3.45a	0.79 ± 0.02c
Alb CO	93.92 ± 2.60a	1.77 ± 0.06a
Glb CO	94.58 ± 2.25a	1.58 ± 0.05b

Mean values with different letters in the same column are significantly different ($p < 0.05$). Albumin (Alb); globulin (Glb); Cuarenton (CU); Colorado (CO).

FLUORESCENCE SPECTROSCOPY

The intrinsic fluorescence spectrum is determined mainly by the polarity of the environment of the tryptophan (Trp) residues and provides a sensitive means of monitoring the conformational changes in proteins and protein-protein as well as ligand-protein interactions (Pallares I , et al, 2004). The maximum fluorescence emission (λ_{max}) suffers a red shift when chromophores become more exposed to solvent and the quantum yield of fluorescence decreases when the chromophores interact with quenching agents, either in a solvent or in the protein itself.

The Glb and Alb fluorescence spectra of both varieties are shown in Figure 3. The λ_{max} for CU and CO Alb, and CU and CO Glb were 342, 349, 340 and 344 nm, respectively. These results suggest that CO protein fractions have tryptophan residues which are located closer to the protein surface relative to a more hydrophobic and less compact structure than the protein fractions of CU.

Moreover, Glb fractions of CU and CO showed higher fluorescence intensities (FI_{max}) than the corresponding Alb fractions. This may be due to the differences in the structure, location and amount of aromatic amino acids present in each fraction or to the different physicochemical conditions in which protein fractions were prepared (aqueous solution and Tris-HCl buffer).

The λ_{max} for CU and CO Glb obtained in this work are coincident with soy β-conglycinin and amaranth 7S globulin (λ_{max}= 344) (Quiroga A, et al, 2011); and higher than 7S globulins (λ_{max}= 331.8; 336.2; 338.0) obtained from three *Phaseolus* varieties (Tang CH & Sun X. A, 2011). The higher fluorescence intensity (FI_{max}) of CU Glb fraction (12,000 mL/mg) are similar to amaranth 7S globulin (12,820 mL/mg) reported by Quiroga et al. (Bera MB, Murkherjee RK, 1989).

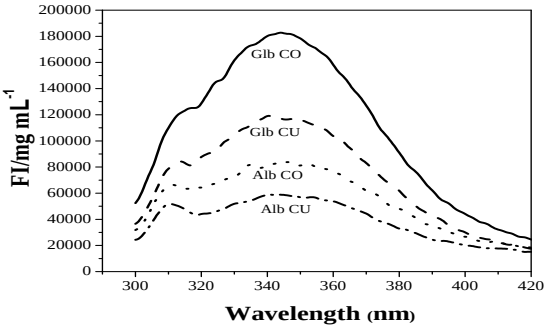

Figure 3: Fluorescence-emission spectra of albumin (Alb) and globulin (Glb) fractions from cowpea standardized to 0.1% (w/v), with excitation at 280 nm. Colorado (CO); Cuarentón (CU).

ULTRAVIOLET SPECTROSCOPY

The Alb and Glb fraction from CO and CU were analyzed by absorption spectrophotometry in the UV region to evaluate the potential conformational changes induced by pH and ionic strength (1 mg/mL dispersions) shown in Figure 4. It should be mentioned that a pH screening was done between pH 2 and pH 10; but in order to show significant changes between extreme pH values (2 and 9) were included in Figure 4 as well as pHs close to isoelectric point (4 and 5).

Regardless of cowpea variety, the Alb fractions exhibited a similar behaviour induced by pH changes; analogous modifications were observed between Glb fractions.

In both varieties, the maximum wavelengths for Alb and Glb fractions were 267 and 278 nm, respectively. Aromatic side chains such as phenylalanine, tyrosine and tryptophan absorb in the range from 270 to 290 nm (Mathews C, et al, 2014). The Alb absorbance values were minor to Glb fraction, which suggests a higher content of aromatic amino acids, as reported by Chan and Phillips (Chan CW, Phillips DR, 1994).

The Alb fraction did not show changes on maximum wavelength value, with absorbance values between 0.75 and 0.85, in the range of the pH analyzed (Figure 4a). On the other hand, the maximum wavelength of Glb exhibited a blue shift (4-5 nm) at pH 4 and 5 with an increase of 0.5 units of absorbance values (Figure 4b), but the maximum wavelength was 278 nm at pH 2 and 9, with optical density values between 0.8-1. The absorbance values obtained at highly acidic and alkaline pH could correspond to protein denaturation (Avanza MV, Añón MC, 2007). On the contrary, the lowest optical density values may be attributed to the isoelectric point nearness (pH 4-5).

