INTRINSIC VISCOSITY AND MARK-HOUWINK PARAMETER OF LUPIN PROTEINS IN AQUEOUS SOLUTIONS

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ABSTRACT

Lupin is a plant, with seeds containing ±40% and has the advantage of growing in marginal soils. Soy protein is successfully used as a functional food ingredient and increased the interest in study the functional properties of other legume proteins, including lupin. It was reported (Cerletti, 1983, Larsen *et al* 1994) that the lupin thermal gelling properties, an important aspect of protein functionality, are inferior to those of soy. To understand why two similar proteins should have different gelling properties, the intrinsic viscosity ([η]) and the Mark Houwink parameter ("a") of the lupin and soy isolates were investigated. These results indicate that ([η]) values were much higher for the soy isolate so the lupin isolate must have a more compact structure. The "a" exponents estimated for the soy and lupin isolates were 0.4 and 0.3 respectively. This may suggest that the protein systems consist of a mixture of coils and rigid particles.

RÉSUMÉ

Le lupin est une plante avec des graines très riche en protéine (±40%) et il pousse en sols pauvres. La protéine de soja a été utilisée avec succés comme ingrédient fonctionnel de l'aliment et a augmenté l'intérêt porté à l'étude d'autres légumineuses pour la même application, le lupin inclu. Cerletti (1983) et Larsen *et al* (1994) ont dit que les propriétés de gélification de la protéine du lupin sont inférieures à celles du soja. Pour comprendre pourquoi deux protéines similaires ont des comportement gélifiant différents, la viscosité instrinsèque et le paramètre de Mark -Houwink des isolats de lupin et de soja ont été étudiés. Les résultats ont indiqués que les valeurs des isolats du soja sont supérieures à celles du lupin, donc les protéines du lupin doivent avoir une structure plus compacte. La valeur estimée pour "a" dans le cas du soja a été de 0,4 et pour le lupin de 0,3. Cela pourrait suggérer que les deux systèmes protéiques sont un mélange de "coils" et particules rigides.

MOTS CLÉ: Lupin protein, intrinsic viscosity, Mark-Houwink parameter.

INTRODUCTION

Soy is the legume protein that is used most extensively as a food ingredient (Morr, 1990). The success of the soy products as functional ingredients in the food industry has encouraged the study of other plant proteins for the same purpose (Wright and Bumstead, 1984). The lupins are legumes with relatively high protein content (± 40%) and are interesting from the agricultural point of view, specially in Australia, the South American and Mediterranean countries (López-Bellido, 1994; Nelson, 1994; Baer, 1994 and Jorge, 1994). The functional properties of the lupin proteins have been previously studied (Riccardi *et al.*, 1983; King *et al.*, 1985) and there is substantial evidence that the gelation and thickening properties of lupin proteins are inferior to soy (Riccardi *et al.*, 1983; King *et al.*, 1985; Larsen *et al.*, 1994). In this work the intrinsic viscosity of the lupin and soy protein isolates was determined to investigate the difference in the hydrodynamic volume of the two protein systems. An attempt to estimate the Mark-Houwink parameter, using a method previoulsy suggested by Lefebvre (1982), was made to support the decision about the best model to describe the rheological behaviour of these proteins in solution. This information will help to understand why the thickening and gelling properties of the lupin isolate are different from the soy isolates.

MATERIALS AND METHODS

MATERIALS

Commercial soy grits were obtained from Iberol-Soc Ibérica de Oleaginosas S A and *Lupinus luteus* seeds from Gonçalves Fonseca C^{a} Lda, both Portuguese suppliers. The water used was re-distilled and de-ionised. The other chemicals used were reagent grade.

ISOLATION OF THE PROTEINS

A hammer mill with a sieve of 1.5 mm aperture diameter was used to reduce the particle size of both the soy grits and whole lupin seeds. The protein isolates were produced by solubilisation of the protein in distilled water (1:10) at pH 9.0 with NaOH and stirring for 2h at room temperature, centrifugation at 5000g for 15 min. with the residue discarded. This was followed by isoelectric precipitation of the protein at pH 4.5 with HCl, centrifugation at 5000g during 30 min., washing of the precipitate twice with warm destilled water, neutralisation with NaOh 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 soy and lupin isolates were (84.6 ± 4.0) % (N x 5.77) and (85.5 ± 4.6) % (N x 5.86), on a dry solids basis, respectively).

