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Water uptake by dehydrated soy protein isolates Comparison of equilibrium vapour sorption and water imbibing methods

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Abstract

Two forms of the hygroscopic behaviour of soybean protein isolates (SPI): liquid water imbibing capacity (WIC) and equilibrium vapour adsorption were tested in non-dialysed native (N) and denatured (D) samples, and in corresponding dialysed isolates (ND and DD). Water activity (a_w) was measured by the optical condensation dew point method. The GAB model adequately predicted the SPI experimental sorption isotherms. The greater number of polar groups exposed during denaturation along with the salt-induced protein aggregation cause the stronger adsorption on D isolate compared with N. No differences between the isotherms up to very high a_w were observed between ND and DD isolates. WIC of isolate D was greater than in N and this correlated with higher moisture adsorbed in equilibrium. Denatured non-dialysed isolates may be stored safely at higher moisture contents, and constitute a suitable ingredient for processing nutritionally-enriched formulated foods requiring high water retention.

Keywords: Soybean protein isolates; Protein denaturation; Functional foods; Water vapour sorption; Water activity; Water imbibing capacity

1. Introduction

Nowadays, soybean protein isolates (SPI) are included in a wide variety of formulated foods because of their desirable functionality, high nutritional value and healthy properties. They are "functional foods" and can also be considered as having nutraceutical properties (Zind, 1998; Hasler, 1998; Riaz, 1999; Milo Ohr, 2000).

Modifications of soybean proteins allows newly formulated foods to be prepared with improved functional properties. For instance, changes induced in the denaturation degree and aggregation state of proteins are reflected both in solubility and water imbibing capacity (WIC) that is, the spontaneous uptake of liquid water (Hermansson, 1977). The WIC is an important index for evaluating their behaviour as ingredient in diverse foodstuffs. It has been shown that the WIC affects not only processing conditions but also final product quality (Pilosof, 2000).

The different treatments applied during SPI extraction and/or processing cause physical and chemical changes in the protein (Wagner, Sorgentini, & Añón, 2000; Puppo, Sorgentini, & Añón, 2000). The structure of isolates would be modified by several treatments to produce suitable functional properties, yielding improved isolates that can be used as ingredients in the food industry. It is well known that thermal treatments induce dissociation, denaturation and aggregation of both soy protein subunits (7S and 11S) (hermansson, 1978; Yamauchi, Yamagishi, & Iwabuchi, 1991), whereas acid treatments lead to denaturation, selective dissociation and unfolding of 11S with minimal protein aggregation (Wagner, Sorgentini, & Añón, 1996; Puppo & Añón, 1999). Thermal-acidic treatments introduce additional modifications such as hydrolysis and deamidation (Wagner & Gueguen, 1995). After neutralisation, a thermal-acidic treated soy isolate produce dispersions of elastic behaviour due to the negatively charged high

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molecular mass protein aggregates, which tend to form a structured dispersion. The presence of these aggregates, responsible for most of the WIC of isolates, shifts the WIC to high values and markedly decreases isolate solubility, producing noticeable changes in viscosity and viscoelastic properties (Puppo et al., 2000).

On the other hand, water activity (a_w) is known to be a fundamental parameter for the stability of foods. For instance, values <0.7 facilitate, from the microbial stability point of view, long term shelf life at ambient temperatures (Cheftel, Cuq, & Lorient, 1993). So, for that reason, the moisture content at $a_w = 0.7$ is generally termed 'safe moisture content'. These values are well-known for cereals, oilseeds (Brooker, Bakker-Arkema, & Hall, 1992) and many foods and foodstuffs (Chirife & Buera, 1994) but scarcely widespread for protein isolates (Hermansson, 1977; Chirife, Timmermann, Iglesias, & Boquet, 1992; Timmermann, Chirife, & Iglesias, 2001) and soy proteins in particular. Knowledge of safe moistures for protein isolates would be useful as contribution for shelf-life information while the measurements of isotherms, the meaningful relationship between the water or moisture content and the water activity at constant temperature, may assist to identify the state of the water associated to the proteins and the more suitable models for sorptional equilibrium. Implications of the equilibrium sorption behaviour are physicochemical phenomena as the vapour pressure of water present in foods, microbiological stability, structural aspects, the chemical stability map (Barbosa-Cánovas & Vega-Mercado, 1996), and technological characteristics, especially its effect on processing time, for instance during spray drying. In this regard, the sorption behaviour permits the driving force available for drying to be determined as the difference of water vapour pressures in isolate and drying air, as well as the energy demand for the operation. The latter feature is based on the heat required to desorb the moisture from the isolate. The safe moisture content concept mentioned above is also used as the drying endpoint so it directly affects drying time.