The UV-spectrum of equal protein fractions showed analogous behaviour at different ionic forces between cowpea varieties; however there were some differences between Alb and Glb behaviour. The UV spectrum of Alb and Glb fractions from CO variety (1

mg/mL dispersions) at different ionic strengths is shown in Figure 4.

The maximum wavelengths of Alb and Glb fractions were not affected significantly by changes in the ionic strength. Alb showed a slight increase in absorbance at 0.5 M NaCl and then a decrease at similar values to those shown in absence of ionic strength (Figures 4c and d). However, Glb showed a slight decrease in absorbance with increasing ionic strength. This finding would suggest that the increased ionic strength modified protein-protein interactions, resulting in an increased aggregation but a reduced denaturation of globulins.

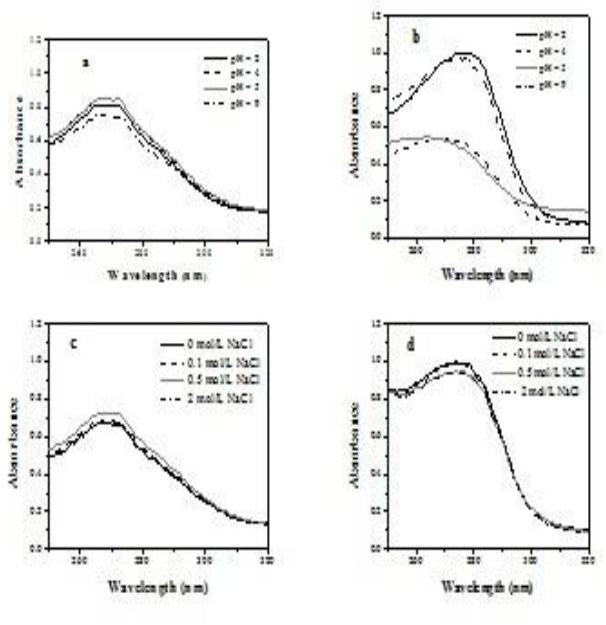

Figure 4: UV-absorption spectra of Colorado (CO) major protein fractions depending on the pH and the ionic strength. (a) and (c) albumin; (b) and (d) globulin.

PHYSICOCHEMICAL PROPERTIES

SURFACE HYDROPHOBICITY

The protein surface hydrophobicity (H_0) is an important structural parameter related to solubility, emulsifying and foaming properties. The aromatic surface hydrophobicity of Alb and Glb fractions was assessed.

Alb presented a lower surface hydrophobicity than Glb in both varieties (Table 2). Alb from CU and CO did not present significant differences in H_0 values ($p > 0.05$). In contrast, the Glb fraction of CO presented a higher H_0 value than the Glb fraction obtained from CU ($p < 0.05$).

The H_0 values were higher than those reported for *Phaseolus vulgaris*, *Vigna radiata* and *Amaranthus hypochondriacus* (Tang CH, Sun X, 2010, Quiroga A, et al, 2011). Kimura et al. have reported that the 7S globulin obtained from cowpea presents a higher surface hydrophobicity value in comparison with the same fraction of other legumes such as soybean, Faba bean, French bean, and pea.

Our results are in agreement with the fluorescence intensities obtained due to the more exposed tryptophan residues present in Glb than those of Alb (section 3.4.2 and Figure 3).

Table 2: Surface hydrophobicity parameters of cowpea major protein fractions

Protein Fraction	Variety	H_0*
Alb	CU	2302 ± 106c
	CO	2987 ± 140bc
Glb	CU	3219 ± 150b
	CO	6276 ± 310a

*Surface hydrophobicity: IF/mg mL^{-1} protein. Different letters in the same column mean significant differences ($p < 0.05$). Albumin (Alb); globulin (Glb); Cuarenton (CU); Colorado (CO)