METHODS

INTRINSIC VISCOSITY

The flow times were obtained using a precision Ostwald capillary viscometer (Schott-Gerate AVS 310) at (25.00 ± 0.01) °C. Measurements were made in a pH 7.0 phosphate buffer (I=0.01) buffer. The concentrations were sufficiently dilute (0.0120 to 0.0620 ± 0.0001 g/ml) to show Newtonian behaviour over a wide shear rate range. The intrinsic viscosity is calculated from the capillary viscosity data by fitting the Huggins and Kraemer equations (see *e.g.* Tanford, 1961 and Harding,

1995). The intrinsic viscosity was corrected 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 (equation 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 1g of protein is added to an infinite volume of the solution, represents the reciprocal of the non-hydrated 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. 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):

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

ESTIMATION OF THE MARK-HOUWINK PARAMETER

The Mark-Houwink "a" parameter comes from the Mark-Houwink equation that relates the molar mass (M) to the intrinsic viscosity $[\eta]$ for a given macromolecule:

$$[\eta] = K\ M^a \quad (3)$$

This gives information on the shape and hydrodynamic behaviour of the polymer. Four different cases can be identified (see *e.g.* Mitchell, 1979, Harding, 1995):

i) the sphere - **'a' = 0**,

i.e., there is no dependence of the intrinsic viscosity on the molecular weight of the polymer for compact spherical particles.

ii) the random coil (equivalent sphere behaviour) - **'a' ≈ 0.5 - 0.8**,

i.e., $[\eta] = K\ M^{0.5 - 0.8}$

the exponent increases with solvent quality;

iii) the random free draining coil - **'a' ≈ 1.0 - 1.2**,

i.e., $[\eta] = K\ M^{1.0-1.2}$

where the exponent also increases with solvent affinity;

iv) the rigid rod - **'a' = 1.8**,

i.e., $[\eta] = K\ M^{1.8}$.

A method for determining the Mark-Houwink parameter 'a' was suggested by Lefebvre (1982). It is based on the Simha concept of the "effective intrinsic viscosity" that reflects the contraction of the coil volume above the critical concentration (c*) plus the calculation made by Graessley (1980) of the coil expansion coefficient in a given solvent. The equation given by Lefebvre (1982) can be written as:

$$\ln \eta_r = 2\ a\ [\eta]\ c^*\ (c/c^*)^{1/2a} - (2\ a - 1)\ [\eta]\ c^* \quad (4)$$

The 'a' value can be calculated if the parameters intrinsic viscosity ($[\eta]$) and critical concentration (c*) are known.

The critical concentration, c*, can be obtained using rotational viscometry. A Bohlin constant stress rheometer with concentric cylinder geometry (C14 and C25) was employed. Measurements were made at 25.00 ± 0.01 °C in a shear rate range from 1 to 80 s^{-1}. The protein suspensions at

concentrations ranging from 0.05 to 0.44 g/ml were prepared with a low ionic strength phosphate buffer (0.01, pH=7.0) to ensure good protein solubility. The suspensions were stirred overnight and allowed to rest for 1h before testing. The zero shear viscosity was calculated by fitting the data to the Cross equation, using the software supplied with the rheometer:

$$\eta = \eta_{\infty} + \frac{\eta_0 - \eta_{\infty}}{1 + (\tau \dot{\gamma})^m} \qquad (6)$$

where η is the shear viscosity, η_0 is the zero shear viscosity or the viscosity of the first Newtonian plateau, η_{∞} is the infinite shear viscosity or the viscosity of the second Newtonian plateau, τ is the Cross relaxation time, $\dot{\gamma}$ is the shear rate and "m" is an exponent which is related to the power law index "n" by the approximate relationship: $m \cong n - 1$. The η_0 values were accepted only when the fit of the data to the Cross equation gave R^2 values above 80%.

RESULTS AND DISCUSSION

INTRINSIC VISCOSITY

The intrinsic viscosity was obtained from the fitting of the Huggins and Kraemer equations to the capillary viscometer data, for several solutions at different concentrations as shown in fig. 1.

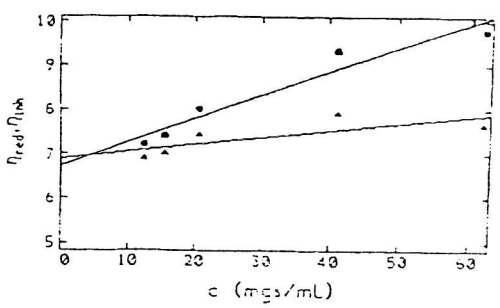


Figure 1 Fitting of the Huggins (circles) and Kraemer (triangles) relationships to the lupin isolate capillary viscometer data.

