Hydrodynamic Characterization of Lupin Proteins: Solubility, Intrinsic Viscosity, and Molar Mass

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The solubility, intrinsic viscosity ([η]), and sedimentation behavior of a lupin protein system extracted from *Lupinus luteus* seeds have been determined. The results were compared with those published in the literature for soy proteins. The relationship between solubility and pH for the lupin protein isolate was similar to that reported in literature for soy isolates. The [η] of the lupin protein isolate was ~7 mL/g. These values are comparable to those obtained by other authors for soy proteins. Sedimentation velocity studies suggested that there are three main globulins in lupin protein with sedimentation coefficients of 13, 7, and 2 S. The molar mass of the major lupin globulin (390 000 g/mol) was slightly higher than reported for the soy 11S globulin (340 000 g/mol), but the lupin 7S component gave a much smaller value (105 000 g/mol) than the soy 7S (180 000 g/mol). The consequences in thickening and thermal gelation properties of the lupin protein system are discussed.

Keywords: *Lupin proteins; solubility; intrinsic viscosity; molar mass*

INTRODUCTION

Lupin belongs to the legume group of plants. The protein content of the lupin seeds is high (±40%), but the presence of quinolizidinic alkaloids prevents the direct consumption of these as food. When the protein is extracted, the resulting protein isolate is free of alkaloids and can be used as a functional ingredient in human food.

There is substantial evidence that the gelation and thickening properties of lupin proteins are inferior to soy proteins (Cerletti, 1983; Riccardi *et al.*, 1983; King *et al.*, 1985; Larsen *et al.*, 1994). Soy protein is used extensively as a food ingredient (Morr, 1990). The objective of our work is therefore to contribute to an understanding of the differences in functional behavior between these two protein systems. To approach this objective, lupin proteins have been characterized in terms of their solubility, intrinsic viscosity, sedimentation behavior, and absolute molar mass. The results obtained for protein extracted from *Lupinus luteus* are compared with reported values for soy protein.

The solubility used to be associated with the native state of the protein, *i.e.*, the more denatured, the less soluble and the poorer the functional properties (Hermansson, 1979), although it was later shown that denaturation and solubility do not always correlate and it has been claimed that sometimes high-solubility data can be obtained from completely denatured proteins (Arrese *et al.*, 1991).

Solubility gives no information as to whether a protein will bind water, but the intrinsic viscosity ([η]), as a measure of the hydrodynamic volume in a given solvent, can provide certain assumptions concerning the protein conformation and an indication of the ability of the protein to swell and take up water. Finally, the sedimentation coefficients (widely used in the nomenclature for seed globulins—see Derbyshire *et al.* (1976)) and absolute molar mass will contribute to the characterization of the lupin proteins and allow better comparison with other related proteins such as soy proteins (Prakash, 1992).

MATERIALS AND METHODS

Materials. Commercial *Lupinus luteus* seeds were obtained from Gonçalves Fonseca C[a] Lda, a Portuguese supplier. The water used was redistilled and deionized. The other chemicals used were reagent grade.

Methods. *Isolation of the Proteins.* A hammer mill with a sieve of 1.5 mm aperture diameter was used to reduce the particle size of whole lupin seeds. The protein isolates were produced by solubilization of the protein in distilled water (1:10) at pH 9.0 with NaOH (1 N), stirring for 2 h at room temperature, and then centrifugation at 5000*g* for 15 min, with the residue discarded. This was followed by isoelectric precipitation of the protein at pH 4.5 with HCl (0.1 N), centrifugation at 5000*g* for 30 min, washing of the precipitate twice with warm distilled water, neutralization with NaOH (0.1 N), and freeze-drying. The dimensions of the freeze-dried isolates were reduced by using pestle and mortar, and the powders obtained were kept at −12 °C.

The protein content of the materials was determined by the Kjeldhal method (the protein content of the lupin isolates was 85.5 ± 4.6% (N × 5.86), on a dry solids basis).

Fractionation of the Proteins. Lupin proteins were fractionated into their main globulin fractions based on differential solubility in NaCl and temperature, as described previously (Suchkov *et al.*, 1990), and purity of the fractions was checked by FPLC and differential scanning calorimetry (DSC) (Sousa, 1993).

