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A N I N T E R N A T I O N A L J O U R N A L

Hydration Properties of Soybean Protein Isolates

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ABSTRACT

Hydration properties of soybean isolates with different processing conditions (heat treatments, pH and protein concentrations) were studied. Degree of denaturation,, solubility in water, in 0.2mol/L NaCl, and in media of different sodium dodecyl sulfate concentrations, viscosity and water imbibing capacity of the different samples were determinated and correlated. Treatments at temperatures higher than 80ºC denatured 11S and 7S proteins, leading to exposure of hydrophobic groups, which produced insoluble aggregates either in water or in high ionic strength media. These isolates possessed high water imbibing capacities and gave rise to viscous dispersions. Significant correlations were obtained between hydration properties and the "m" coefficient as calculated by a power law equation relating viscosity with the protein concentration of the dispersion. This "m" coefficient also correlated with the denaturation enthalpy of the protein isolates. On the basis of these results, it might be suggested that the "m" coefficient - dependent of the hydrodynamic behaviour of the particles - was a good estimator of the degree of protein denaturation.

Key words: Soybean proteins, hydration properties, protein isolates functional properties

INTRODUCTION

Soy isolates are the most highly refined soybean proteins commercially available.Effects of proccesing conditions on the functional properties of protein isolates have been studied by many authors (Pour El, 1981; Remondetto, 1997; Kinsella, 1979; Shiga & Nakamura, 1987; Arce *et* al., 1989). Nevertheless, studies regarding pilot plant scale are scarce (Delgado *et al.*, 1989; González *et al.*, 1995), except for those registered as patents (Calvert, 1995; Potito De Paolis, 1972; Potito De Paolis, 1973). This type of investigation becomes important to obtain information about the necessary processing conditions required for the industrial manufacture of protein isolates with given charasteristics. Most of the studies relating functional properties to structural and physicochemical properties analyze isolates prepared in a laboratory scale and modified by thermal, chemical or enzymatic treatment after its preparation (Kinsella, 1979; Urbansky *et al.*, 1983; Fiora *et al.*, 1990; Sorgentini *et al.*, 1991; Sorgentini *et al.*, 1995; Petruccelli & Añón, 1994 (I); Petruccelli & Añón, 1994 (II)).

The objetive of our study was to evaluate the hydration properties of soybean protein isolates obtained in a pilot plant and to correlate them with the extent of denaturation undergone by the different protein components ocuring in the samples.

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MATERIALS AND METHODS

Raw Material: Deffated soybean flakes were supplied by Guipeba S.A., Santa Fe, Argentina. Composition of flakes, in g/100g, were as follows: 45.0 protein (N x 6.25), 13.0 moisture, 1.5 oil, 5.8 ash, protein dispersability index (PDI) was 78% (AACC, 1969)

Preparation of soybean protein isolates: A modification of the procedure described by Circle and Smith (1972) was used for preparing protein isolates (Fig.1).

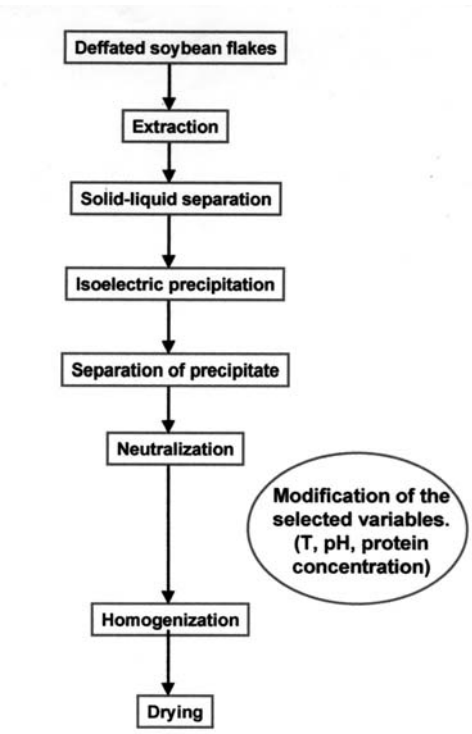

Figure 1 - Diagram of the experimental procedure used in the preparation of soybean protein isolates in the pilot plant.