The values of the intrinsic viscosity, Huggins and Kraemer constants for the different studied materials are listed in Table 1.

TheKraemer equation always gave higher values for the standard error but the values obtained using this and the Huggins equation were very similar.

For the lupin isolate the intrinsic viscosities were 7.03 ± 0.18 and 7.47 ± 1.80 ml/g, using the Huggins and the Kraemer equations respectively. As the intrinsic viscosity is a measure of the hydrodynamic volume of the particles in solution, it is apparent from the respective values for the soy isolate (12.3 ± 0.49; 13.8 ± 0.66 ml/g) that the hydrodynamic volume of the soy proteins in aqueous solutions is almost double that of the lupin. This will obviously have repercussions on the thickening ability of these isolates. The soy dispersions would be expected to show higher viscosities at all concentrations.

The calculated values for the intrinsic viscosity of the lupin proteins are not far from those reported as characteristic values for globular proteins (2.5 to 6 ml/g) by Rha and Pradipasena (1986). One explanation for the high values of the intrinsic viscosity of the soy proteins is the occurrence of partial denaturation. This probably happened during the industrial processing of the grits (as the samples are subjected to heat during solvent extraction and drying). In fact values of 13.6 and 17.4 ml/g were reported by Diep *et al.* (1982) for the soy 11S and 7S soy globulins in a 0.5 μ, pH 11.0 buffer, at high pH values (pH>9) the proteins would be expected to be denatured.

Table 1 Intrinsic viscosity of lupin and soy isolates.

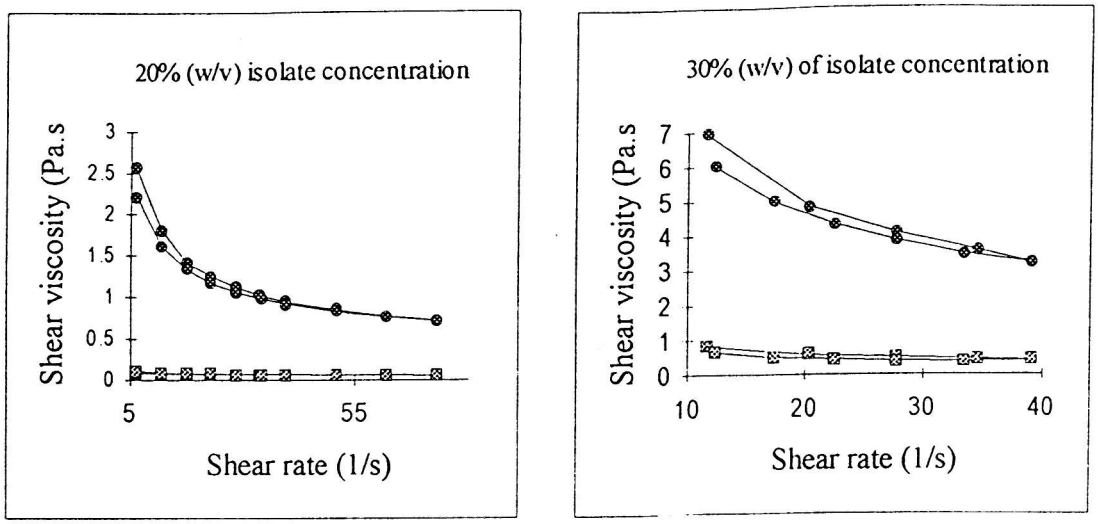
MATERIALS	DATA FROM HUGGINS' PLOT		DATA FROM KRAEMER PLOT	
	Huggins' $[\eta]$ ($cm^3.g^{-1}$)	Huggins' constant	Kraemer's $[\eta]$ ($cm^3.g^{-1}$)	Kraemer's constant
Lupin Isolate in 7.0, 0.01 buffer	7.03 ± 0.18	1.75	7.47 ± 1.80	0.52
Soy Isolate in 7.0, 0.01 buffer	12.30 ± 0.49	1.75	13.80 ± 0.66	0.31

The Huggins (k_H) and Kraemer (k_K) constants are theoretically related ($k_H + k_K = 1/2$) but in practice this is often not the case and our results are another example of $k_H + k_K \neq 1/2$. Launay *et al.*, (1986) advise the use of both equations to verify that they lead to similar values of $[\eta]$. Our lupin protein intrinsic viscosity values, obtained from the two data, are not significantly different although for the soy proteins the difference is significant ($0.02<P<0.05$). As the concentrations used in the determinations were comparable, this may reflect the shear dependence at the higher concentrations used for the soy suspensions as soy protein shows a hydrodynamic volume twice as high as the lupin protein.