Solubility. The solubility of the lupin protein isolate, in aqueous solution at different pH values, was determined following a method developed by Shen (1981). In this method, dilute (0.9%) aqueous protein solutions with pH appropriately adjusted using HCl (0.1 N) or NaOH (0.1 N) are shaken for 2 h at 120 rpm at 25 °C and centrifuged at 42000*g* for 40 min; the protein left on the supernatant is then quantified by the Kjeldhal method.

Intrinsic Viscosity. The flow times were obtained using a precision Ostwald capillary viscometer (Schott-Gerate AVS 310) at 25.00 ± 0.01 °C. The intrinsic viscosity is calculated from the capillary viscosity data fitting the Huggin's and

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S0021-8561(95)00516-4 CCC: $12.00

Kraemer equations in a double-extrapolation plot (see, *e.g.*, Tanford (1967) and Harding (1995)), taking into account the kinematic viscosity - dynamic viscosity correction factor for the density of the solutions (Tanford, 1955), which includes the density of the buffer ρ_0 and the partial specific volume $\bar{v}$ of the protein (eq 1)

$$[\eta] = [\eta]' + [(1 - \bar{v}\,\rho_0)/\rho_0] \quad (1)$$

where $[\eta]'$ is the kinematic intrinsic viscosity and $[\eta]$ the dynamic intrinsic viscosity.

The partial specific volume, *i.e.*, the volume increase when 1 g of protein is added to an infinite volume of the solution, represents the reciprocal of the nonhydrated density of the particle. The $\bar{v}$ values were calculated from the densities determined in an Anton Paar (Graz, Austria) digital precision density meter DMA 02C at 25.00 ± 0.01 °C (Kratky *et al.*, 1973). The partial specific volume is related to the density of the solution (ρ), the density of the solvent (ρ_0), and the concentration of the macromolecule (c) (in g/mL) by the equation (Kratky *et al.*, 1973):

$$\bar{v} = 1/\rho_0(1 - \partial\rho/\partial c) \quad (2)$$

Sedimentation Analysis. The sedimentation coefficients and the absolute molar mass were evaluated in an analytical ultracentrifuge Optima XL-A (Beckman Instruments Inc.) with double-sector centerpiece cells and an absorbance optical system. Solvent in dialysis equilibrium with the protein solution (samples were dialyzed against buffer, for at least 2 days, at 5 °C) was used as the reference. All the measurements were performed at 20.0 °C, and distributions of solute were recorded with the absorption optical system at a wavelength of 278 nm. Sedimentation velocity experiments for sedimentation coefficient evaluations (in Svedberg units S = 10^{-13} s) were performed at 37 000, 45 000, and 47 000 rpm. The time interval between the scans was 480 s. Measurements were made simultaneously with samples of three different concentrations ranging from 0.5 to 5 mg/mL. Sedimentation coefficients were corrected to standard conditions (density and viscosity of water at a temperature of 20.0 °C) in the usual way (van Holde, 1985) to give $s_{20,w}$ values.

For absolute molar mass measurements, *i.e.*, the sedimentation equilibrium study, the low-speed method (Creeth and Harding, 1982) was employed, at a rotor speed of 8000 rpm. Because of the low concentrations used (0.5−1.0 mg/mL), no correction for thermodynamic nonideality was deemed necessary. It was considered that equilibrium was achieved when two consecutive scans, recorded several hours apart, appeared identical. Solute distributions at sedimentation equilibrium were analyzed using the FORTRAN program MSTARA (Harding *et al.*, 1992). Whole-cell weight-average molar masses (M^0_w) were extracted by using the limiting value at the cell base of the M^* (point-average molar mass) function (an independent estimate for the initial loading concentration was not required) as described by Creeth and Harding (1982).

RESULTS AND DISCUSSION

Solubility at Different pH Values. The solubility of the lupin isolate in distilled water at different pH values (Figure 1) follows the same solubility profile previously reported by other authors (see, *e.g.*, Shen (1976a) or Arrese *et al.* (1991)) for soy proteins. Similar results were obtained for the solubilities in 0.2 M NaCl solutions (Hermansson, 1979). Subsequent measurements were made at pH 7.0 where the solubility is high.

