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Physicochemical and structural characterization of Lima Bean (*Phaseolus lunatus*) globulins

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Abstract

A globulin fraction from *Phaseolus lunatus* have been isolated and characterized. The fractionation scheme used allowed to obtain a Globulin partially purificated. Globulin purification by ultracentrifugation allowed to isolate three peaks with different sedimentation coefficient, while gel filtration chromatography yielded two species, I and II, which were later identified as 11S and 7S globulins, respectively. Species II, 7S globulin from *P. lunatus*, seems to have a molecular mass of 72 kDa and to be constituted by polypeptides of 34–36, 25–27 and 16–18 kDa without intermolecular disulfide bonds. These polypeptides would present certain heterogeneity, as suggested by the results of isoelectric focusing and electrophoresis analyses. This protein exhibited some particular features, including a lower molecular mass and higher thermal stability than typical vicilins. Species I, with a molecular mass of 336 kDa, would be constituted by subunits of 53–55 and 40–41 kDa formed by smaller polypeptides (around 30 and 20 kDa) linked by disulfide bonds. These features, together with the existence of basic and acid polypeptides in the molecule, would confirm that species I is a globulin of the legumins, 11S globulins or α-conglutin family.

Keywords: *Phaseolus lunatus*; Globulins; Protein characterization

1. Introduction

Legume seeds are important from a nutritional point of view because of their high protein content ($200-400\,g\,kg^{-1}$), higher than that of other plants, such as cereals ($70-140\,g\,kg^{-1}$). They are also a source of proteins for farm birds and other monogastric animals, which supply food for human consumption. Although there are thousands of legume species, some are infrequently consumed, due mainly to their content of antinutritional components, leaving about 10 species for massive consumption (Sotelo, 1998).

Globulins are the main storage proteins in legume seeds, accounting for 35–72% of the total protein content, and even 90% in some cases, such as soy (Utsumi, 1992). The remaining protein fraction is constituted by albumins (Machuca, 2000), usually from the 2S fraction, which frequently have a physiological role. Albumins have been classified as storage proteins in some cases (Segura-Nieto & Jiménez-Flores, 1999).

According to their sedimentation coefficient, globulins from legume seeds are classified into 7S (7.1S–8.75S) or vicilins, and 11S (10.1S–14S) or legumins, which are proteins with common characteristics. The 7S globulins are usually glycosilated, their molecular mass is lower than that of 11S globulins, and they can form trimers arranged in a flat disk stabilized by ionic bonds, as in soy (Fukushima, 1991; Petruccelli & Añón, 1995). In many

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vegetables, including soy, barley, sesame, bean, pea and colza (Fukushima, 1991; Guéguen & Cerletti, 1994), the 11S protein has been described as an hexamer. In contrast, it forms a dimer: 2(An-S-S-Bn) in rice and pumpkin (Fukushima, 1991; Utsumi, 1992). This globulin has a highly packed structure whose rigidity stems not only from the disulfide bonds that link the two polypeptide chains that form their subunits, but also from the hydrophobic interactions between the basic chains (Utsumi, 1992). The molecular mass of 11S globulins is usually higher than 300 kDa, although masses as low as 208 and 112 kDa have been informed for the cocosin from *Cocos nucifera* and the globulin from *Cucurbita* sp., respectively (Dieckert & Dieckert, 1985).

The seeds from *Phaseolus lunatus* have a high protein content ($210-260\,g\,kg^{-1}$) and a high carbohydrate content ($550-640\,g\,kg^{-1}$), but low levels of fat ($10-23\,g\,kg^{-1}$) and fiber ($32-68\,g\,kg^{-1}$). They also have a high content of minerals such as K, Zn, Ca and Fe, and low levels of Na and P. Methionine has been regarded as the limiting amino acid in their proteins (Oshodi & Adeladun, 1993).

Since the current tendency is to increase the use of plant products as functional ingredients for foods, both because of economical reasons and to improve the nutritional quality of products (Sanchez-Vioque, Clemente, Vioque, Bautista, & Millan, 1999), *P. lunatus* appears as a promising source of nutrients. For this reason, we have started to analyze the structure and properties of its seed proteins, to determine their best use from a nutritional and functional point of view. The main goal of this work was to characterize the globulins from *P. lunatus* and to test the physicochemical properties of globulins.

2. Materials and methods

2.1. Flour preparation

P. lunatus seeds were harvested in the state of Campeche in Mexico, from February 1998–2000. The best seeds were selected and manually cleaned to eliminate impurities. Seeds were initially ground in a Mikrós impact device to obtain a flour that could traverse a 20 mesh, and