Comparison between the WIC and the equilibrium vapour sorption of different proteins was studied by different researchers (Hermansson, 1977; Elisalde, Pilosof, & Bartholomai, 1996). No information on the relationship between WIC and equilibrium water sorption expressed as sorption isotherms was found in modified SPI. For this reason, the objective of this work was to compare two forms of the hygroscopic behaviour of native and denatured soy protein isolates, either dialysed or not: WIC, and vapour sorption. A second objective is related to verifying the suitability of the two-stage hygrometric method to determine water activity: the first stage consists of generating samples of different water activity (a_w) and the second is to measure the a_w in a hermetic chamber by the hygrometric method based on the optical determination of the dew point temperature (General Eastern Instruments, 1994). The two-stage hygrometric method should take less time than the gravimetric method, and does not require mathematical extrapolation to find the equilibrium weight of the sample, as in the so-called 'dynamic vapour sorption' (DVS) method (Teoh, Schmidt, Day, & Faller, 2001).

2. Materials and methods

2.1. Preparation of soybean protein isolates

The soy protein isolates used in this study were prepared from defatted soyflour provided by Santista Alimentos, S.A (Brazil). Native isolates (N) and those denatured by acidic (pH 1.6) thermal treatment at 90 °C for 30 min (D), were obtained by the following laboratory procedure (Puppo et al., 2000).

The defatted soyflour was dispersed in water (1:10 w/v) and brought to pH 8. After centrifugation at 7000 g for 30 min, the supernatant, which was the part retained, was conditioned to pH 4.5 using HCl, so the proteins are precipitated in their isoelectric point and separated by centrifugation. This precipitate was resuspended in water, and brought to pH 8 at a protein concentration of 5% w/v. One part of this solution was frozen (N) and a second part thoroughly dialysed against water (ND). A third part of the solution was used to obtain denatured isolates: the dispersion was brought to pH 1.6 with HCl, then heated to 90 °C for 30 min and subsequently conditioned to pH 8 and frozen (D). Part of this isolate was thawed and thoroughly dialysed against water (DD). All prepared samples were then freeze dried and stored until used. To prepare the starting material for the adsorption experiments, the porous freeze-dried isolates were ground with a mortar, screened and subsequently dehydrated further in vacuum oven set at 40 °C for 10 days, using P_2O_5 as desiccant.

2.2. Testing the thermal behaviour of soy protein isolates

The extent of thermal denaturation was evaluated by differential scanning calorimetry (DSC) measurements (Puppo & Añón, 1999) in a Polymer Laboratories, Rheometric Scientific (UK) DSC calorimeter. The equipment was calibrated at a heating rate of 10 °C/min, using indium, lauric acid and stearic acid as standards. Tests were performed in hermetically-sealed aluminium capsules, where sample and reference were gradually heated between 30 and 120 °C, at 10 °C min^{-1} heating rate. Samples consisted in aqueous dispersions at 20% w/v of protein isolates. Capsules were punctured after each run and kept overnight in a conventional oven to determine dry matter content. Denaturation temperature (Td) and transition enthalpy (ΔH) were obtained by

analysing the thermograms with a Software Plus V5.41 (UK).

