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Physical and Chemical Changes Resulting from Heat Treatment of Soya and Soya Alginate Mixtures

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SUMMARY

The effect of water content and hydrocolloid inclusion on the denaturation behaviour and water binding properties of soya protein has been investigated mainly by using differential scanning calorimetry. The most interesting observation made was that when soya isolates containing high mannuronate alginates are heated there is a substantial increase in the amount of volatiles (probably water) formed. The dependence of this effect on water activity (a_w) suggests that it is the result of browning or condensation reactions. This effect is not seen to the same extent with other added hydrocolloids and may be the reason why alginate addition reduces the viscosity of the soya 'melt' during soya extrusion. The inclusion of high mannuronate alginate appears to raise the onset denaturation temperature of the 7S globulin and at low water contents reduces the heat change associated with the transition. It is possible that the former effect may be associated with the production of water during heating, which makes it difficult to define the water content at which denaturation is taking place. Measurements of freezable water and sorption isotherms suggest that alginate addition increases the water binding ability of soya isolate after denaturation.

INTRODUCTION

It is well established that the addition of alginates containing a high proportion of mannuronate residues decrease torque and product expansion during soya extrusion (Smith *et al.*, 1982; Boison *et al.*, 1983; Berrington *et al.*, 1984; Imeson *et al.*, 1985). These effects can be explained by a reduction in the viscosity of the soya melt on alginate addition. The objective of the investigation described in this paper was to establish the mechanism for this viscosity reduction. To achieve this we have investigated the protein–water relationships in heated soya systems in the presence and absence of added polysaccharides.

Relatively little information is available about the physical and chemical changes that occur when proteins are heated at the low moisture contents encountered in extrusion cooking. It is, however, well-established that the denaturation characteristics of proteins are very dependent on the moisture content of the system. The temperature of denaturation invariably increases with decreasing water content whereas the enthalpy of denaturation (ΔH_D) generally decreases (Hagerdal & Martens, 1976; Ruegg *et al.*, 1975; Fujita & Noda, 1981; Sheard *et al.*, 1986). Tropocollagen, however, is unusual as it exhibits an increase in ΔH_D as the water content decreases from 60 to 28% wet sample basis (w.s.b.), although a further reduction in water content causes a rapid decrease (Luesher *et al.*, 1974). The 7S and 11S globulins of soya exhibit the expected increase in stability with decreasing water content (Sheard *et al.*, 1986), but the dependence of ΔH_D on moisture content is not known.

It is known that proteins can undergo several types of degradative and condensation reactions at elevated temperatures, the reactions being most rapid at low moisture contents (Ledward, 1978). Although the conditions necessary to bring about these changes are far more severe than those for protein denaturation, they may well be achieved during the extrusion processing of soya. The volatiles, including water, that are generated could significantly modify the rheology of the melt. This is an aspect of extrusion behaviour that has received little attention.

In this paper, differential scanning calorimetry (DSC) is used to monitor the temperature and enthalpy of denaturation of the 7S and 11S globulins in soya isolate with and without added hydrocolloids. The generation of volatiles is measured after heating in the calorimeter and confirmation of the trends found is obtained by Karl–Fischer analysis of separately heated samples. Information about the state of water in the systems is obtained from sorption isotherms and the measurement of the freezing and melting of water by DSC.

MATERIALS AND METHODS

Native soya isolate was prepared as outlined by Hermansson (1978) from a 70% PDI flour. Manucol DM (an alginate with a high mannuronic acid content) and Manugel GMB (an alginate with a high guluronic acid content) were kindly donated by Kelco International Ltd, UK. Xanthan gum, gum arabic, guar gum and pectin were obtained from the Sigma Chemical Co. (UK) and carboxymethyl cellulose (CMC) from BDH Ltd (UK).

Scanning calorimetry

Soya isolate or soya isolate plus hydrocolloid in the ratio of 50:1 were:

(i) mixed with the appropriate amount of distilled water and allowed to equilibrate at room temperature for 24 h; or
(ii) stored over distilled water and removed at various times, up to 3 days; or
(iii) equilibrated over silica gel or solutions of known water activity at 298 K to constant weight.

About 5 g of the dehydrated or hydrated sample were sealed in weighed 'volatile' aluminium pans and the weight accurately determined. The lids of some of the sample pans were punctured and the apparent moisture content of the samples determined by drying *in vacuo* at 343 K for 48 h.

The remaining samples were subjected to calorimetric analysis. The denaturation profiles of the soya proteins were determined, at a scan rate of 5 K min^{-1}, in a Perkin Elmer DSC-2 scanning calorimeter, previously calibrated against indium. The samples were reweighed to ensure no weight loss had occurred and the pans punctured and the apparent moisture content determined by drying *in vacuo* at 343 K for 48 h. The melting profiles of the water associated with the macromolecules were determined at a heating rate of 10 K min^{-1}, after cooling as rapidly as possible to 233 K. Distilled water was used to calibrate the instrument for the freeze–thaw measurement. When all the water had melted the samples were heated at 5 K min^{-1} until all the thermal transitions were complete (temperature 423–443 K). The samples were then cooled again as rapidly as possible to 233 K and the melting transitions re-examined. Following calorimetric analysis, the moisture content of the samples was determined by puncturing the pans and heating at 343 K for 48 h *in vacuo*.

Freeze–thaw and denaturation measurements were performed separately as freeze–thawing appeared to affect the enthalpy of the 7S transition.