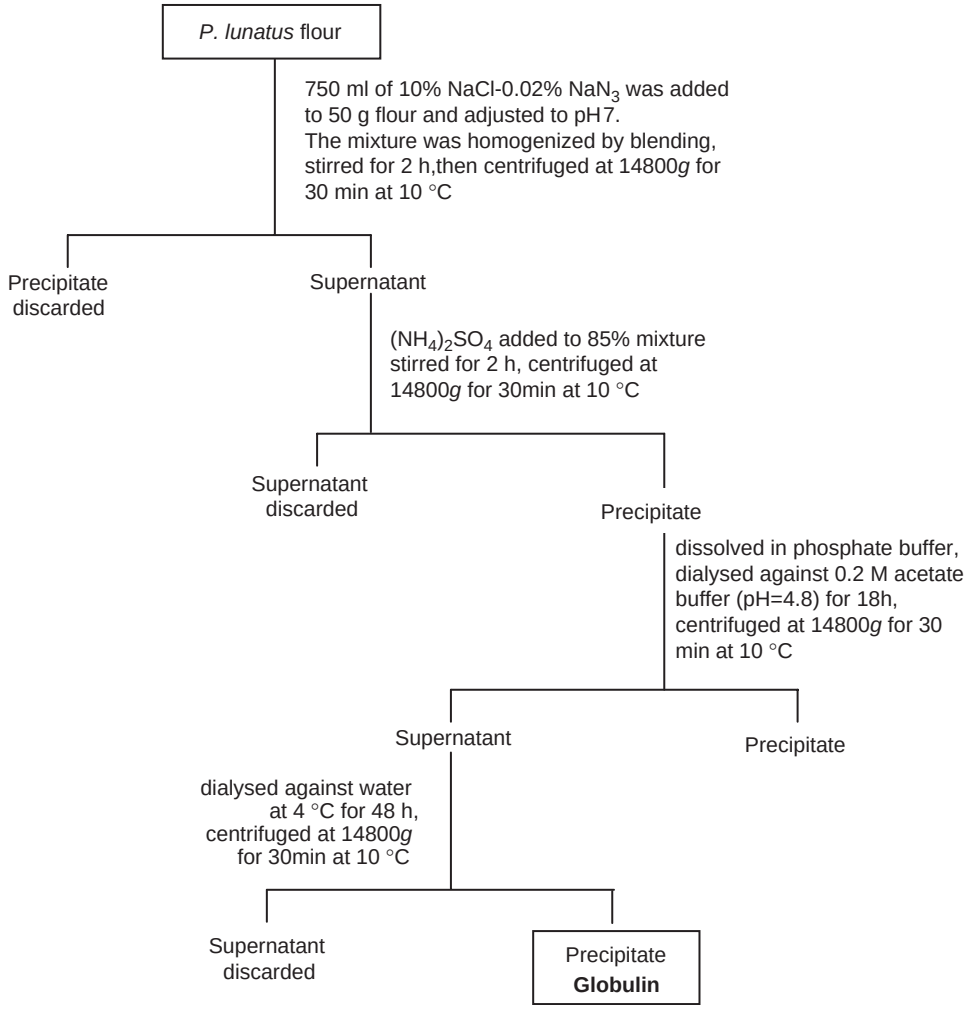

Fig. 1. Scheme for extraction of globulins from *P. lunatus*.

subsequently in a Cyclotec 1093 mill (Tecator, Sweden) to obtain a flour of smaller particle size (60 mesh).

2.2. Globulin extraction

The method used for extraction was adapted from Blagrove and Gillespie (1975). Technical details of the extraction procedure are described in Fig. 1.

2.3. Characterization of P. lunatus globulins

The proteins obtained were subjected to the following tests:

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). This was performed using the procedure described by Martínez, Castellani, and Añón (1997). Runs were carried out with 60−120 or 120 $g\,l^{-1}$ acrylamide gels. The following continuous buffer system was used: 0.375 M Tris-HCl, pH 8.8, 1 $g\,l^{-1}$ SDS for the separating gel; 0.025 M Tris-HCl, 0.192 M glycine and 1 $g\,l^{-1}$ SDS, pH 8.3 for the running buffer and 0.125 M Tris-HCl, pH 6.8, 200 $ml\,l^{-1}$ glycerol, 10 $g\,l^{-1}$ SDS, and 0.5 $g\,l^{-1}$ bromophenol blue as sample buffer. For runs in reducing conditions the sample buffer contained 50 $ml\,l^{-1}$ 2-mercaptoethanol (2-ME) and samples were heated for 60 s in a boiling-water bath. The following protein molecular mass standards were used: phosphorylase b (94 kDa); bovine serum albumin (67 kDa); ovalbumin (45 kDa); carbonic anhydrase (30 kDa); trypsin inhibitor (20.1 kDa); α-lactalbumin (14.4 kDa). Gels were fixed and stained with Coomassie Brilliant Blue Stain. Gels images were acquired with the Gel Doc 1000 Image Analysis System (Bio-Rad, Richmond, California, USA) and analyzed with the Molecular Analyst Software (Bio-Rad).

Native gel electrophoresis (PAGE). This was performed using an adaptation of the method of Laemmli (1970), in which SDS was replaced with distilled water. Polyacrylamide gels were prepared at 80 $g\,l^{-1}$ concentration. Molecular mass standards included bovine albumin (66 kDa), fructose 6-P kinase (84 kDa), phosphorilase B (94 kDa), ß-galactosidase (116 kDa) and myosin (205 kDa). Gels were fixed and stained with Coomassie Brilliant Blue Stain. Gels images were acquired with the Gel Doc 1000 Image Analysis System (Bio-Rad).

Two-dimensional gel electrophoresis: Two kinds of bidimensional electrophoresis were carried out. In one case PAGE in the first dimension and SDS-PAGE with 2-ME in the second was carried out (Fig. 2). In the other SDS-PAGE in the first dimension and SDS-PAGE with 2-ME in the second (Fig. 6) were performed. Each lane of the first dimension slab gel was treated with 10 volumes of treatment buffer (62.5 mM Tris-HCl pH 6.8, 10 $g\,l^{-1}$ SDS and 200 $g\,l^{-1}$ sucrose, 0.2 M 2-ME) at 55 °C for 30 min. The procedure was repeated twice, changing the treatment buffer. Immediately, this portion of the gel was placed on top of the gel used for the second dimension. Runs were performed under the same conditions mentioned for the one-dimensional gel. Gels were fixed and stained with Coomassie Brilliant Blue Stain. Gels images were acquired with the Gel Doc 1000 Image Analysis System (Bio-Rad).