To study the relationship between structure-function and water absorption properties, the denaturation degree of protein isolates, studied in a previous work (Puppo et al., 2000) was experimentally verified. Native isolate (N) presented its two typical endotherms corresponding to 7S at 78.9 °C and 11S at 94.2 °C with a denaturation enthalpy equal to 16.4 J/g dry isolate, a typical value for fully native isolates (Puppo & Añón, 1999). No endotherm was observed for the denatured isolate (D), which reflects a complete denaturation state induced by the thermal-acidic treatment.

2.3. Water imbibing capacity

The WIC of all isolates was measured in a Baumann apparatus according to a method modified by Wagner et al. (1996). The method determines the spontaneous uptake of liquid water by a protein powder at a given temperature. Wetted filter paper is placed on a funnel connected to a graduated water pipette filled with distilled water. A 30 mg sample of dehydrated isolate was screened and placed as thin layer on the filter paper while closing the funnel mouth with a hermetic lid. For each material, the water uptake over time was recorded at 20 °C until equilibrium was reached. Practical equilibrium conditions were attained when uptake readings in the plateau zone repeated within 0.01 ml (Elisalde et al., 1996). This criterion was used to define the WIC, the asymptotic amount of liquid water (ml or g) absorbed from the pipette per g of original isolate, and the approximate imbibing time (t_i), the shortest period elapsed that comply with the equilibrium criterion.

2.4. Preparation of samples with various water contents for equilibrium vapour sorption

2.4.1. Stage 1. Vapour adsorption by the isolates

To bring the totally dehydrated isolates to different water contents, the procedure used was vapour sorption. The method was based on placing one native and one denatured sample, 1 g each, together with a saturated salt solution of given a_w. The saturated salt solutions were used as means to moisten the isolates very slowly by vapour adsorption, but, as true equilibrium takes a prohibitive length of time, moistening times were limited from 7 to 10 days where samples were close to the equilibrium asymptote. After these moistening period, the native and the denatured sample were in equilibrium one another but not necessarily reaching the a_w of the salt. The following saturated salt solutions were used at 20 °C to condition the samples (Lang, McCune, & Steinberg, 1981; Giner, 1999): LiCl, $a_w = 0.11$; $K(CH_3COO)$, $a_w = 0.23$; $MgCl_2$, $a_w = 0.33$; K_2CO_3, $a_w = 0.44$; $NaNO_2$, $a_w = 0.65$; NaCl, $a_w = 0.75$; K_2CrO_4, $a_w = 0.86$; $BaCl_2$, $a_w = 0.91$ and K_2SO_4, $a_w = 0.97$. The true a_w reached by the samples were measured by the hygrometric method, as described next.

2.4.2. Stage 2. Hygrometric, dew point-based determination of a_w in the samples

Isolate samples N, ND, D and DD conditioned by vapour adsorption to different water contents as mentioned in stage 1, were removed from the conditioning containers and rapidly transferred under isothermal conditions to a thermostatised chamber placed in a constant temperature room set to 20 °C. The dry bulb temperature (T_{db}) inside the chamber was measured with a Cu–Ct thermocuple covered with a protective copper shield and connected to a Solomat MPM 2000 Module for continuous monitoring. The air dew point temperature (T_D) in the chamber was measured by an optical condensation chilled mirror dew point meter (General Eastern Instruments, 1994), using a 1111 *H–SR* sensor connected to a Hygro M4 console with continuous display of the measured value. After reaching a stable reading of T_D, the relative humidity (rh) of the air over the sample, assumed equal to the a_w of the isolate, was calculated as $\mathrm{rh} = p_s(T_D)/p_s(T_{db})$, were p_s was the saturation vapour pressure of water. Even for a dry bulb temperature T_{db} of 20 °C, dew point values at low water activities may be below the freezing point (i.e, 'frost points'), so two correlations of p_s, one above liquid water

$$p_s = 100 \times 6.1121 \exp\left[\frac{17.502T_c}{240.97 + T_c}\right] \quad (1)$$

and one above ice

$$p_s = 100 \times 6.1115 \exp\left[\frac{22.452T_c}{272.55 + T_c}\right] \quad (2)$$

should be available. In both equations, T_c is the temperature in °C, and p_s is given in Pa (General Eastern Instruments, 1994).