Heat treatment of dry powders

About 0·3 g of soya isolate or soya isolate containing 2% polysaccharide was sealed in glass ampoules ($\simeq 2$ cm^3), accurately weighed, and placed in a fan oven at 185°C for 35 min. The samples were allowed to cool to room temperature and, 12–24 h after removal from the oven, their apparent moisture content was determined by Karl–Fischer titration. The ampoules were broken under 10 ml of formamide and stirred for 30 min to ensure complete solubilisation of the powders. Prior to titration with Aqua-Fi titrant, 10·0 ml of Aqua-Fi solvent was mixed with the formamide for 10 min.

Sorption isotherms

Soya isolate, premoistened to about 35% water, was denatured by retorting at 394 K for 30 min in sealed cans. Calorimetric analysis indicated that denaturation was complete.

Native soya isolate, the denatured material and native and denatured soya isolate containing 2% Manucol DM were dried by heating in a vacuum oven at 343 K for 48 h. Samples of approximately 1 g of the dried materials were equilibrated at 298 K over saturated salt solutions of known a_w (Rockland, 1950). Equilibration was complete within 48 h at $a_w < 0{\cdot}90$ and within 9 days at $a_w > 0{\cdot}90$. The moisture content of the equilibrated samples was determined by drying *in vacuo* at 343 K.

All moisture contents are quoted on a wet weight basis, i.e. g H_2O per 100 g wet solids.

RESULTS

Effect of water content

At all water contents the thermograms of the isolate exhibited two major endothermic peaks (Fig. 1): that occurring at the lower temperature was assumed to correspond to denaturation of the 7S globulin and that at the higher temperature to the 11S globulin (Hermansson, 1978). Enthalpies were calculated per unit weight of dry sample. As the 7S globulin only constitutes about 30–35% of the isolate weight, the true values are obviously higher than quoted.

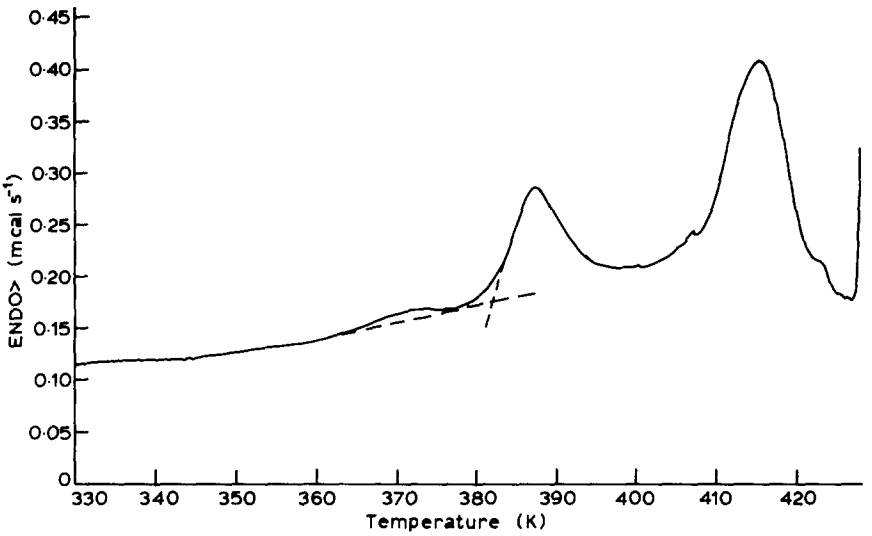


Fig. 1. Thermogram of 12·5 mg of soy isolate, containing 24% water.

The effect of water content on the enthalpy and temperature of the transition, for soya 7S globulin, is shown in Fig. 2. It is seen that there is a rapid increase in T_{onset} with decreasing water content; similar results have been reported by Sheard *et al.* (1986). The enthalpy associated with the transition for the 7S globulin increased with increasing water content up to about 31% water, with a subsequent decrease as the water content increases further to about 55%. At higher water contents the enthalpy was approximately independent of the water content.

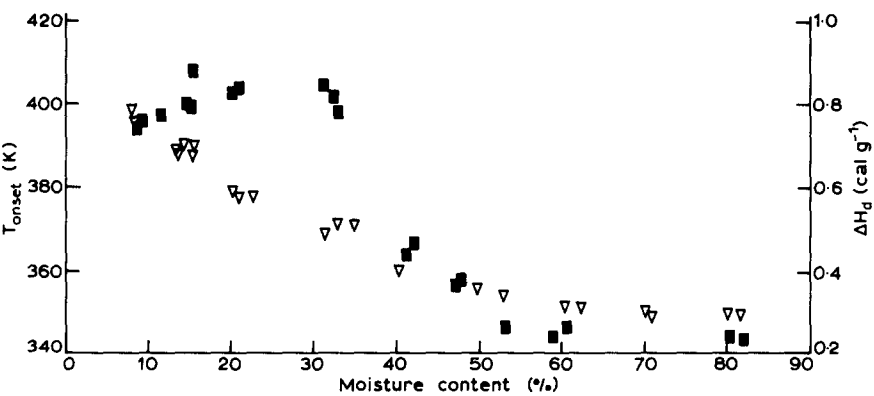


Fig. 2. Heat of transition ΔH_D (■) and temperature onset of the transition (T_{onset}) (▽) of the 7S globulin of soy isolate as a function of final water content (wet weight basis). ΔH_D is quoted per gram of dry sample.