Extraction was performed in water at pH 9 and at 50ºC with a solid/liquid ratio 1/10, using anchor type stirrer (60 rpm) for 1h. Solid- liquid separation was performed in a basket type centrifuge with filtering material. Isoelectric precipitation of the extract was carried out at pH 4.5 by adding HCl, 3 mol/L. Separation of the precipitate was carried out in a SAADH 205 model Westfalia centrifuge (Westfalia, Oelde, Germany). This procedure was used in all samples, variations of the solid concentrations, pH and temperature were done during neutralization. Modifications of these variables were done using an agitation tank with an anchor type stirrer (90 rpm) and heating jacket.

An experimental design consisting of 3 variables at 3 levels (Box & Behnken, 1960) was used. Fourteen treatments including twelve combinations of the three variables with a central point were obtained. Duplicate determinations were performed throughout (Table 1). The three levels of solid concentration used (7.0 – 9.0 and 11.0, in g/100g) were obtained by dilution of the washed precipitate.

Table 1 - Treatment conditions used.

Sample	(%) Solids concentration	Temperature (ºC)	pH
1	11	65	10
2	7	65	10
3	9	65	11.5
4	9	65	8.5
5	11	80	11.5
6	11	80	8.5
7	7	80	11.5
8	7	80	8.5
9	9	80	10
10	9	80	10
11	11	95	10
12	7	95	10
13	9	95	11.5
14	9	95	8.5

Alkaline treatment performed to reach each pH level (8.5, 10 and 11.5) was carried out at room temperature by adding NaOH, 3mol/L; pH was maintained for 10 min, and then the sample was neutralized immediately.

Three types of thermal tratment were used (65, 80 and 95ºC), by heating the dispersion with circulating hot water through the heating jacket, at a temperature 4 ºC higher than that planned, each temperature level was maintained for 15 min.

After thermal treatment, all samples were cooled to room temperature and homogenized before spray drying. A Manton Gaulin two-stage homogeneizer was used; pressures in the first and second stage were 2 x 10^5 and 5 x 10^5 Pa, respectively. Drying was perfomed in a Niro Atomizer dryer (Niro-Atomizer, Copenhague, Denmark), with inlet an outlet temperature of 130 and 90 °C, respectively.

Determination of viscosity: Dispersions within the

range 4.5-8.0 (g of protein/100g) of the different protein isolate samples obtained by stirring at 90 rpm for 1 h at room temperature were prepared; the dispersions were allowed to stand for 2 h before each measurements. Rheograms were obtained at 30ºC with a Haake Rotovisco RV2 viscosimeter (Haake, Karlsruhe, Germany) with a 50-500 rotor and NV system. Rotor velocity was between 0 and 50 rpm (shear rate range from 0 to 270 s^{-1}) using an acceleration rate of 25 rpm/min.

Velocity was maintained until a constant reading was obtained and then decreased to 0 rpm at the same rate of the ascending part. Viscosity was calculated from the lower curve. The exponent "m", corresponding to non-linear relationship between the viscosity expressed in centipoise (at 270 s^{-1}), and the protein concentration expressed in g/100g protein, were estimated by regression:

$$\eta_{app} = A\, C^{m} \quad [1]$$

Where A and m are characteristic constants, C is the protein concentration of the dispersion.

***Solubility*:** Solubility of the different samples obtained was determined in distilled water, NaCl, 0.2 mol/L and in two different SDS concentration (0.1 and 0.236, g/L). Dispersions containing 0.2 g/L of protein in each solvent at pH 7.0 were prepared; these were stirred with magnetic stirrer for 1 h at room temperature, centrifuged at 2000 x g for 30 min at room temperature and the content of soluble nitrogen in the supernatant was determined by the Kjeldahl method. Results were expressed as (g.soluble N/g total N)x 100.

Water-Imbibing Capacity (WIC): It was determined by means of the device described by Baumann and modified by Torgenson and Toledo (Torgenson & Toledo, 1997). Determinations were carried out at room temperature and the maximum (or the equilibrium value) was obtained; results were expressed as ml water/g isolate.

Differential Scanning Calorimetry,(DSC): The extent of denaturation of the different protein isolates was evaluated by DSC. A 910 Du Pont calorimeter (Du Pont, Wilmington, DE) with 7046 B Hewlett Packard recorder (Hewlett-Packard, Palo Alto, CA). Twenty percent dispersions (w/v) in distilled water of the different isolates obtained were analyzed. Fifteen to 20 mg samples were placed in air-tight aluminum capsules. An empty capsule was used as reference, rate of heating was 10ºC/min. Capsules were punctured at the end of scanning and the dry matter content was determined by heating at 105 ºC until a constant weight was reached. Calibration of temperature and cell constant were determined using Indium as a standard (ASTM, 1979; ASTM, 1980). The area under the curve of each thermogram was measued by means of Morphomat 34 Zeiss image analyzer (Zeiss, Wetzlar, Germany); this value was used to calculate denaturation enthalpy.