2.5. Determination of water content

Moisture content (W) of protein isolates was determined by dehydration in an atmospheric oven set at 130 °C and kept until constant weight.

2.6. Solubility

Isolates were dispersed in distilled water to a concentration of 1% w/v for 1 h at 30 °C, under periodic stirring, and then centrifuged at 18,000 × g for 30 min. The supernatant was the part retained to determine soluble protein by the Biuret method (Wagner et al., 2000).

3. Results and discussion

3.1. Hygroscopic behaviour of isolates

3.1.1. Water imbibing capacity

Fig. 1 shows the kinetics of liquid water absorption by the dry isolates. Concerning the effect of denaturation in dialysed and non-dialysed samples, the water uptake by denatured isolates is observed to be greater than in corresponding native samples; the cause of this may be attributed to the protein unfolding caused by denaturation, which exposes more water-binding sites. Both in non-dialysed and in dialysed samples, denaturation increase the WIC by around 4 g water per g isolate. These binding sites are stronger in the presence of salts: the WIC of non-dialysed isolates, i.e. of samples whose salts were not removed, is some 2 g water/g isolate above those in dialysed isolates. In any protein isolate, however, the WIC asymptote should always correspond to high water activities; for instance the lower WIC, corresponding to the dialysed native sample, is close to 2 g water/g isolate, that is, a proportion of about 60% w/w of water. This spontaneous uptake, absorption or retention of water combines sorption characteristics, i.e, the attachment of water molecules to specific low-energy active sites of individual isolate powder particles with other physicochemical properties as capillarity and surface tension. Moreover, the uptake may even be related with powder properties as particle size and void fraction, as well as the degree of powder packing. Therefore, values of WIC may include some loosely held water, placed in interstitial spaces between particles.

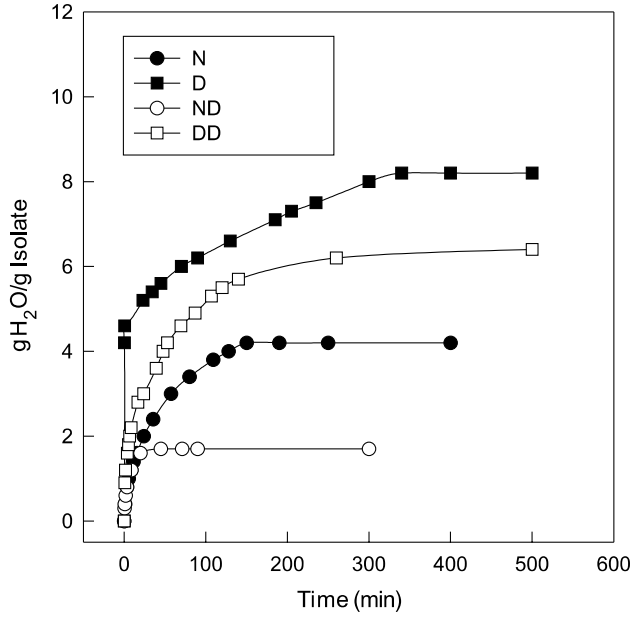

Fig. 1. WIC of SPI Non-dialysed isolates: native, N (●), denatured, D (■). Dialysed isolates: native, ND (○), denatured, DD (□).

3.1.2. Equilibrium vapour adsorption

The experimental vapour adsorption isotherm of native non-dialysed isolate obtained by the hygrometric method for measuring a_w by the optical condensation dew point meter, was in agreement with those gravimetrically measured by Hermansson (1977) for commercial soy protein isolate (Promine-D) (Fig. 2).