Isoelectric focusing: Protein fractions (1 mg) were dissolved in 50 μl of sample buffer (10 μl of anpholine Sigma A-4549, pH 3-10, and 90 μl of glycerol 250 $ml\,l^{-1}$) and were heated at 95 °C for 120 s with shaking. After centrifuging (3900 × *g*, 10 °C, 15 min) samples were loaded on top of a 50 $g\,l^{-1}$ polyacrylamide gel. pI standards (BioRad 161-0310, pH = 4.46–9.6) were used. Gels were run for 3 h at

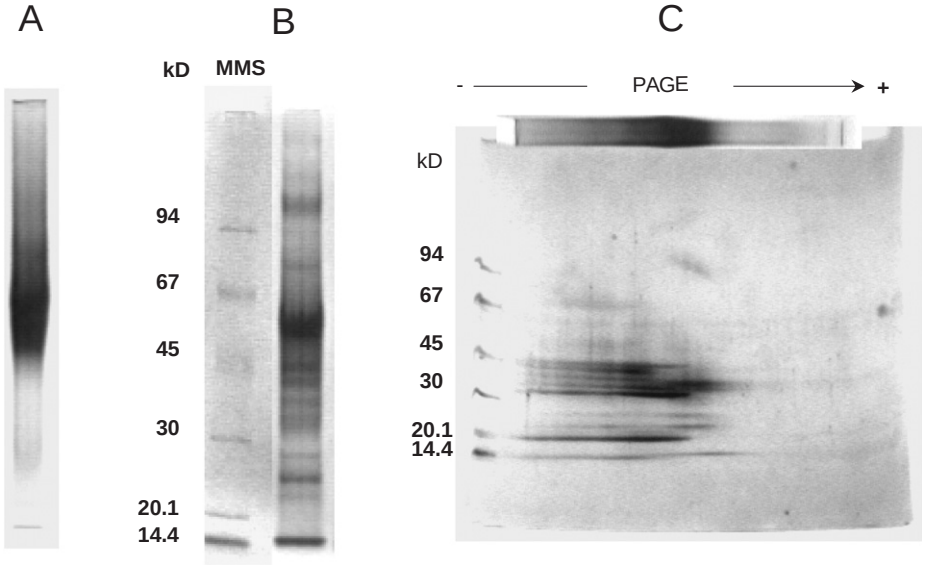

Fig. 2. A: PAGE, B: SDS-PAGE, and C two-dimensional electrophoresis (PAGE and SDS-PAGE + 2-ME) of globulin from *P. lunatus*. Standard molecular mass proteins *MMS*. Gels were stained with Coomasie Blue.

200–400 V (O'Farell, 1975). Gels were fixed and stained with Coomasie Brilliant Blue Stain. Gels images were acquired with the Gel Doc 1000 Image Analysis System (Bio-Rad).

Differential scanning calorimetry (DSC): Protein fractions were analyzed by DSC according to the method of Martínez and Añón (1996). Samples were prepared in distilled water (final concentration 25% w/w). Aluminum hermetic capsules (Perkin-Elmer No. 0219-0062) were loaded with 8 mg of the 250 g l^{-1} dispersions and allowed to stabilize for at least 30 min at room temperature (25 °C) before testing. A Perkin Elmer DSC-6 Pyris device was employed, using a heating rate of 10 °C min^{-1} between 35 and 130 °C.

Surface hydrophobicity (So): Values of So were determined using the hydrophobic fluorescence probe 1-anilino-8-naphthalene-sulphonate (ANS) according to the method described by Hayakawa and Nakai (1985). Fluorescence intensity (FI) was measured at 365 nm (excitation) and 484 nm (emission) using a Perkin-Elmer 2000 fluorometer (Perkin-Elmer Corp. Norwalk, CT, USA). The initial slope of the FI versus protein concentration plot (calculated by linear regression analysis) was used as an index of protein hydrophobicity (So). Protein concentration was determined by the method of Lowry, Rosenbrough, Farr, and Randall (1951). At least two determinations were performed for each sample.

2.4. Globulin purification

Ultracentrifugation (UC): The method described by Guéguen and Barbot, as cited by Barba De La Rosa, Guéguen, Paredes-López, and Viroben (1992), was used. A Beckman 15-65 B device with a Ti SW 40 rotor was employed. The sample (0.6 ml) was layered on top of a sucrose gradient (50−200 g l^{-1}) in 50 mM Tris-HCl buffer containing 0.3 M NaCl, and was centrifuged at 219 000 × *g* for 20 h at 4 °C. When run was complete, 0.5 ml aliquots were read at 280 nm in a Beckman UV-Vis spectrophotometer. The following proteins were used for gradient calibration: bovine serum albumin (4.4S), γ-globulin (7.9S) and catalase (11S).

Globulin isolation by UC: Ultracentrifugation was performed on a sucrose gradient (100−300 g l^{-1}, 1.2 ml)

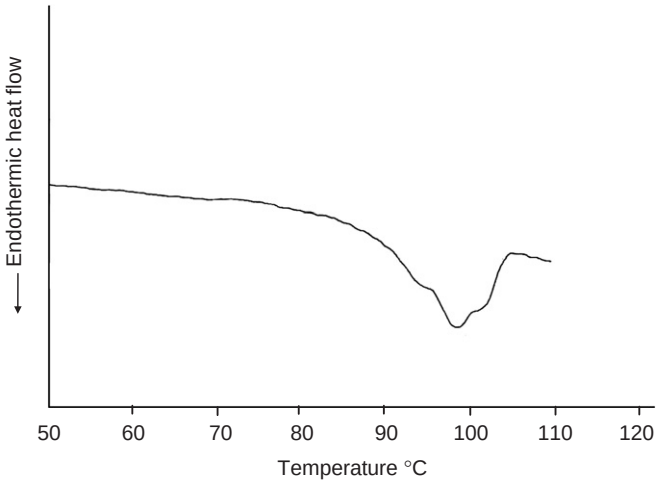

Fig. 3. Thermograms of globulin isolated from *P. lunatus*.