Table 2 - Properties of the soybean protein isolates obtained in a pilot plant

Sample	ΔH (J/g)	WIC (mL/g)	Sol.H_2O (%)	Sol.NaCl (%)	Sol.SDS (%)	η_{app} (cP)	M
1	7.10	5.0	56.0	44.0	96	37	2.57
2	10.84	3.8	86.5	60.0	96	25	2.29
3	8.36	4.4	54.0	41.5	92	37	2.64
4	8.86	3.5	83.0	53.0	96	35	2.13
5	3.99	6.7	15.0	13.0	82	23	4.01
6	4.24	7.6	31.5	25.0	86	124	3.10
7	1.72	7.8	22.0	19.5	83	112	3.56
8	6.81	5.2	41.0	32.0	96	94	2.53
9	7.60	5.5	60.0	49.0	93	32	2.54
10	4.11	5.7	35.0	30.0	94	41	3.32
11	1.88	6.3	15.5	15.0	72	25	3.68
12	--	10.1	19.0	13.0	66	197	3.69
13	--	8.1	15.6	12.5	68	77	4.05
14	--	6.3	20.6	17.0	69	87	3.77

ΔH: Denaturation entalphy; WIC: Water - Imbibing; Sol. H_2O: Solubility in water;
Sol. NaCl: 0.2 mol/L; Sol. SDS: solubiity in Sodium Dodecyl Sulphate, 0.1 g/L;
η_{app}: apparent viscosity of a 8% protein dispersion ; m: Coefficient.

RESULTS AND DISCUSSION

The model describing the relationships between each property and process variables were discussed elsewhere (Remondetto,1997; González *et al.*, 1995).

Table 2 shows the experimental results of all evaluations corresponding to the soybean protein isolates obtained. Samples were grouped according to the treatment temperature. DSC analysis is shown in Fig 2.

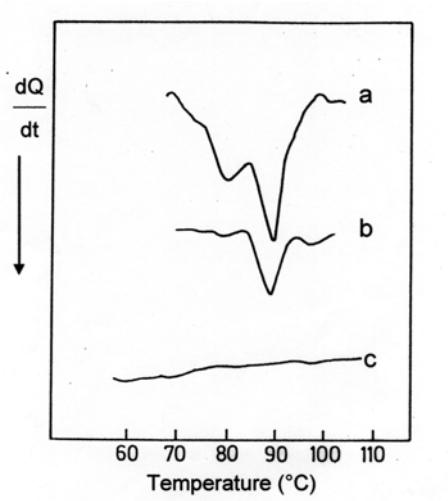

Figure 2 - Typical DSC thermograms of soybean protein isolates treated at 65°C (a); 80°C(b) and 95°C(c)

Thermograms of samples treated at 65ºC showed the two endotherms corresponding to 7S and 11S fractions. Calculated values of enthalpy (ΔH_T: 10.84 – 7.10 J/g) were lower than those corresponding to native isolates (ΔH_T: 15 – 20 J/g), (Petruccelli & Añón, 1995), indicating partial denaturation of the proteins. The extent of denaturation reached was maximum at the highest pH at which the thermal treatment was performed, in agreement with previous results obteined at the laboratory scale (Petruccelli & Añón, 1994 (I); Petruccelli & Añón, 1995). Samples treated at 80ºC contained almost totally denatured β-conglicinin and partially denatured glycinin (ΔH_T: 7.6 – 1.2 J/g); while samples heated at 95ºC were completely denatured, with the exception of sample 11. This was probably due to a protective effect of the higher solids concentration level (11 %).

Solubility in the different solvents used led to show up the possible types of particle-particle and particle-solvent interaction predominating in dispersion behaviour (2). Solubility in 0.2mL/L NaCl showed a high linear correlation with solubility in water (r = 0.98) (Fig 3). An increase of ionic strength lowers solubility; thus decrease becomes larger as the extent of protein denaturation decreases (Table 2). This behaviour was in accordance with that described by Wagner and Añón (1990) with regard to commercially available soybean protein isolates. It is likely that the less denatured proteins expose a larger number of charged hydrophilic groups upon which NaCl would provide a protective effect, thus making possible closer proximity of molecules and therefore a higher extent of aggregation

Presence of 0.1 g/l SDS markedly increased the solubilities of all samples. Values varied between 96 and 66. As expected, samples treated at 95ºC, exhibited the lowest solubility values (66-72) (Table 2). These results suggested that insolubility caused by the treatments was due to formation of aggregates largely estabilized by hydrophobic bonds. It has shown that both the thermal treatment and alkaline pH favor exchange SH/SS reactions (Petruccelli & Añón, 1995). Solubilities lower than 100 g/100 g would then be expected in a medium containing SDS (in the absence of a reducing agent), mainly in the case of isolates treated at 95ºC.