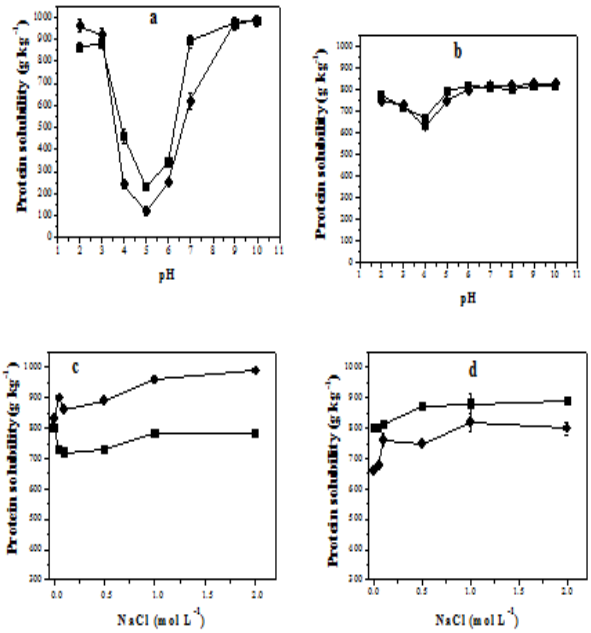

Figure 5: Solubility of albumin (Alb) and globulin (Glb) from cowpea. Depending on the pH: a) Glb and b) Alb. Depending on the ionic strength: c) Glb and d) Alb. (■) Colorado: CO; (●) Cuarentón: CU.

SOLUBILITY

Protein solubility, which can be understood as the equilibrium between the protein solvent (hydrophilic) and the protein-protein (hydrophobic) interactions, is critically important for protein functionality (Avanza MV, *et.al*, 2012).

The solubility profile of Alb and Glb obtained from both varieties at different pHs and ionic strengths, are shown in Figure 5. Glb and Alb of both varieties showed similar pH behaviour. The lowest solubility value for Alb was observed at pH 4 (65%); for Glb this value was observed at pH 5 (20%), mainly in protein fractions of CU which could be related to the nearness of isoelectric points

of legume proteins (Tang CH, Sun X, 2011). The Glb fraction exhibited a high solubility (90-100%) at pH 2-3 and 9-10. In contrast, the Alb fraction presented the highest solubility (85%) in the range of pH 6-10. Our results are consistent with those reported by Avanza et al. (Avanza MV, et al, 2012) in cowpea flour obtained from CO and CU varieties. Similar results were reported by Tang and Sun (Tang CH, Sun X, 2011) for three vicilins of *Phaseolus* and Rangel et al. (Rangel A, et al, 2003) for vicilins from cowpea and pea (Figures 5a and b).

Even though the Glb from CU was the most soluble, the solubility profile of Glb from CO at different ionic strengths showed slight variations. The low values of Glb solubility from CO at low ionic strengths is in accordance with the presence of protein aggregates (AAMM > 600 kDa) (Figure 2a).

In contrast, Alb improved its solubility in the presence of NaCl (Figures 5c and d). The Alb fraction obtained from the CO variety presented the highest values of solubility; however Alb from CU showed the highest solubility increase depending on the ionic strength. This could be ascribed to a higher density molecular charge on this protein fraction, facilitating intermolecular protein-water interactions.

CONCLUSION

Physicochemical and conformational properties of Glb and Alb fractions from cowpea seeds were characterized. In both varieties, globulins are the major seed protein fractions. CU globulins showed an increased in vitro protein digestibility than Alb fraction, whereas CO globulins and albumins showed similar values.

Physicochemical properties varied considerably between globulin and albumin fractions, where the globulins showed a higher content of aromatic amino acids, polypeptides bound by disulfide bonds, high molecular aggregates (only on CO globulins) and a major thermal stability. The presence of aggregates showed on the latter fraction could be attributed to the minor in vitro protein digestibility.

Conformational studies showed that the globulin fraction was more hydrophobic than albumins despite the fact that no significant differences were found between solubilities at pH 7.0.

These results suggested the existence of close relationships among the physicochemical properties and conformational features of cowpea proteins. This type of preliminary study is useful to understand the properties of NEA-cowpea storage proteins, thus facilitating their utilization in nutrition and health food formulations. Although these features, further analysis should be done on this fractions basically related to biological activities done *in vitro* and *in vivo*.

ACKNOWLEDGEMENTS

The authors would like to thanks the financial support from the Agencia Nacional de Promoción Científica y Tecnica (PICT-2341/10) and the Universidad Nacional del Nordeste (PI-003/11). M V. Avanza and M. C. Añón are members of the Research Career of the ConsejoNacional de Investigaciones Científicas y Técnicas (CONICET). paula Aphalo is member of Comisión de Investigaciones Científicas (CIC-PBA).

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