ROTATIONAL VISCOSITY

The results (fig. 2.) showed that the viscosity of the soy dispersions were considerably higher than the viscosity of the lupin dispersions at the same concentration.

The soy isolate showed a higher thickening potential with a shear viscosity about 10 times higher than the lupin isolate dispersions. This is not surprising if the intrinsic viscosity values for the isolates are considered. The soy also exhibits a more pronounced shear thinning behaviour (a viscosity decrease with shear rate): the calculated values for the flow index (n) were around 0.4 for the soy dispersions (R^2= 0.97 for the power law equation fit) and above 0.5 for the lupin (R^2= 0.95).



A B

Figure 2 Viscosity *versus* shear rate for lupin (squares) and soy (circles) isolates at 20% (A) and at 30% (B) concentration in pH 7.0/I=0.01 buffer dispersions.

MARK-HOUWINK PARAMETER

The critical concentration c* of a polymer is the concentration above which there is a more pronounced increase in viscosity with concentration because the macromolecules in solution are close to each other and entanglements and other interactions make a major contribution to the viscosity.

If the equation 4 is to be used to determine the Mark-Houwink parameter ("a"), the values of the intrinsic viscosity [η] and critical concentration c* of the polymer in the considered solvent needs to be known.

c* can be either obtained from the intrinsic viscosity using equation 5 or from a double logarithmic plot of the zero shear viscosity against (concentration x intrinsic viscosity) the latter is shown for both protein systems in fig.2. The data can be approximated by two straight lines with R^2 values greater than 0.90 for the soy but these values are lower for the lupin protein. The straight lines fit for the proteins are given below (Table 2). The slopes of the straight lines can be compared to those obtained by Morris *et al.* (1981) for a wide range of polysaccharides with slopes for the dilute concentrations ≈ 1.4 and for the higher concentrations ≈ 3.3 and transition from dilute to concentrated solution occurred at $c[\eta] \approx 4$ and $\eta_{sp} \approx 10$.

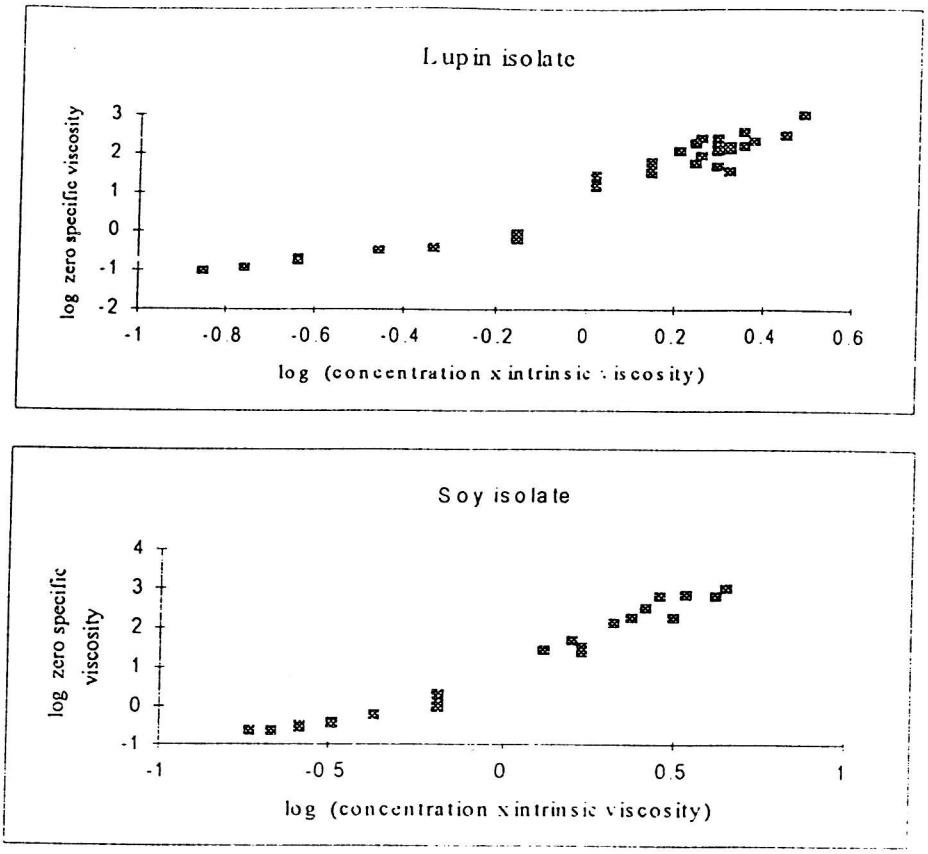


Figure 2. Curves to estimate c* for lupin and soy isolates in pH=7.0/I=0.01 phosphate buffer