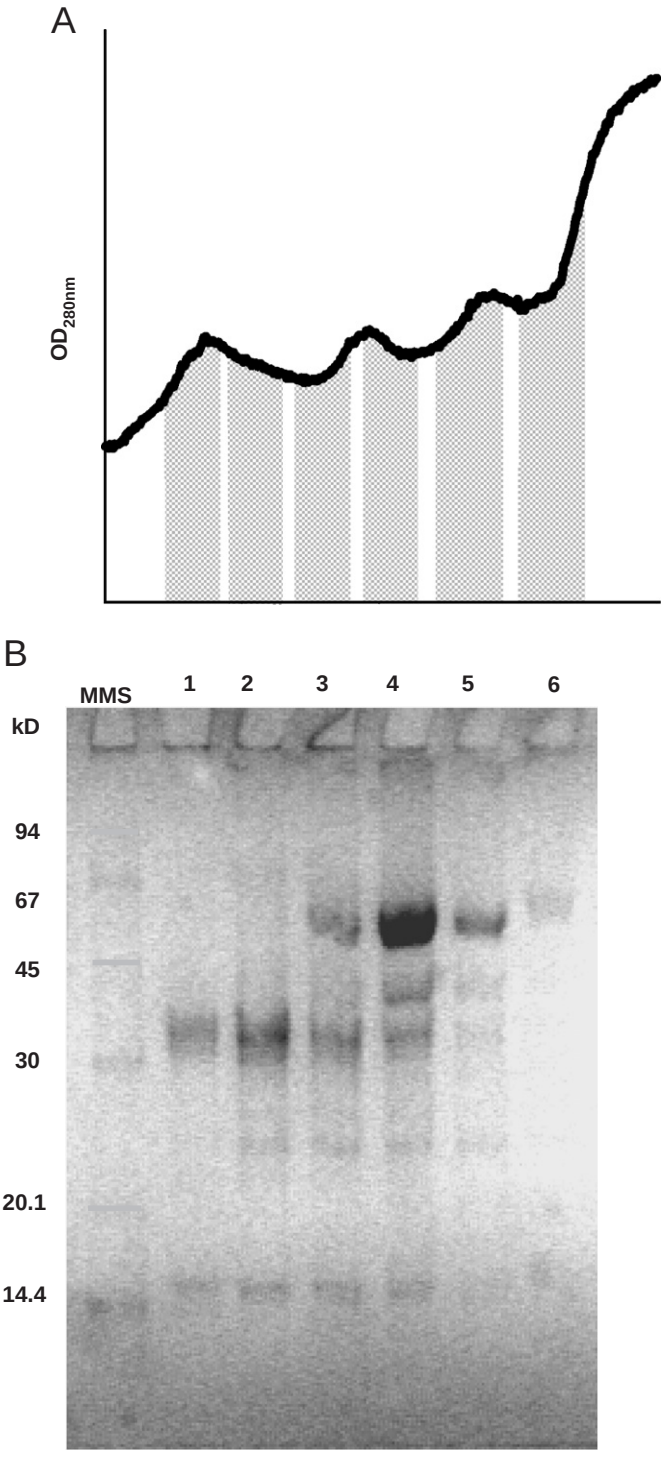

Fig. 4. (A) Ultracentrifugation profile of globulin. (B) SDS-PAGE of ultracentrifugation fractions. MMS: Standard molecular mass proteins. Gels were stained with Coomasie Blue.

in a Beckman Optima TL device with a TLS-55 rotor. Samples (0.1 ml, containing approximately 0.3 mg protein) were layered on top of the gradient and runs were performed at 55000 × *g* for 5 h at 10 °C.

Molecular exclusion chromatography: A Superose 6B HR 10/30 column was employed using a Pharmacia LKB, FPLC System (Uppsala, Sweden). Samples (approx. 2 mg protein) dissolved in buffer NaH_2PO_4 0.15 M, pH 7 were injected and eluted with the same buffer at 0.2 ml min^{-1}. Absorbance at 280 nm was monitored and 0.3 ml fractions were collected. The column was calibrated with blue dextran (for void volume, $V_0 = 7.64$ ml), tyroglobulin (669 kDa), apoferritin (443 kDa) α-amylase (200 kDa), alcohol dehydrogenase (150 kDa), and carbonic anhydrase (29 kDa). The molecular masses of the fractions were calculated using the equation

$$\log \mathrm{MM} = 4.5 - 5.1\ K_{\mathrm{AV}},$$

where $K_{\mathrm{AV}} = (V_e - V_0)/(V_T - V_0)$, V_e is the elution volume of the resolved species, and V_T is the total volume of the column ($V_T = 25.09$ ml). Data from three different analyses were used.

The fractions from each of the two peaks in exclusion chromatography were analyzed by non-denaturing electrophoresis, denaturing electrophoresis (SDS-PAGE), denaturing-reducing electrophoresis (SDS-PAGE + 2-ME), two-dimensional electrophoresis, DSC, ultracentrifugation and isoelectric focusing, according methods cited previously.