Densimetry and Viscometry. The values of the partial specific volume, $\bar{v}$, of the lupin and soy isolates in the two different solvents are displayed in Table 1. There were no relevant differences in the $\bar{v}$ values determined for the soy and lupin isolates, and their values are within the range (0.70−0.75 cm^3 g^{-1}) reported for proteins (Tanford, 1968; Rha and Pradipasena, 1986).

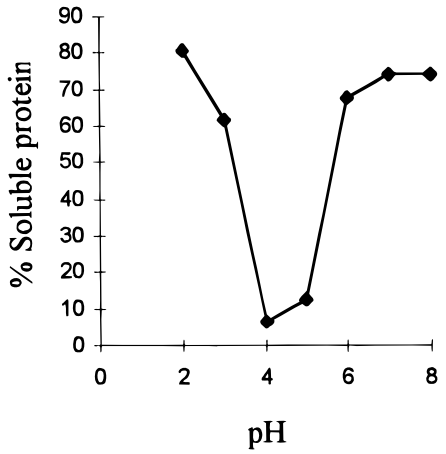

Figure 1. Solubility profile of lupin protein isolate in water at different pH values. The solubility is given as percentage of soluble protein on the basis of the initial protein content.

Table 1. Values of the Partial Specific Volume of Lupin and Soy Isolates, Calculated in a High and Low Ionic Strength Solvent

	phosphate buffer pH 7.6, I = 0.5 M, n = 5	phosphate buffer pH 7.0, I = 0.01 M, n = 5
lupin isolate	$\bar{v}$ = 0.736 (0.005)[a] cm^3 g^{-1}	$\bar{v}$ = 0.759 (0.002)[a] cm^3 g^{-1}

[a] Values in parentheses are standard errors of the mean.

Table 2. Intrinsic Viscosity of Lupin and Soy Isolates

	$[\eta]$ (σ)[a] (cm^3 g^{-1})	
lupin isolate	Huggins	Kraemer
in pH 7.6, 0.5 phosphate buffer	6.73 (0.29)	6.90 (1.30)
in pH 7.0, 0.01 phosphate buffer	7.03 (0.18)	7.47 (1.80)

[a] n = 5.

Table 3. Sedimentation Coefficients ($s^0_{20,w}$) for Lupin Proteins[a]

	$s^0_{20,w}$ (10^{-13} s)		
sample/buffer	1st component	2nd component	3rd component
lupin proteins			
in pH 7.0, 0.01 phosphate buffer	13.6	7.2	2.0
in pH 7.6, 0.5 phosphate buffer	13.7	7.3	2.2

[a] Calculations were based on at least five determinations.

The intrinsic viscosities obtained for lupin protein isolate in two different buffer systems are displayed in Table 2. The values obtained for the lupin proteins ranged from 6.7 to 7.5 mL/g, and there were no significant differences between the values at the low and high ionic strength buffers. These values are comparable to those previously observed for soy and its fractions, ranging from 5.2 to 7.8 mL/g (Wolf *et al.*, 1963; Koshiyama, 1968; Shen, 1976b; Diep *et al.*, 1982; Varfolomeyeva *et al.*, 1986).

Ultracentrifugation. Sedimentation velocity analysis of the lupin protein isolate revealed the presence of three components with sedimentation coefficients listed in Table 3. The presence of three components was also found in DSC studies (Sousa *et al.*, 1995) and are in agreement with results from the DSC thermograms earlier reported by Wright *et al.* (1980) for other lupin species.

Table 4. Molar Masses of the Lupin Main Globulins

sample/buffer	M_w[a]	M^0_w[b]
lupin 11S globulin		
in pH 7.0, 0.01 phosphate buffer	300 000−400 000	390 000
in pH 7.6, 0.5 phosphate buffer	200 000−500 000	400 000
lupin 7S globulin		
in pH 7.0, 0.01 phosphate buffer	80 000−110 000	95 000
in pH 7.6, 0.5 phosphate buffer	50 000−150 000	115 000

[a] Point-average molar mass range. [b] Whole-cell weight-average molar mass.

Sedimentation coefficients are concentration dependent; the extrapolation to zero concentration of the results obtained at several concentrations gives the $s^0_{20,w}$ value (with values of $R^2 > 0.93$).

The sedimentation coefficient for the heavier lupin globulin (13.7 ± 0.4 S) is within the range reported (14.7−10.1 S) in the literature (Derbyshire *et al.*, 1976; Prakash, 1992) for plant globulins.