Fig. 3 show the adsorption isotherms of non-dialysed native and denatured isolates while Fig. 4 exhibits those for dialysed isolates. In Fig. 3, the experimental data indicate negligible moisture content differences in the low a_w range. For water activity values above 0.5, however, the moisture content adsorbed in denatured isolates is observed to be consistently higher than in native samples. On the moisture content at $a_w = 0.7$, also called 'safe' moisture content from the microbial stability viewpoint, Fig. 3 shows that denatured isolates may be stored at higher moistures than native ones, so the drying requirements would be lower for the former. A practical criterion for evaluating differences at the same a_w can be proposed as the relationship between the oven moisture determination error and the difference of adsorbed moistures between denatured and native isolates. Such ratio falls below 10% for $a_w > 0.64$, keeps below 2% for $a_w > 0.7$ to reach almost zero at the highest a_w measured (i.e, 0.92). The sorptional behaviour in Fig. 4, i.e, dialysed isolates, show negligible differences between the two isotherms up to higher a_w values compared with non-dialysed samples. Notwithstanding, for the two highest a_w measured, i.e., 0.91 and 0.92, the criterion mentioned above is met so the mois-

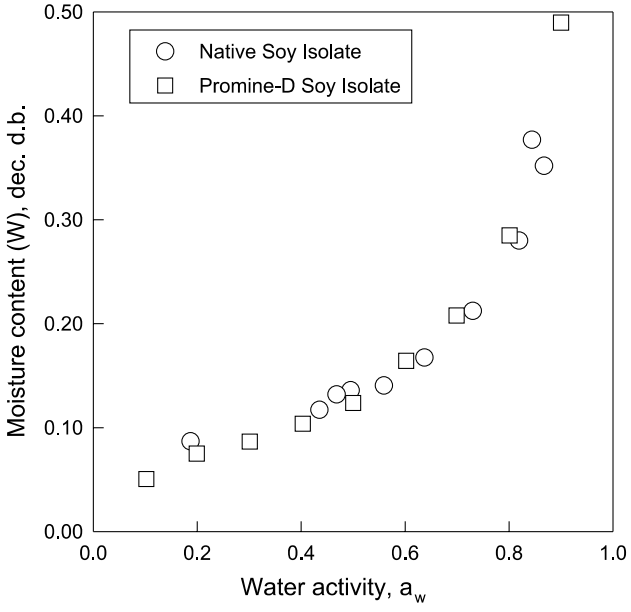

Fig. 2. Experimental data of moisture content vs. water activity measured at 20 °C of non-dialysed native soybean isolate from this work, N (○) and of Promine-D soybean isolate from Hermansson (1977), (□).

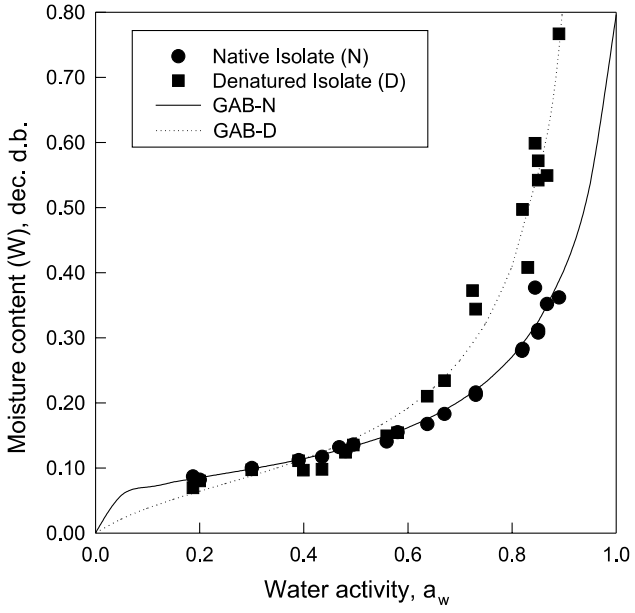

Fig. 3. Water vapour sorption isotherms (20 °C) for non-dialysed isolates: native, N (●) and denatured, D (■). GAB model curves: (—) N, (- - -) D.