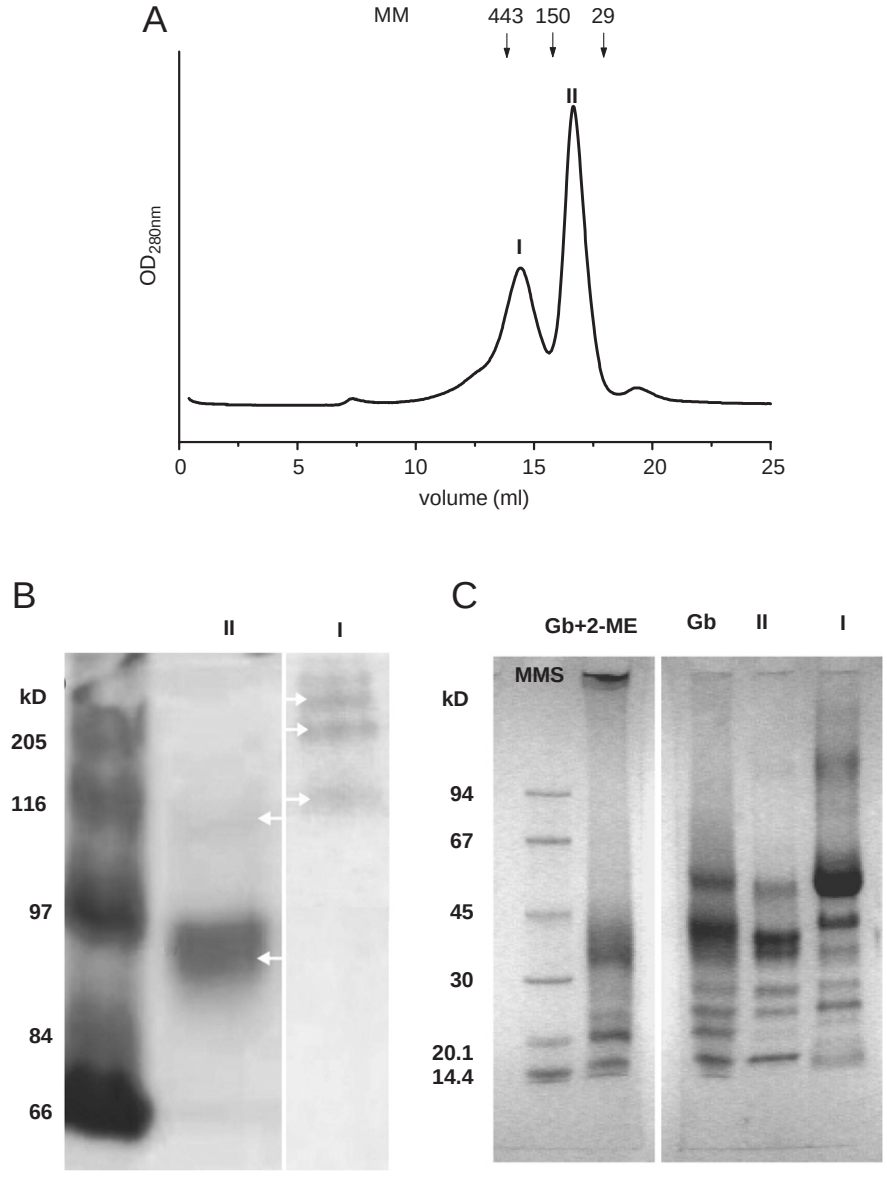

Fig. 5. (A) Exclusion chromatography (FPLC) of globulin. (B) PAGE of FPLC fractions. (C) SDS-PAGE of FPLC fractions. MMS: Standard molecular mass proteins. Gels were stained with Coomasie Blue.

Every determination was performed at least twice. Data correspond to mean ± standard deviation.

3. Results and discussion

3.1. P. lunatus globulin extraction

A globulin fraction was obtained following the protocol described for *Lupinus angustifolius* (Blagrove & Gillespie, 1975) (Fig. 1).

3.2. Globulin characterization

Globulin was analyzed by non-denaturing and denaturing electrophoresis (Fig. 2). Under non-denaturing conditions, Globulin presented two bands (the one of higher mobility being more wide and intense than the one of lower mobility).

When globulin was analyzed under denaturing conditions (Fig. 2B) the bands of higher intensity corresponded to polypeptides of 114–110 kDa, 54 ± 1 kDa, and 16 ± 1 kDa while less intense bands corresponded to polypeptides of 43.8 ± 0.6 kDa, 40 ± 1 kDa, 34 ± 1 kDa, 31.2 ± 0.8 kDa, 26.0 ± 0.8 kDa, and 23.0 ± 0.5 kDa.

The patterns of two-dimensional electrophoresis PAGE→SDS-PAGE + 2-ME of globulin are shown in Fig. 2C. The globulin split into three bands (two of them with very similar mobility) in the first dimension, since three zones of different polypeptide composition were observed in the two-dimensional electrophoresis. The one with the highest mobility would be formed by polypeptides of 33–35, 21–25 and 16–14 kDa, the intermediate by 35 and 20 kDa polypeptides, and the one with the lowest mobility by polypeptides of 38–40, 33 and 20 kDa.

The polypeptide of molecular mass higher than 94 kDa would correspond to aggregates (Fig. 2B, molecular mass 114 ± 2 kDa). The absence of this band in the second dimension of Globulin (Fig. 2C) together with its presence under non-reducing conditions (Fig. 2B) would indicate that these aggregates are stabilized by disulfide bonds.

This globulin showed high surface hydrophobicity. This value, 212, was similar to amaranth globulins 190 ± 15 and higher than soybean glycinin, 95 ± 10, respectively (Castellani, 2000).