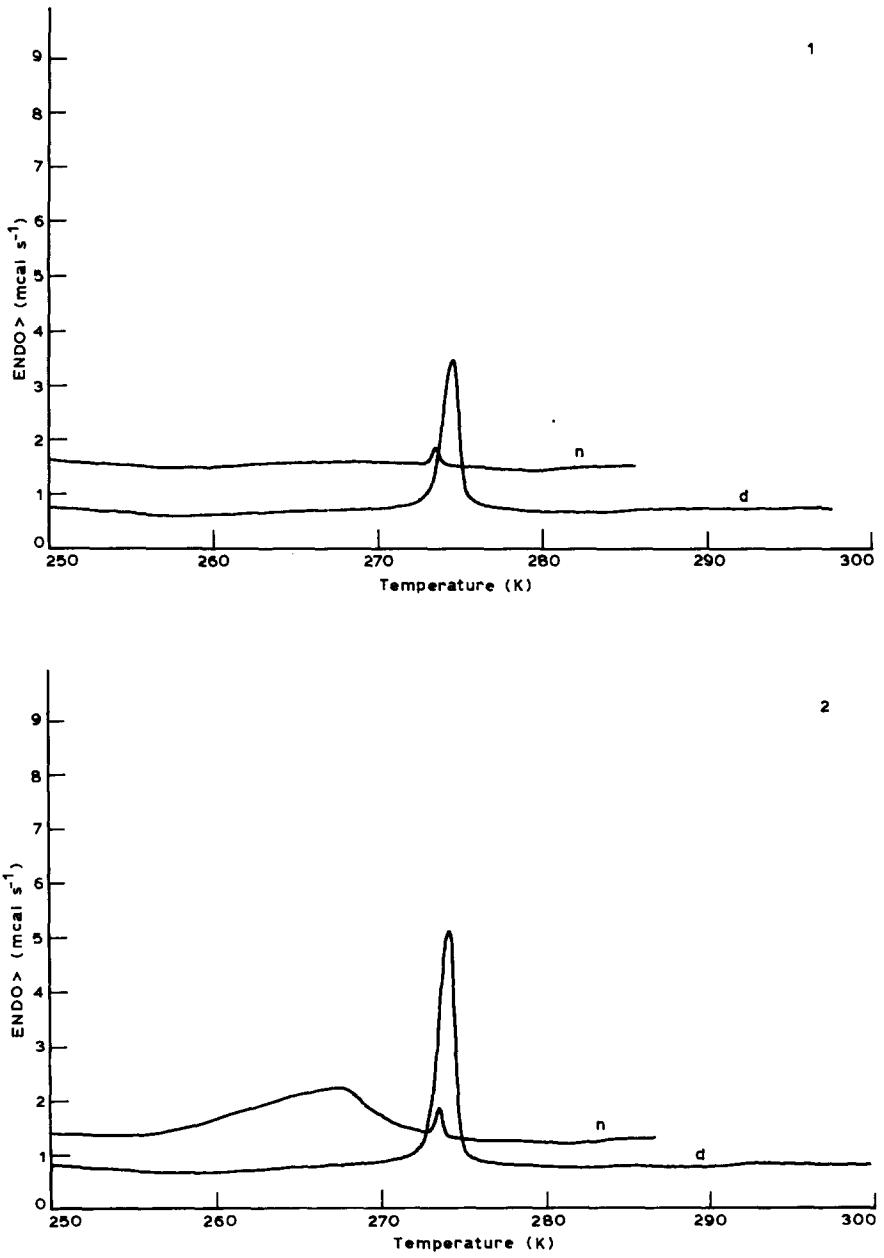


Fig. 3. Typical thermograms of the water associated with soya protein at initial water contents of (1) 38%, (2) 43%, (3) 55% and (4) 65%; sample weights are 7·13, 7·54, 9·66 and 11·48 mg, respectively (n = native protein, d = denatured protein).