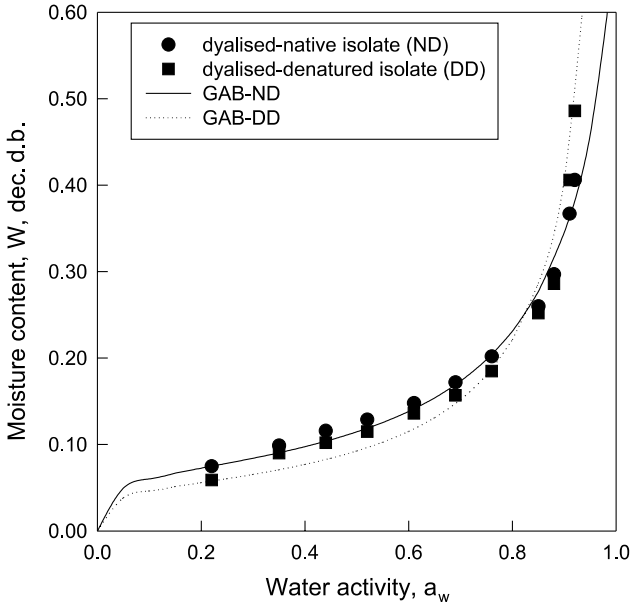

Fig. 4. Water vapour sorption isotherms (20 °C) for dialysed isolates: native, ND (●) and denatured isolate, DD (■). GAB model curves: (—) ND, (- - -) DD.

ture adsorbed by denatured isolates is again higher. Consequently, the results of WIC from Fig. 1 and those of equilibrium vapour adsorption, Figs. 3 and 4, are congruent: higher WIC corresponds to higher moisture adsorbed in equilibrium. However, more experimental data on vapour adsorption is required to obtain conclusive evidence on this behaviour from the statistical point of view.

Hermansson (1977) has worked with different proteins (soy protein isolate, milk whey concentrate and sodium caseinate) and, for low and intermediate water activities (a_w between 0.2 and 0.6), has also found that isolates of higher WIC positively correlated with vapour sorption. In our work, measurements were performed over a wider a_w and moisture contents ranges compared with the work of Hermansson.

In order to apply a physicochemically-based model to the experimental values of adsorbed data, water content (W) vs a_w, the GAB expression was selected, which predicts:

$$W = \frac{W_m C_G k_G a_w}{(1 - k_G a_w)(1 - k_G a_w + C_G k_G a_w)} \tag{3}$$

the parameters being: W_m, monolayer moisture content (decimal, dry basis), a value for which all adsorption active sites are covered by one water molecule; C_G and k_G, dynamic equilibrium sorption parameters. In particular, as k_G values approach unity, the GAB model tends to its simpler version, the two-parameter BET model (Gregg & Sing, 1967; van den Berg, 1985), and higher values of moisture are predicted at high a_w. The parameters obtained, as well as the goodness of fit indicators, are listed in Table 1. For each isolate sample, the GAB model behaves very well with high coefficients of determination (r^2) and low standard deviations of the estimate, s_y. The value of s_y, given in moisture content units, was calculated as follows:

$$s_y = \sqrt{\frac{\sum_{i=1}^{N} (\text{predicted}_i - \text{experimental}_i)^2}{N - p}} \tag{4}$$

where N is the number of experimental data points and p the number of fitting parameters ($p = 3$ in GAB). The results show, therefore, that GAB model assumptions of multilayer physical adsorption in non-porous adsorbents, functionally represents the vapour adsorption characteristics of protein isolates in the a_w range

Table 1
GAB constants fitted at 20 °C for water vapour sorption in soy protein isolates