The thermogram of the globulin fraction showed three endotherms (Fig. 3), which could correspond to species of different thermal stability and/or to different domains of a single protein, as suggested by Myers (1990) and by Petruccelli and Añón (1996). The denaturing temperatures of endotherms were 94.1 ± 0.2, 98.8 ± 0.1 and 101.2 ± 0.2 °C, and the ΔH of the process was 15.7 ± 0.4 J g^{-1}.

3.3. Globulin purification

Since globulin is an heterogeneous fraction, was the starting material for the purification of globulins.

3.3.1. Globulin purification by ultracentrifugation

Globulin ultracentrifugation in sucrose gradient (100−300 g l^{-1}) yielded three peaks with different sedimentation coefficient (Fig. 4A). Fractions 1 and 2, included in the peak with the lowest sedimentation coefficient, contain polypeptides of 35 ± 1 and 18 kDa, while fractions 3 and 4

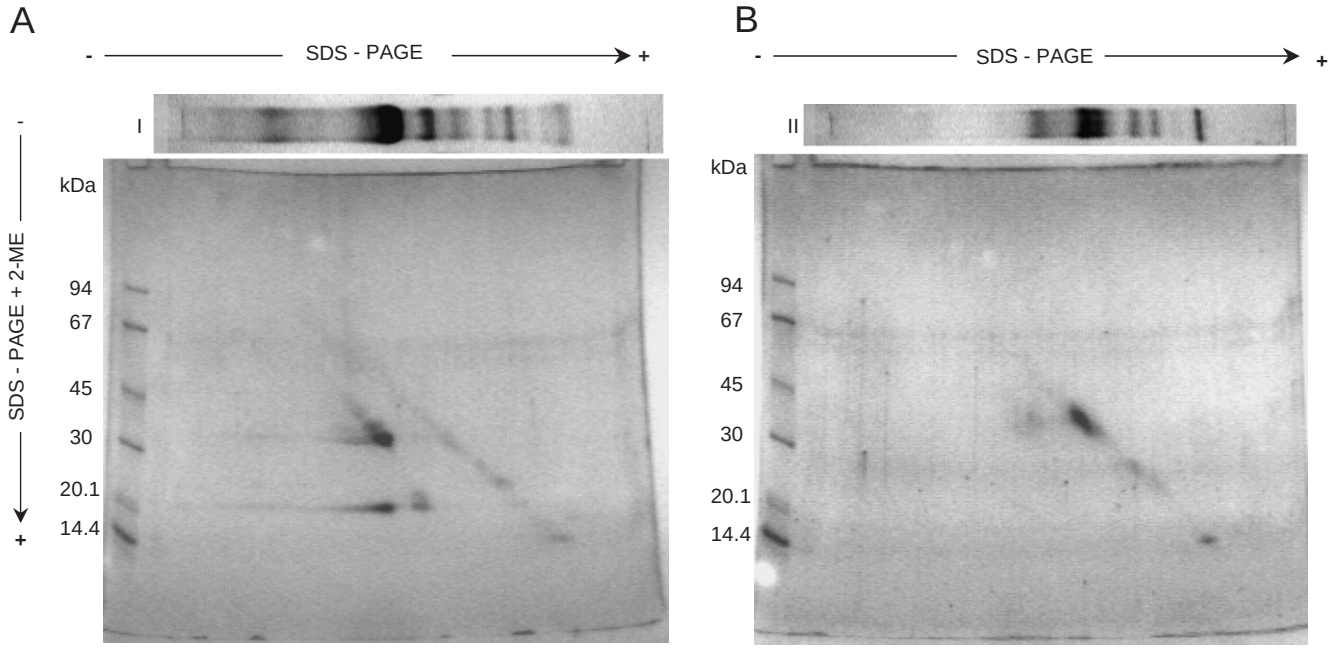

Fig. 6. Two-dimensional electrophoresis (SDS-PAGE and SDS-PAGE + 2-ME) of protein species I (A) and II (B). Gels were stained with Coomasie Blue.

contain polypeptides of higher molecular weight (54 ± 1 kDa and 40 ± 1 kDa), more intensely defined in fraction 4. The 54 kDa band predominates, although weakly, in the peak with the highest sedimentation coefficient. These results confirm the heterogeneity of globulin fraction. While a complete separation was not obtained, the first peak would correspond to dissociated subunits, the central one to the 7S globulin, and the third to 11S globulin.

3.3.2. Globulin purification by molecular exclusion chromatography

To separate the fractions included in globulin, exclusion chromatography was performed (Fig. 5A). Two peaks were obtained, the first (I) corresponding to proteins of 336 kDa and the second (II) corresponding to proteins of 72 kDa.

The electrophoretic patterns of species I and II are shown in Figs. 5B and C. Under non-reducing conditions (Fig. 5B), species I showed several bands of low mobility, while species II exhibited only one band of higher mobility than the former. Species I (Fig. 5C) was constituted mainly by two polypeptides of 54 ± 1 kDa and 40 ± 1 kDa, and in a lower proportion by a polypeptide of 114 ± 2 kDa. Other bands, with molecular masses lower than 40 kDa and coincident with those found in species II, were also observed. The electrophoretic pattern of species II revealed two main bands of 40 ± 1 and 35 ± 1 kDa, and minor bands of 26.0 ± 0.8, 23.0 ± 0.5 and 16 ± 1 kDa. A 54 kDa polypeptide, coincident with the one observed in species I, was also observed in species II, although with low intensity.