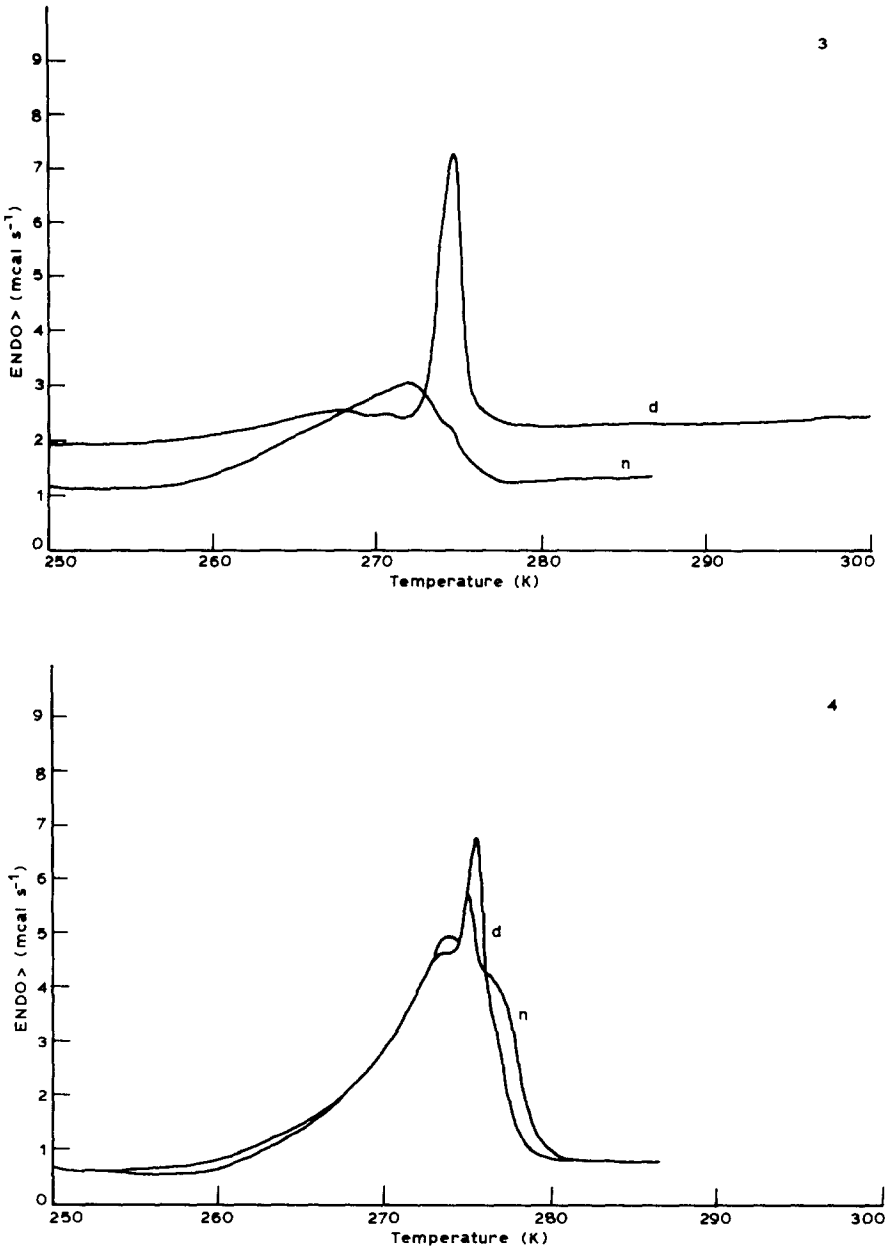

3
ENDO > (mcal s⁻¹)
Temperature (K)
d
n
4
ENDO > (mcal s⁻¹)
Temperature (K)
d
n

Fig. 3 — *contd.*

It was also observed, in agreement with the results of Sheard *et al.* (1986), that the temperature associated with the denaturation of the 11S globulin increased with decreasing water content (T_{onset} increased from 364 to 431 K on decreasing the water content from 81 to 8%). It was not possible to estimate the enthalpy associated with this process, as in many cases the baseline on the thermogram was difficult to determine following denaturation of the 11S globulin (Fig. 1).

Typical thermograms for the fusion of water associated with native and denatured soya isolate over the temperature range 243–310 K are shown in Fig. 3. Below about 30% water no peaks corresponding to water melting were observed, but at higher water contents the native isolate showed two peaks. The sharp peak, with T_{onset} around 273·5 K, corresponds to the melting of the 'free' or bulk water of the system. The broad transition centred around 266·5 K is presumably due to the melting of water whose characteristics are modified by association with the protein. With increasing water content both peaks increased in area, a result also found by Muffet & Snyder (1980). In these native systems a small exothermic peak was consistently present in the thermograms, centred around 280 K (Fig. 3).

Following denaturation, the lower melting peak virtually disappeared and the area of the peak centred at about 273·5 K increased dramatically. This suggests that water is liberated from association with the

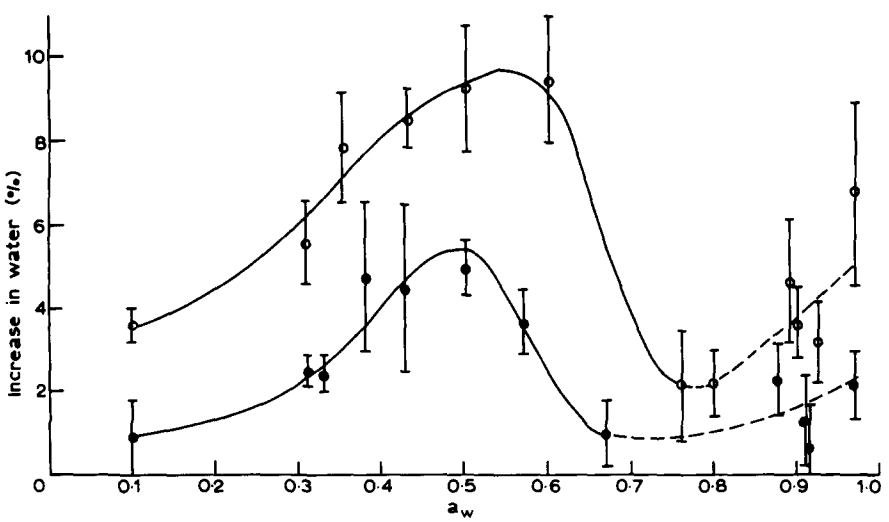


Fig. 4. Increase in apparent water content (determined by drying *in vacuo* at 343 K) as a function of initial water activity (a_w) for soya isolate + 2% Manucol DM (○) and soya isolate alone (●). All values are the means ± standard errors of the differences.

protein following denaturation. No exothermic transition at 280 K was seen in these systems.