It should be pointed out that soybean protein isolates, at the laboratory level which had undergone thermal tretments, had SDS-soluble aggregates. These aggregates were sensitive to the action of β-mercaptoethanol and were formed by the α and α‘ subunits of β-conglycinin and A and B glycinin polypeptides (Petruccelli & Añón, 1994 (I)). Morover, the pH range used (8.5 to 11.5) favors dissociation of the AB-11AS subunit, thus facilitating the formation of β-7S/B-11S aggregate), (Petruccelli& Añón,1995).

Isolates containing a higher content of denatured proteins and with lower solubility exhibited a higher capacity for water absorption (Table 2). Eight percent dispersions of these samples with the exception of samples 5 and 11 also showed viscosity values higher than those corresponding to isolates which underwent less severe thermal treatments. Sample 11, treated at 95ºC contained a certain amount of native glicinin (ΔH_T: 1.88 J/g – Table 2) and a vey slight solubility in water. The presence of undenatured 11S protein in the insoluble fraction would appear as a decrease of WIC (Sorgentini *et al.*, 1991). On the other hand,

conditions employed to obtain this isolate sample (high solids concentration, high pH and high temperature) could lead to the formation of more compact aggregates, producing lower viscosity. The same considerations may be extended to sample 5. These results are in agreement with those previously reported for both commercial soybean protein isolates and isolates at the laboratory scale (Sorgentini *et al.*, 1991; Petruccelli & Añón, 1994 (I); Petruccelli & Añón, 1994 (II); Wagner & Añón, 1990).

González *et al*. (1995) had previously shown that the viscosity, measured at a 270 s^{-1} velocity gradient of dispersions of different protein concentrations of soybean isolates could be expressed as: $\eta_{app} = A.C^m$, "m" being linearly correlated with the temperature of the thermal treatment. This type of relationship has been applied to many food systems containing hydrated particles (Holdsworth,1971).

Wagner *et al.* (1992) reported that the viscosity of soybean protein isolates dispersion could be expressed by the power law and that furthermore, it may be expressed by an equation of the type

$$\eta_{app} = \eta_0 + a[P]^n \qquad [2]$$

where η_{app} is the initial viscosity, [P] is the concentration of the protein dispersion and a and n are constants whose values depend on the nature of the polymer, the solvent and the temperature. This relationship allowed them to make evident the transition from a quasi-newtonian behaviour to a pseudoplastic one whenever the protein concentration increased. This transition was evident by the abrupt slope change. The change occurred at a protein concentration of approximately 3-4 %.

Analysis of the possible relationships existing between the evaluated properties of the isolates indicates good linear correlation for some of them and power law type correlation for others (Figures 3, 4, 5, and 6).

Figure 4 shows negative correlation between WIC and Sol H_2O (r = -0.84) and a significant increase was observed of WIC when Sol H_2O decreased below 40. As it was mentioned before the WIC was directly related to the degree of desnaturation, so solubility was a good indicator of degree of desnaturation.

Similarly there was a good negative correlation between the solubility in water and coefficient "m" (r = 0.97) (Fig 5).

Taking into account that solubility decreases with increasing temperature of the thermal treatment – therefore with an increase of the degree of denaturation undergone during treatment- it may be concluded that, for the group of samples analyzed, there existed a good correlation between "m" and ΔH as measured by DSC (r =-0.914) (Fig 6). It could be also noticed that all isolates that underwent mild thermal treatments - in addition to higher ΔH values - showed "m" values below 3. The value of this coefficient "m", associated to the hydronamic behaviour of the system, might be a good parameter to estimate the degree of protein desnaturation, as well as of the degree of particle-particle interaction. It should be emphasized that, although the system studied consisted of macromecules having complex quaternary structures, a behaviour parallel to those of models of macromolecules in solution could be thought (Rha & Pradipasena, 1986).