To determine the molar mass of the lupin globulins, these were fractionated and the measurements were made by sedimentation equilibrium in the two solvents. The results shown in Table 4 indicate that the lupin major globulin has a molar mass ~50 000 higher than the highest value found in the literature (Varfolomeyeva *et al.*, 1986) for the respective soy globulin, 340 000, determined by ultracentrifugation from an 11S soy fraction purified by another method, but using the same high ionic strength (pH 7.6, $I = 0.5$ buffer). For the soy 7S globulin, the reported value for the molar mass determined by ultracentrifugation is 180 000 (Koshiyama, 1983), which is almost double the determined molar mass for the respective 7S lupin globulin in this study.

The sedimentation equilibrium data suggest that the samples showed more heterogeneity in the high ionic strength buffer. This is consistent with the results of Duranti *et al.* (1988), who showed that the globulins from *Lupinus albus* underwent dissociation that was dependent on the ionic strength, later confirmed by Prakash (1992) on other seed proteins.

Conclusions. The solubility of lupin proteins, isolated under mild conditions, compares well with the solubility of soy proteins. The average partial specific volumes and intrinsic viscosity values for the lupin proteins are also similar to those reported by other authors for the soy and other seed storage proteins.

The main difference was found in the molar mass of the main globulins (13S and 7S). The heavy globulin showed an excess of almost 50 000 units when compared to the reported values for soy 11S, and the second globulin studied showed almost half the weight reported for the soy 7S. These findings can have implications on the hydration rate of the protein. With a substantial amount of smaller components, lupin proteins need more water to be fully hydrated, since they have a higher surface area to volume ratio. It was also found (Sousa *et al.*, 1995) that the lupin 7S globulins are more heat stable than the soy 7S globulins. These differences can be reponsible for the inferior gelation and thickening properties of lupin proteins when compared to soy proteins.

LITERATURE CITED

Arrese, E. L.; Sorgentini, D. A.; Wagner, J. R.; Añón, M. C. Electrophoretic, solubility and functional properties of commercial protein isolates. *J. Agric. Food Chem.* **1991**, *39*, 1029−1032.

Cerletti, P. Lupin seed proteins. In *Developments in Food Proteins-2*; Hudson, B. J. F., Ed.; Applied Science Publ.: London 1983.

Creeth, J. M.; Harding, S. E. Some observations on a new type of point average molecular weight. *J. Biochem. Biophys. Methods* **1982**, *7*, 25 −34.

Derbyshire, E.; Wright, D. J.; Boulter, D. Review. Legumin and vicilin, storage proteins of legume seeds. *Phytochemistry* **1976**, *15*, 3−24.

Diep, O.; Boulet, M.; Castaigne, F. Effect of extreme pH and salt concentration on intrinsic viscosity and Huggins' constant of 7S and 11S soybean globulins. *Can. Inst. Food Sci. Technol. J.* **1982**, *15*, 316−318.

Duranti, M.; Guerrieri, N.; Takahashi, T.; Cerletti, P. The legumin-like storage protein of *L. albus* seeds. *Phytochemistry* **1988**, *27*, 15−23.

Harding, S. E. On the hydrodynamic properties of macromolecules. *Biophys. Chem.* **1995**, in press.

Harding, S. E.; Horton, J. C.; Morgan, P. J. A Fortran program for the model independent molecular weight analysis of macromolecules using low or high speed sedimentation equilibrium. In *Analytical Ultracentrifugation in Biochemistry and Polymer Science*; Harding, S. E., Rowe, A. J., Horton, J. C., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1992.

Hermansson, A.-M. Methods of studying functional characteristics of vegetable proteins *J. Am. Oil Chem. Soc.* **1979**, *56*, 272−279.

King, J.; Aguirre, C.; Pablo, S. Functional properties of lupin protein isolates. *J. Food Sci.* **1985**, *50*, 82−87.

Koshiyama, I. Soybean proteins V: Chemical and physical properties of a 7 S protein in soybean globulins. *Cereal Chem.* **1968**, *45*, 394−404.

Koshiyama, I. Storage proteins of soybean. In *Seed Proteins*; Gottschalk, W., Muller, H. P., Eds. ;Martinus Nijhoff: The Hague, London, 1983.