Following heat denaturation of soya protein samples there is an apparent increase in the water content of the system when determined by drying at 343 K *in vacuo* (Fig. 4). The increase in 'apparent' water following denaturation is evident over the entire range of water activities examined (0·1–0·97). At 'initial' a_w values above about 0·65 the increase is not significantly dependent on a_w (mean 1·46%), but below this apparent liberation of water increases to a maximum at an a_w of about 0·5. At lower a_w values the magnitude of the increase steadily diminishes (Fig. 4). To ensure denaturation of the 11S globulin, the samples of lower a_w were heated to slightly higher temperatures than those of higher a_w. Bursting of pans due to a build-up of pressure made heating the samples of high moisture to 443 K difficult.

Effect of hydrocolloid inclusion

The effect of water content on the enthalpy and temperature of the transition of soya 7S globulin following the inclusion of 2% Manucol DM is shown in Fig. 5. The profiles for both the enthalpy and T_{onset} of the transition are similar to those found for soya alone (Fig. 2). At any given water content T_{onset} is elevated by 2–3 K following the inclusion of Manucol DM. This effect is specific to Manucol DM, as other hydro-

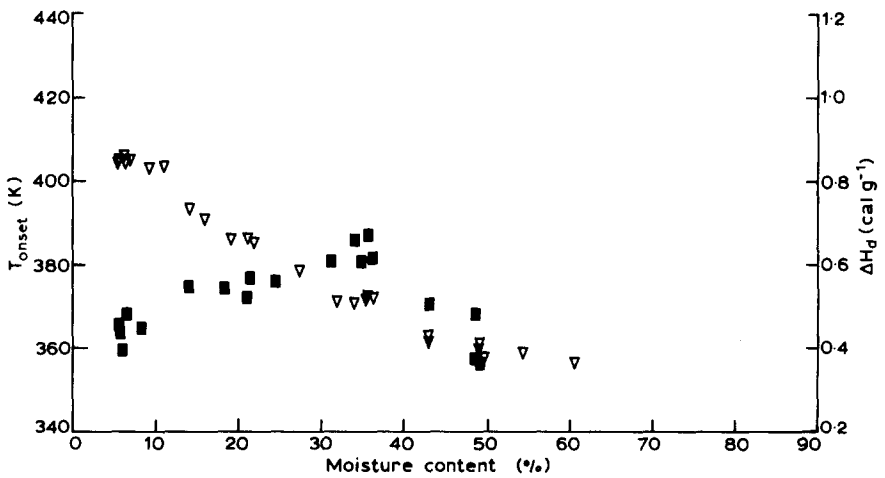


Fig. 5. Heat of transition, ΔH_D (■) and temperature onset of the transition (T_{onset}) (▽) of the 7S globulin of soya isolate containing 2% Manucol DM as a function of final water content. ΔH_D is quoted per gram of dry sample.

TABLE 1
The Transition Temperature (T_{onset}) of the 7S Globulin in Samples of Soya Isolate and Soya Isolate plus Hydrocolloid, at a Water Content of 50·0 ± 1·5% Measured after Heating

Sample	T_{onset} *(K)*
Soya isolate	355·9 ± 1·2
Soya isolate + 2% Manucol DM	360·4 ± 0·4
Soya isolate + 2% Manugel GMB	356·2 ± 0·1
Soya isolate + 2% pectin	356·7 ± 0·1
Soya isolate + 2% xanthan gum	356·2 ± 0·4
Soya isolate + 2% guar gum	355·3 ± 0·4
Soya isolate + 2% gum arabic	356·1 ± 0·2

colloids examined have little or no effect upon T_{onset} or reduce it slightly at the water content examined (50%) (Table 1).

Typical thermograms for the native and denatured soya isolate over the range 243–310 K and containing 2% Manucol DM are shown in Fig. 6. The thermograms of the native samples are very similar to those obtained in the absence of polysaccharide (Fig. 3). However, in the denatured samples containing polysaccharide the endothermic peak centred around 266·5 K, although decrease in area is still far more in evidence than in the hydrocolloid-free system (Fig. 3), suggesting that more of the freezable water is associated with the macromolecules in these systems following denaturation of the protein.

For samples containing 2% Manucol DM there is an increase in apparent water content following denaturation (Fig. 4). At all water activities the increase is far greater than in the sample not containing this polysaccharide (Fig. 4). This increased formation or liberation of water is unique to Manucol DM as other hydrocolloids examined did not exhibit such an increase. If anything, less water is formed than in the soya-only samples. Thus at 5·5% moisture ($a_w = 0·38$) soya isolate containing 2% guar gum, 2% gum arabic or 2% xanthan increased its apparent moisture content (mean ± standard error) by only 0·7 ± 0·5, 1·4 ± 0·6 or 0·6 ± 0·5%, respectively. Soya isolate alone gave an increase of 4·8 ± 0·43 at this a_w, whilst Manucol DM inclusion led to an increase of 5·5 ± 1·1%. At 9·0% initial moisture ($a_w = 0·52$), soya isolate containing 2% CMC increased its apparent moisture by 1·9 ± 1·3%, whilst isolate containing 2% pectin decreased its apparent moisture by 0·7 ± 0·6%. With 2% Manugel GMB the apparent increase in moisture content under these