Table 2 Straight lines fitted to the double log plot (fig.2) obtained for lupin and soy systems.

Protein systems	First part of the curve $\log(c[\eta]) \leq -0.2$	Second part of the curve $\log(c[\eta]) > -0.2$
Lupin	$y = 0.352 + 1.72\ x$ $R^2 = 0.85$	$y = 1.024 + 4.07\ x$ $R^2 = 0.68$
Soy	$y = 0.236 + 1.27\ x$ $R^2 = 0.98$	$y = 0.906 + 3.48\ x$ $R^2 = 0.92$

Deviations from the Morris and co-workers slopes have been reported and Launay *et al.* (1986) reviewed the published data. Reported values for the first slope are within the range of 1.1 to 1.4 and for the second slope the range is between 2.7 and 5.1.

Our values for soy of 1.27 and 3.48 would seem to fit with the published polysaccharide data. The values obtained for lupin of 1.72 and 4.07 seem high, but the regression coefficients were not so good for this protein. This change in slope is thought to be due to polymer-polymer interaction, *i. e.*, entanglements and even polymer aggregation (Launay *et al.*, 1986).

The c* values determined from fig.2 were 0.04 g/ml for soy and 0.07 g/ml for lupin. These values are slightly lower in both cases than those based on the equation 5 (0.09 and 0.05 g/ml, respectively).
The high concentration required for the lupin to reach the critical concentration, where the molecules begin to overlap, is of course the reason for the low viscosities recorded for this protein.

Calculated 'a' values

Using for c* the values determined from fig.2, the Mark-Houwink parameter can be calculated using equation 4.. The estimated 'a' values were optimised by adjusting the calculated (equation 4) η_{rel} to the experimental η_{rel} at different concentrations by the least square method. The Mark-Houwink parameter for the soy protein was 0.4 and for the lupin protein was 0.3. These results fall between the 'a' values of the model of the compact sphere (a=0) and of the equivalent sphere model (a=0.5 - 0.8).
The simplest interpretation of these results is that the lupin and soy macromolecules have an approximately globular conformation as expected but are not compact spheres. An alternative interpretation is that we have a mixture of molecular species, *i. e.*, native protein obeying the compact sphere picture and denatured protein following the equivalent sphere model. The molecules may have a considerable amount of solvent entrapped inside the globular structure, and the soy protein may have more solvent bound, *i. e.*, is a less compact molecule than the lupin protein. This could be the reason the viscosity of the soy is higher than the lupin at equivalent concentrations.

CONCLUSIONS

Measurements of intrinsic viscosity for the lupin and soy protein suspensions indicated much higher values for the soy isolate (≈13 ml/g) compared to the lupin isolate (≈7 ml/g). The higher values for the soy reflects a higher hydrodynamic volume of this protein in aqueous media, probably with a more random like conformation rather than a more compact globular conformation shown by the lupin.
Viscosity of dispersions of the soy and lupin at high concentrations again showed the better thickening properties of the soy. The shear viscosity of soy dispersions was about ten times higher than the lupin protein dispersions for concentrations around 0.2, 0.3 g/ml.
The critical concentration (c*), which denotes the concentration required for the particles to overlap and start to entangle, was obtained by two different approaches and both indicated that the soy values (0.04 - 0.05 g/ml) were considerably lower than those for the lupin (0.07 - 0.09 g/ml). The estimated values for "a" were 0.3 for the lupin and 0.4 for the soy proteins and this was interpreted as the isolates being composed of a mixture of molecular species, *i. e.*, native protein obeying the compact sphere picture and denatured protein following the equivalent sphere model. The soy protein may have more solvent bound, *i. e.*, is a less compact molecule than the lupin protein. This will have implications on the gelation behaviour of these protein systems, *i. e.*, lupin protein would be expected to gel at higher concentrations than soy protein as is observed.

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