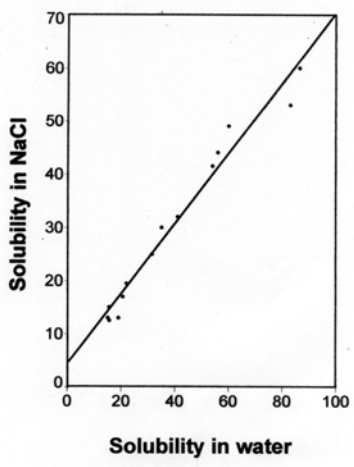

Figure 3 - Relationship between solubility in NaCl 0.2 mL/L and solubility in water of the soybean protein isolates under study.

CONCLUSION

The hydration properties of soybean isolates obtained at pilot plant scale and different processing variables showed good correlation between one another and also with the degree of denaturation. For these types of treatments, the water solubility and WIC were good estimators of the protein denaturation degree. The "m" coefficient was an empirical measurement of the particle-particle interactions and it had also a high correlation with denaturation degree.This

coefficient could be considered as a good indicator of the hydrodinamic volume of the particles or aggregates.

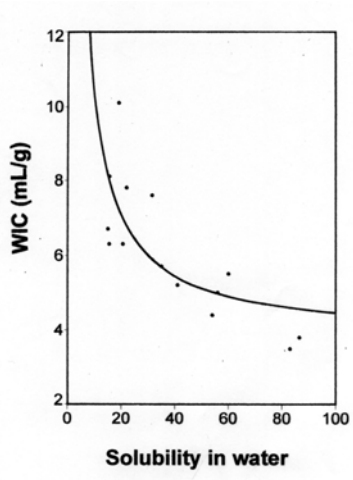

Figure 4 - Relationship between water imbibing capacity and solubility in water of the soybean protein isolates under study.

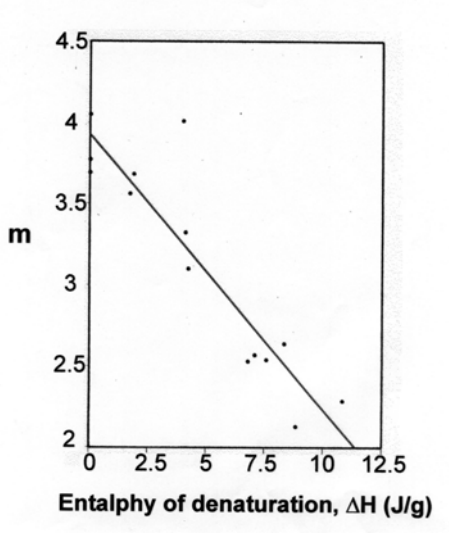

Figure 5 - Relationship between "m" coefficient and solubility in water of the soybean protein isolates studied.

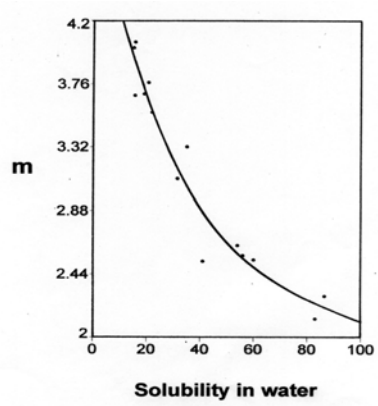

Figure 6 - Relationship between "m" coefficient and entalphy of denaturation of the soybean protein isolates under study.

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RESUMO

Estudaram-se as propriedades de hidratação de isolados de soja com diferentes condições de procesamento (tratamentos térmicos, pH e concentração de proteínas). Para diferentes amostras determinaram-se e correlacionaram-se o gráu de desnaturalização, a solubilidade em água, em 0,2mol/L NaCl e em diferentes concentrações de dodecil sulfato de sódio, viscosidade e capacidade de absorção de água. Os tratamentos a temperaturas superiores aos 80°C desnaturaram as fracções 11S e 7S, provocando a exposição de grupos hidrofóbicos os que produziram agregados insolúveis, em água como em solução com alta força iónica. Estes isolados posuiam alta capacidade de absorção de água e dispersões com alta viscosidade. Achou-se uma correlação significativa entre as propriedades de hidratação e o coeficiente "m", calculado através da função de potência que relaciona a viscosidade com a concentração proteica da dispersão. Este coeficiente "m" também correlacionou com a entalpia dos isolados. Sobre a base destes resultados poderia-se sugerir que o coeficiente "m" – dependente do comportamento hidrodinâmico das partículas – foi um bom estimador do grau de desnaturação proteica.

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