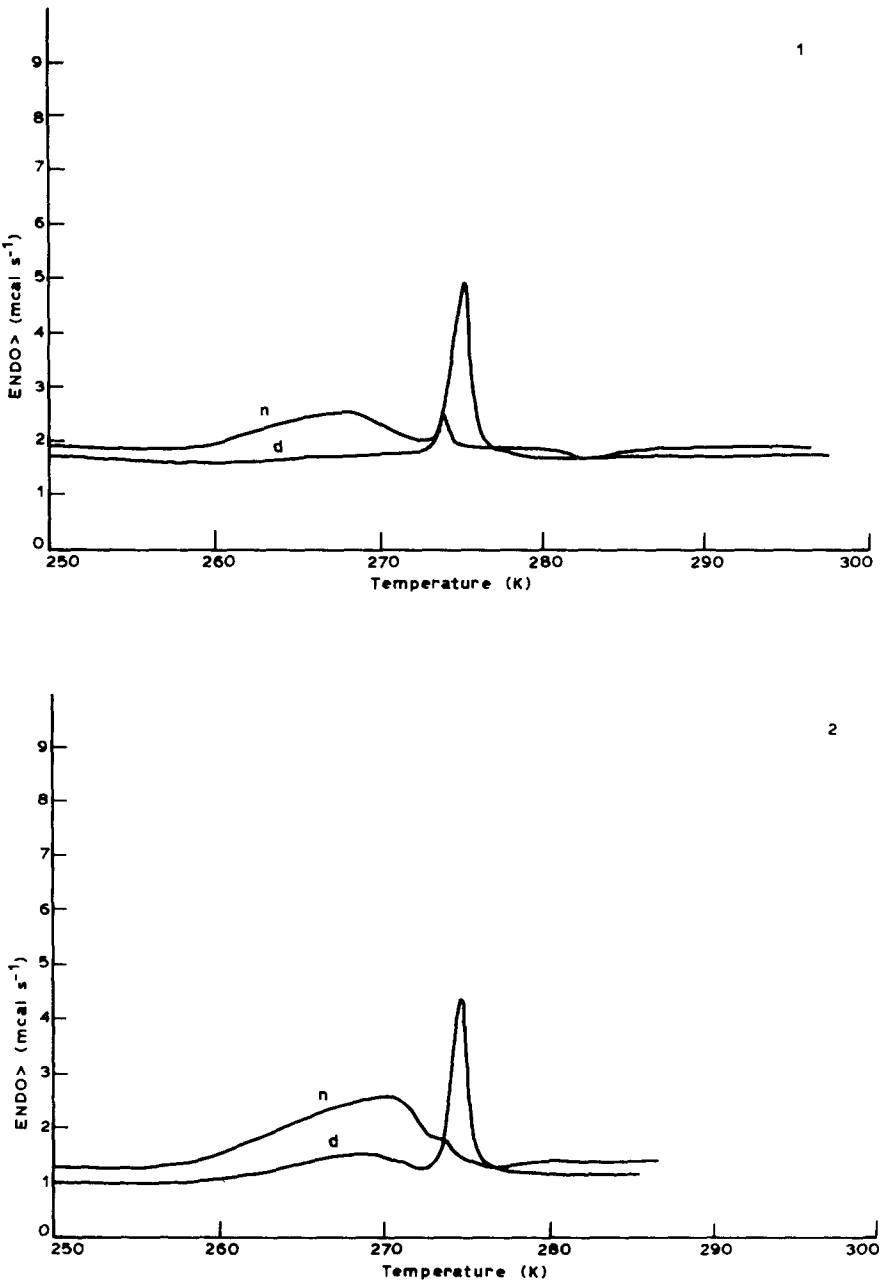
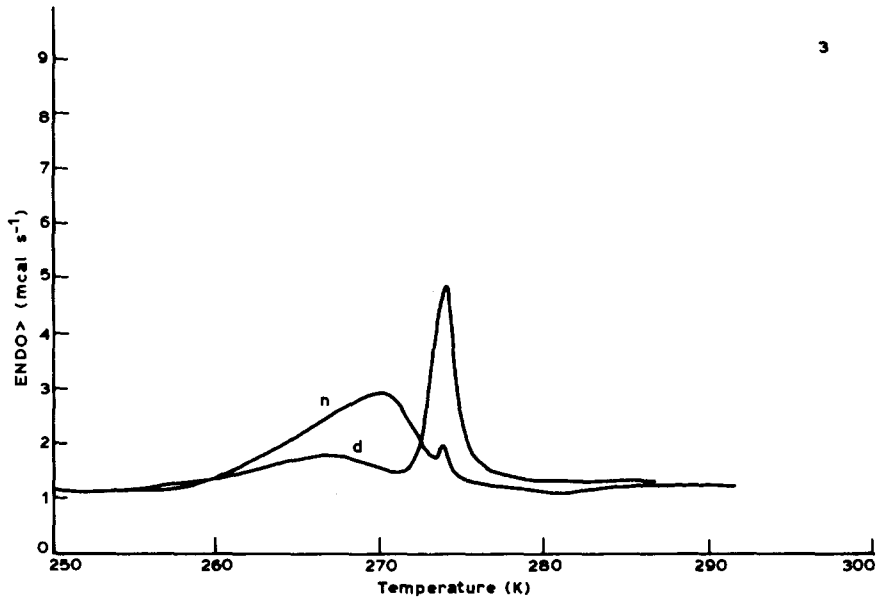