Kratky, O.; Leopold, A.; Stabinger, H. The determination of the specific volume of proteins by the mechanical oscillator technique. In *Methods in Enzymology*; Hirs, C. H. W., Timasheff, S. N., Eds.; Academic Press: London, 1973.

Larsen, L. B.; Sousa, I. M. N.; Beirão da Costa, M. L. The use of the Maillard reaction to improve gelation of soy and lupin proteins. In *Advances in Lupin Research*; Neves Martins, J. M., Beirão da Costa, M. L., Eds.; ISAPress: Technical University of Lisbon: Lisbon, Portugal, 1994.

Morr, C. V. Current status of soy protein functionality in food systems. *J. Am. Oil Chem. Soc.* **1990**, *67*, 265−271.

Prakash, V. Sedimentation analysis of seed proteins. In *Analitycal Ultracentrifugation in Biochemistry and Polymer Science*; Harding, S. E., Rowe, A. J., Horton, J. C., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1992.

Rha, C.; Pradipasena, P. Viscosity of proteins. In *Functional Properties of Food Macromolecules*; Mitchell, J. R., Ledward, D A., Eds.; Elsevier Applied Science Publ.: London, New York, 1986.

Riccardi, A.; Cerletti, P.; Bertolotti, G. Emulsifying and gelling properties of the proteins from lupin seed. In *Perspectives for Peas and Lupins as Protein Crops*; Thompson, R., Casey, R., Eds.; Martinus Nijhoff Publ.: London, 1983.

Shen, J. L. Soy protein solubility: the effect of experimental conditions on the solubility of soy protein isolates. *Cereal Chem.* **1976a**, *24*, 784−788.

Shen, J. L. Solubility profile, intrinsic viscosity, and optical rotation studies of acid-precipitated soy protein and commercial soy isolate. *J. Agric. Food Chem.* **1976b**, *24*, 784−788.

Shen, J. L. Solubility and viscosity. In *Protein Functionality in Foods*; Cherry, J. P., Eds.; ACS Symposium Series 147; American Chemical Society: Washington, DC, 1981.

Sousa, I. M. N. Functional Properties of Lupin Proteins. Ph.D. Thesis. University of Nottingham, Nottingham, U.K. 1993.

Sousa, I. M. N.; Mitchell, J. R.; Ledward, D. A.; Hill S. E.; Beirão da Costa, M. L. Differential scanning calorimetry of lupin and soy proteins. *Z. Lebensm. Unters. Forsch.* **1995**, *201*, 566−569.

Suchkov, V. V.; Popello, I.; Grinberg, V. Ya.; Tolstogusov, V. B. Isolation and purification of 7S and 11S globulins from broad beans and peas. *J. Agric. Food Chem*. **1990**, *38*, 92−95.

Tanford, C. Intrinsic viscosity and kinematic viscosity. *J. Phys. Chem*. **1955**, *59*, 798−799.

Tanford, C. Protein denaturation. *Adv. Protein Chem*. **1968**, *23*, 121−282.

Tanford, C.; Kawahara, K.; Lapanje, S. Proteins as random coils. I. Intrinsic viscosities and sedimentation coefficients in concentrate guanidine hydrochloride. *J. Am. Chem. Soc*. **1967**, *89*, 729−731.

van Holde, K. E. *Physical Biochemistry*, 2nd ed.; Prentice Hall: Englewood Cliffs, NJ, 1985.

Varfolomeyeva, E. P.; Danielenko, A. N.; Bikbov, T. M.; Grinberg, V. Ya.; Leontiev, A. L.; Tolstogusov, V. B. The rheological properties of diluted solutions of 11S globulin isolated from soybeans by using selective thermal denaturation of 2S and 7S globulins. *Nahrung* **1986**, *30*, 487−500.

Wolf, W. J.; Eldrige, A. C.; Baboock, G. E. Physical properties of alcohol-extracted soybean proteins. *Cereal Chem*. **1963**, *40*, 504−514.

Wright, D. J.; Boulter, D. Differential scanning calorimetric study of meals and constituents of some food grain legumes. *J. Sci. Food Agric*. **1980**, *31*, 1231−1241.

Received for review September 11, 1995. Accepted July 9, 1996.[⊗] This work was partially funded by an E.U. SCIENCE grant (JNICT BD 600 90 I.E.).

JF950516F

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1996.