Two-dimensional electrophoresis of species I and II were carried out (Fig. 6). These results revealed that the polypeptides with molecular masses 54 ± 1 and 40 ± 1 kDa found in species I (Fig. 6A) are constituted by polypeptides linked by disulfide bonds. Results suggested the existence of more than one 54 kDa polypeptide, which would be constituted by polypeptides of 30 and 20 kDa. The 40 kDa polypeptide would be constituted by 20 kDa polypeptides. The 114 kDa polypeptide, formed by polypeptides of 30 and 20 kDa, would correspond to aggregates of the 54 kDa subunit. This polypeptide composition is typical of 11S globulins.

Polypeptides included in species II (Fig. 6B) are monomers, since they appear in the diagonal of the two-dimensional gel.

These results suggest the presence of two globulins: species I, which according to its size and polypeptide composition would correspond to a legumin, and species II, which is constituted by monomeric polypeptides and could correspond to a 7S globulin or a vicilin with a molecular size somewhat lower than those usually described.

To complete this purification, species I and II were subjected to sucrose gradient sedimentation ($100-300\ g\ l^{-1}$, Fig. 7A). The sedimentation coefficient of species I was higher than that of species II. This result correlates with that found by exclusion chromatography, in which the molecular size of species I was higher than that of species II (336 and 72 kDa, respectively). Although ultracentrifugation peaks were wide, the polypeptide composition of the species was the same in all the fractions collected (species II: major polypeptides of 34–36 kDa and weaker polypeptides of 26 and 23 kDa; species I: 54 and 40 kDa), which shows that each species was pure (Fig. 7A, inset).

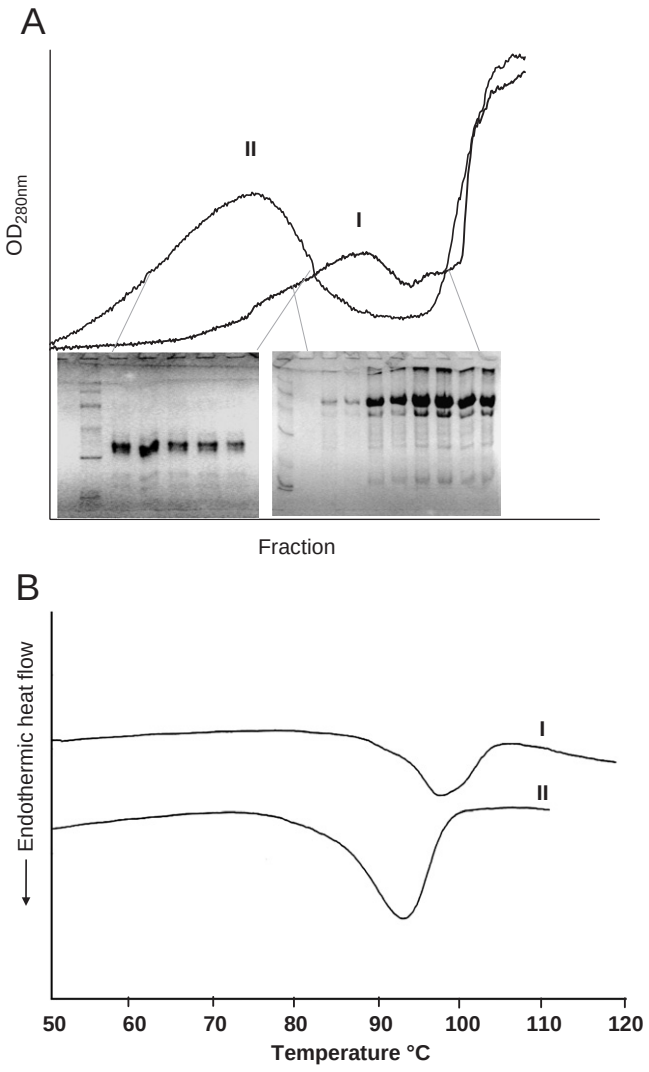

Fig. 7. A: Ultracentrifugation profile of protein species I and II. Inset (A) SDS-PAGE of ultracentrifugation fractions. (B) Thermograms of protein species I and II.

The pI of the polypeptides present in species I and II was determined. Species I contained several acid polypeptides and two basic polypeptides, while species II seemed to be formed by a lower number of polypeptides, all of them acidic, with pI values different from those of species I (Table 1).

The calorimetric analysis revealed the existence of a single endotherm for each species (Fig. 7B), which differs from the results obtained with crude fractions (Fig. 3).

Table 1
pI of polypeptides of protein species I and II

		Species I	Species II
pI	Basic	7.2 7.1	–
	Acid	5.9 5.5 5.2–4.9 4.3 4.2	5.2 5.1 5.0 4.9–4.6

The denaturation temperature of species II was $93 \pm 1\,^{\circ}C$, while that of species I was $97.6 \pm 0.9\,^{\circ}C$. If one considers that species II seems to belong to the family of vicilins, ß-conglutin or 7S proteins, the thermal stability of this species was high, specially when compared with the ß-conglycinin from soy (74−78 °C) (Myers, 1990) or the vicilin from bean (83 °C) (Duranti, Sessa, Scarafoni, Bellini, & Dallocchio, 2000). However, this value is similar to that reported by Myers (1990) for vicilins from bean (92 °C), and that informed by Meng and Ma (2001) for vicilins from *P. angularis* (87.7−94.1 °C) and *V. unguiculata* (94 °C). The Td of species I was higher than that of species II, in agreement with the higher thermal stability of legumins relative to vicilins (Meng & Ma, 2001).