Fig. 6. Typical thermograms of the water associated with soya protein plus 2% Manucol DM at the initial water contents of (1) 42%, (2) 45%, (3) 51% and (4) 64%; sample weights 5·71, 7·94, 9·26 and 10·72 mg, respectively (n = native protein, d = denatured protein).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Temperature (K)
n
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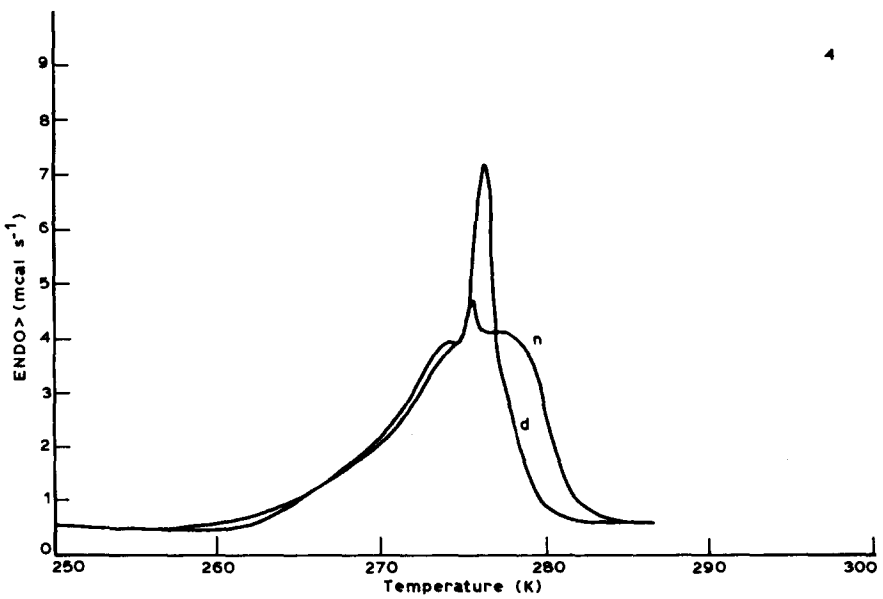
4
ENDO> (mcal s^{-1})
Temperature (K)
n
d

Fig. 6. — *contd.*

conditions ($a_w = 0{\cdot}52$) was $0{\cdot}9 \pm 0{\cdot}8\%$. Under these conditions Manucol DM increased the apparent moisture content by 9–10% whilst soya alone increased its apparent moisture content by 4–5% (Fig. 4).

The increase in apparent water content of the different systems on denaturation was confirmed by Karl–Fischer analysis (Table 2). It was also noted that a small number of ampoules burst during heating, these invariably being those containing Manucol DM, suggesting the formation of more volatiles in these systems. Of all the other polysaccharides investigated, only 2% pectin yielded any evidence of increased 'water' formation.

TABLE 2
Pooled Percentage Moisture Content Determination (mean ± *SD*) of Soya Isolate and Soya Isolate + Hydrocolloids[a]

Sample	*Moisture (g per 100 g solid)*
Soya isolate	$12{\cdot}2 \pm 1{\cdot}2$
Soya isolate + 2% Manucol DM	$20{\cdot}4 \pm 6{\cdot}6$[b]
Soya isolate + 2% Manugel GMB	$12{\cdot}0 \pm 0{\cdot}7$[c]
Soya isolate + 2% CMC	$12{\cdot}2 \pm 6{\cdot}3$[c]
Soya isolate + 2% pectin	$15{\cdot}8 \pm 3{\cdot}5$[d]

[a] Probability of no significant difference from the control (soya isolate alone) determined by Dunnet's test.
[b] $P < 0{\cdot}01$.
[c] No significant difference.
[d] $P < 0{\cdot}05$.

Figure 7 shows the sorption isotherms at 25°C for native soya isolate, native soya isolate + 2% Manucol DM, denatured soya isolate and denatured soya isolate + 2% Manucol DM. With the exception of the denatured isolate, the isotherms for all samples are identical within experimental error. At all water activities the denatured soya exhibited reduced water binding.

DISCUSSION

Role of water

The enthalpy of denaturation as a function of water content has been determined for several proteins. For chymotrypsinogen-A (Fujita & Noda, 1981), β-lactoglobulin (Ruegg *et al.*, 1975) and myoglobulin

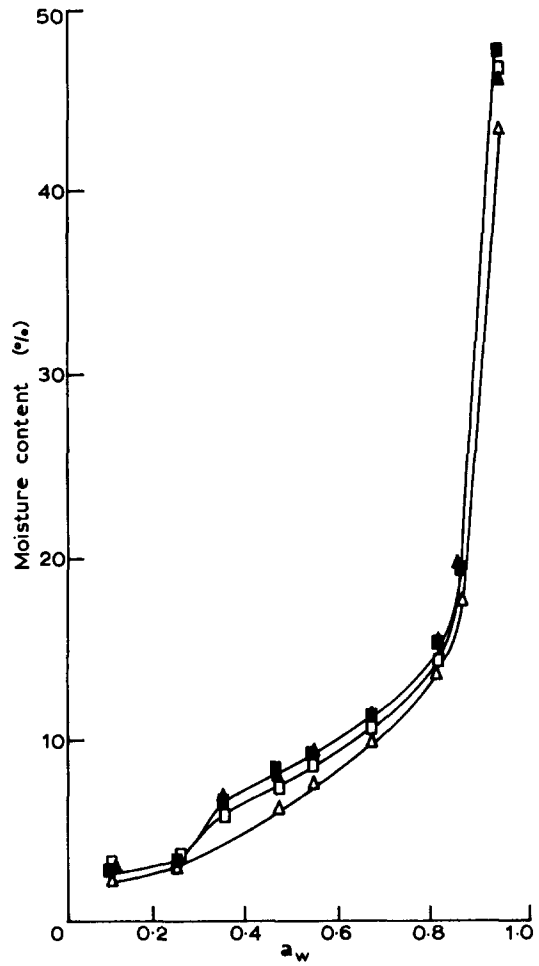

Fig. 7. Water sorption isotherm for soya isolate (▲), soya isolate + 2% Manucol DM (■), denatured soya isolate + 2% Manucol DM (□) and denatured soya isolate (△).