Isolate type	W_m dec., d.b.	C_G	k	r^2	s_y dec., d.b.
N	0.075 ± 0.006	60.0 ± 144	0.906 ± 0.019	0.978	0.016
D	0.099 ± 0.021	3.136 ± 2.672	0.979 ± 0.029	0.971	0.040
ND	0.064 ± 0.004	60.00 ± 67.87	0.906 ± 0.012	0.991	0.012
DD	0.048 ± 0.005	60.00 ±. 98.51	0.970 ± 0.015	0.976	0.024

measured here. Being $k_G < 1$ the sorption behaviour of adsorbed multilayers is intermediate between those of pure water and monolayer moisture (Timmermann et al., 2001). The k_G of denatured isolates (Table 1) are higher than for natives, and closer to unity, reflecting the tendency of denatured isolates to adsorb more moisture at high a_w. On a different point, values of $C_G > 2$, as in Table 1, indicates that the isotherm is sigmoid. Gely and Giner (2000) have deduced that values of $C_G > 20$ are less relevant to predict the shape of sigmoid isotherms because, mathematically, they tend to drop out from the GAB equation. This is reflected by the high uncertainty of C_G values in three out of the four isolates listed in Table 1, despite the high r^2 and low s_y found in all cases.

The monolayer moisture content (W_m) was higher in non-dialysed isolates than it was in dialysed ones because of the stronger water adsorption caused by the presence of salts (Table 1). Concerning the comparison of denatured (D) and native (N) non-dialysed isolates, the greater moisture adsorption observed at high water activities by the isolate D is congruent with its higher W_m, an adsorption parameter of the low a_w zone. In dialysed isolates, however, such improved adsorption properties of the denatured isolate (DD) are much less notorious being, as mentioned above, observed only at the two highest a_w measured. For this reason, W_m values of DD and ND isolates are comparable. In all isolates, the order of magnitude of W_m values are within the range reported by Timmermann et al. (2001) for proteins. In this regard, these authors have found a correlation between the GAB monolayer moisture content and the number of polar groups for various proteins. If this correlation is used here for the results of Table 1, it would be said that isolate D has more polar groups exposed to water than isolate N. The word "exposed" was written deliberately because some polar groups in isolate *N* may not be accessible for water sorption due to steric constraints. The denaturing thermal acidic treatment creates an enhanced surface water adsorption structure. The amount of water linked by a protein shall depend on its aminoacid composition, of the number of exposed polar groups, on protein conformation and of surface polarity (Kinsella, Whitehead, Brady, & Bringe, 1989). On the other hand, non-covalent bonds stabilise secondary and tertiary structures, and heat-induced denaturation leads to rupture of such bonds. This process takes place in various ways, for instance heating may cause dissociation of a complex macromolecule followed by partial unfolding, so the amount of sorbed water can increase (Cheftel et al., 1993).

3.2. Comparison of water imbibing and vapour adsorption in soy protein isolates

To compare equilibrium vapour adsorption and uptake of liquid water, the moisture adsorbed at several water activities (0.4, 0.7, and 0.9) is shown in Table 2, together with solubility, WIC and an approximate imbibing time (t_i), as defined in 2.3. Reverting to equilibrium vapour adsorption, denatured non-dialysed isolate (D) adsorb more moisture at intermediate and high water activities. In dialysed proteins (DD) such differences occur to a lesser extent and is only noticeable at very high a_w. Therefore, the presence of salts is observed to amplify the differences of sorption behaviour between denatured and native isolates. Moreover, in Table 2, the safe moisture content ($a_w = 0.7$) is higher in D, so these isolates have the improved functional property of absorbing more water (higher WIC) and, by the same mechanism, could be stored with more retained water still keeping their microbial stability. In fact the safe moisture content of isolate D is of 0.265 dec., d.b., much higher than in cereals (Brooker et al., 1992). To illustrate the difference, data from a survey by Sun and Woods (1993) were used here to average the safe moisture content of whole wheat grains used in breadmaking. The result was 0.168 dec., d.b., clearly below the value for isolate D. Salts affect the forces involved in protein–protein or protein–water interactions (Kinsella, 1984), because they do not only adsorb water, but also affect protein structure by interacting with the proteins themselves and/or with the water that surrounds the protein (Damodaran & Kinsella, 1982). Protein structure and their solubility are affected by salt ions both by electrostatic interaction of ions with charged or polar groups of protein and by altering hydrophobic forces and so the water structure associated to the protein. The removal of salt during dialysis exposes less hydrophilic