Denaturation enthalpies of species I and II (3 ± 1 and $4.7 \pm 0.4\,J/g$, respectively) were low relative to that of globulin ($15.7 \pm 0.4\,J/g$), indicating that a fraction of proteins is denatured during the purification process.

In summary, these results indicate that the protocol used to isolate the globulin fractions of *P. lunatus* was adequate. The purification of globulin by exclusion chromatography allowed the isolation of two species, I and II, which were composed of 11S and 7S globulins, respectively. Our results show that species II, or 7S globulin of *P. lunatus*, has a molecular mass of 72 kDa and is constituted by polypeptides of 36–34, 26–23 and 16 kDa, without intermolecular disulfide bonds. These polypeptides would have certain heterogeneity, as suggested by the results of isoelectric focusing. This protein exhibited some particular features, such as a molecular mass lower than that of typical vicilins and a high thermal stability.

Species I, with a molecular mass of 336 kDa, would be constituted by subunits of 53–55 kDa and 39–41 k Da formed by smaller polypeptides (of about 30 and 20 kDa) linked by the disulfide bonds. These characteristics, together with the existence of acid and basic polypeptides as revealed by isoelectric focusing, would confirm that species I is a globulin of the legumin or 11S globulin family.

Acknowledgments

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References

Barba De La Rosa, A., Guéguen, J., Paredes-López, O., & Viroben, G. (1992). Fractionation procedures, electrophoretic characterization, and amino acid composition of amaranth seeds proteins. *Journal of Agricultural and Food Chemistry*, *40*, 931–936.

Blagrove, R. J., & Gillespie, J. M. (1975). Isolation, purification and characterization of the seed globulins of *Lupinus angustifolius*. *Australian Journal Plant Physiology*, *2*, 13–27.

Castellani, O. (2000). *Caracterización estructural y fisicoquímica de la globulina-P de amaranto*. Tesis, Universidad Nacional de La Plata, Argentina.

Dieckert, J. W., & Dieckert, M. C. (1985). The chemistry and biology of seed storage proteins. In *Food science and technology. New protein foods* (pp. 1–180). New York: Academic Press.

Duranti, M., Sessa, F., Scarafoni, A., Bellini, T., & Dallocchio, F. (2000). Thermal stabilities of lupin seed conglutin g protomers and tetramers. *Journal of Agricultural and Food Chemistry*, *48*, 1118–1123.

Fukushima, D. (1991). Structures of plant storage proteins and their functions. *Food Reviews International*, *7*, 356–381.

Guéguen, J., & Cerletti, P. (1994). Proteins of some legume seeds: Soybean, pea, fababean and lupin. In B. Hudson (Eds.), *New and developing sources of food proteins* (pp. 145–197). London, UK: Chapman & Hall.

Hayakawa, S., & Nakai, S. (1985). Relationships of hydrophobicity and net charge to the solubility of milk and soy proteins. *Journal of Food Science*, *50*, 486–491.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature*, *217*, 680–685.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin-fhenol reagent. *Journal of Biological Chemistry*, *193*, 265–275.

Machuca, J. (2000). Characterization of the seed proteins of Velvet Bean (*Mucuna pruriens*) from Nigeria. *Food Chemistry*, *68*, 421–427.

Martínez, E. N., & Añón, M. C. (1996). Composition and structural calorimetric study. *Journal of Agricultural and Food Chemistry*, *44*, 2523–2530.

Martínez, E. N., Castellani, O. F., & Añón, M. C. (1997). Common molecular features among amaranth storage proteins. *Journal of Agricultural Food and Chemistry*, *45*, 3832–3839.

Meng, G., & Ma, C. Y. (2001). Thermal properties of *Phaseolus angularis* (Red Bean) globulin. *Food Chemistry*, *73*, 453–460.

Myers, C. D. (1990). Study of thermodynamics and kinetics of protein stability by thermal analysis. In *Thermal analysis of foods* (pp. 16–50). New York: Elsevier Science Publishing Co.

O'Farell, P. H. (1975). High resolution two-dimensional electrophoresis of proteins. *Journal of Biological Chemistry*, *250*, 4007–4012.

Oshodi, A. A., & Adeladun, M. O. A. (1993). Proximate composition, some nutritionally valuable minerals and functional properties of three varieties of lima bean (*Phaseolus lunatus linn*) flour. *International Journal of Food Science and Nutrition*, *43*, 181–185.

Petruccelli, S., & Añón, M. C. (1996). pH-induced modifications in the thermal stability of soybean proteins isolates. *Journal of Agricultural and Food Chemistry*, *44*, 3005–3009.

Petruccelli, S., & Añón, M. C. (1995). Soy protein isolate components and their interactions. *Journal of Agricultural and Food Chemistry*, *43*, 1762–1767.

Sanchez-Vioque, R., Clemente, A., Vioque, J., Bautista, J., & Millan, F. (1999). Protein isolates from chickpea (*Cicer arietinum L.*): Chemical composition, functional properties and protein characterization. *Food Chemistry*, *64*, 237–243.

Segura-Nieto, M., & Jiménez-Flores, R. (1999). Genetic modifications of plant seed storage proteins for food production. In O. Paredes López (Ed.), *Molecular biotechnology for plant food production* (pp. 411–474). Lancaster, PA, USA: Technomic Publishing Co. Inc.

Sotelo, L. (1998). Leguminosas, fuente natural de proteínas. *Tecnología de Alimentos*, *33*, 22–23.

Utsumi, S. (1992). Plant food protein engineering. In E. Kinsella (Ed.), *Advances in food and nutrition research* (pp. 89–208). San Diego, CA, USA: Academic Press.