Table 2
Moisture content (W) by equilibrium adsorption of soy isolates for various water activities (a_w), solubility (S), and WIC after an imbibing time (t_i)

Sample	W, dec., d.b.			S%	WIC kg water/kg isolate	t_i (min)
	Low ($a_w = 0.4$)	Microbial stability ($a_w = 0.70$)	High ($a_w = 0.9$)			
N	0.1143	0.2031	0.4048	78	4.20	150
D	0.1139	0.2654	0.8372	47	8.20	340
ND	0.0975	0.1733	0.3454	nd	1.70	45
DD	0.0770	0.1517	0.4059	nd	6.40	260

nd: not determined.

groups, promoting the formation of aggregates with lower WIC (Damodaran & Kinsella, 1982), as verified here in Fig. 1. A mechanism of increasing water uptake by proteins was proposed by Hermansson (1977). During protein hydration, voids created between molecules would give rise to high intermolecular forces that reduce their surfaces. At low a_w unfolding and crosslinking of proteins enlarges the exposed interfaces that increase the driving force for water sorption, and then capillarity and osmotic forces would lead to swelling. These effects continue at very high a_w until protein is fully solvated.

Previous work has shown that thermal-acidic denaturation imparts the isolate higher surface hydrophobicity (H_0), lower solubility (S) and greater WIC compared to the native isolate (Puppo et al., 2000). Petruccelli and Añón (1994) have also found that isolates of higher WIC, also present lower solubility. Table 2 also shows that the isolates of higher WIC present a higher amount of adsorbed water in equilibrium at high a_w, so a good correlation is observed between these static equilibrium and dynamic water sorption methods. Our results, substantially coincident with those observed by Hermansson (1977) in Promine-D-soy isolate, and in other proteins as caseinate and whey protein concentrate, show that the greater the WIC, the greater the equilibrium adsorption reached at high a_w. On the other hand, solubility was lower in non-dialysed denatured isolate (D) than it was in native (N), because of protein aggregation induced by the thermal treatment.

4. Conclusions

The soy protein isolates prepared by diverse treatments showed distinct hydration characteristics. The WIC of denatured soy protein isolates was between two and three times greater than in native samples, both for non-dialysed and dialysed samples. In turn, the presence of salts in non-dialysed samples also increased the WIC (by 50–100%) though not as strongly as denaturation.

The determination of a_w by the dew-point based hygrometric method did not require calibration, was accurate and faster to construct equilibrium vapour adsorption isotherms compared to the gravimetric method.

The experimental adsorption isotherms, sigmoid in all samples, were very similar in the low a_w range. For $a_w > 0.5$, however, the isotherms of denatured non-dialysed isolates departed upwards, showing stronger water-retention characteristics compared with the corresponding native samples. In dialysed isolates, this difference was found only at the two highest a_w values measured (around 0.9). The GAB sorption model was very accurate in all cases, and, for denatured non-dialysed isolates, the monolayer moisture content was higher, possibly in congruence with its higher number of exposed polar groups.

Non-dialysed denatured isolates are likely to permit storage at ambient temperatures ($a_w = 0.7$) with moisture contents of about 0.25 d.b., and this potential for reducing the energy load during isolate drying should be investigated further. In turn, this type of isolate can be used in nutritionally-enriched formulated foods which require high water retention capacity.

The denaturation degree and the salt content strengthened the hydration properties of soy isolates, and this was so both for liquid water uptake and equilibrium vapour adsorption. Denatured isolates adsorbed more water (WIC) than the native because of the higher number of groups exposed during unfolding. This effect was stronger in the presence of salts owing to a more intense protein–protein interaction leading to an aggregation state of the protein matrix.

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