(Hagerdel & Martens, 1976) the enthalpy of the transition increases with increasing water content up to ~ 50% water. At water contents above 50% there is little or no change in the measured enthalpy of the transition. However, Luesher *et al.* (1974) found a very similar profile for the enthalpy of the denaturation of tropocollagen, as a function of water content, to that exhibited by the soya 7S globulin. Although the reasons for these differences are not obvious, chymotrypsinogen-A, β-lactoglobulin and myoglobulin are all relatively small globular proteins which denature in a relatively co-operative manner (Privalov, 1982),

whilst tropocollagen is a rigid rod which melts with a far lower degree of co-operation (Privalov, 1982). The soya 7S globulin is also a relatively complex aggregate of six subunits which may also be expected to have complex denaturation kinetics. Thus the non-co-operation of the denaturation process appears to relate to the more complex moisture dependence of ΔH_D. In scanning calorimetry the heat absorbed is of course a composite of the heat evolved and absorbed by all the reactions taking place over this temperature range. Even if denaturation of the protein was the only reaction taking place, it would only be equal to the enthalpy of denaturation at constant pressure. However, differences due to aggregation phenomena and differences in pressure are unlikely to be of major significance.

For the 7S globulin the moisture dependence of ΔH_D can be divided into three relatively distinct regions. At water contents below about 30% there is a slight decrease in ΔH_D as the moisture content decreases, although T_{onset} increases quite markedly. This type of relationship is true for all proteins and may well represent hydration levels insufficient to allow complete unfolding of the protein. At water contents below this threshold value, Bull & Breese (1968) have proposed that inter- and intra-molecular interactions must occur to prevent the formation of 'voids' within the protein complex. Evidence for such bond formation has been shown by infra-red spectroscopy (Careri *et al.*, 1979). The formation of these bonds will lead to an increase in thermostability and, due to incomplete unfolding, the enthalpy of the process will decrease.

Evidence for this explanation is afforded by the thermograms of the ice–water transition, as at water contents below about 30% no 'freezable' water is present in the system. Thus, at these moisture contents the primary hydration of the protein is probably incomplete. At 30% moisture ΔH_D is maximal as complete unfolding can occur, but the *inter-* and *intra*-molecular bonds in the protein aggregates are still strong. Further hydration leads to an increase in both the bound and free water (Fig. 3) and consequently a decrease in the strength of the protein–protein bonding and consequent decrease in ΔH_D. Increased hydrophobic interactions would lead to an increase in stability but a decrease in ΔH_D, thus increased or decreased formation of these bonds cannot explain the observed dependence on moisture content in the range 30–50%, although such an explanation would account for the behaviour at moisture contents below 30%. However, if all the water is bound it is difficult to envisage how such interactions, involving the structuring of water, can occur. Not unexpectedly, above a certain hydration level ($\simeq$60% moisture) the denaturation process is independent of the total water content.

Figure 3 shows that, as with other proteins (Lumry, 1973), the secondary water associated with the protein (i.e. the water melting several degrees below 273 K) is released following denaturation. This reduced water binding ability of the denatured protein is confirmed by the experimentally determined sorption isotherms for the native and denatured samples (Fig. 7).

Hydrocolloid inclusion

The denaturation behaviour of the 7S globulin appears to be affected by the inclusion of a high mannuronic acid alginate (Manucol DM) in two ways. First, the onset temperature of denaturation is significantly increased by the addition of the polysaccharide (Table 1) and, secondly, at water contents below 40% ΔH_D is reduced (compare Figs 2 and 5). The latter effect is not specific to alginates, as it has been observed with several other polysaccharides (Oates *et al.*, unpublished results). As mentioned previously, the enthalpy is the sum of the heat changes associated with several processes, so it is not possible at the present time to assign it to any specific interaction.

In interpreting the apparently specific increase in T_{onset} with alginate addition, it should be borne in mind that the moisture contents quoted are those obtained after heating. If, as the data in Table 2 and Fig. 4 suggest, heating of soya alginate mixtures results in the generation of substantial water, which may well be produced after denaturation, then the apparent increase in T_{onset} may reflect the fact that the true water content is lower for the alginate-containing sample than for soya alone or the samples containing the other polysaccharides. Thus the different value for T_{onset} found with alginate may be regarded as some confirmation of an unusual release of additional volatiles (probably water) on heating this system. Alginate addition appears to have some influence on the final state of the water in the system after denaturation. Thus the denatured soya containing alginate has a higher content of secondary water (compare Figs 3 and 6) and greater water binding ability (Fig. 7) than soya alone.

The addition of extra volatiles on heating soya/alginate mixtures is probably the reason for the decrease in the viscosity of the soya 'melt' observed when alginate is added to an extruder feed. This effect has also been shown to be far greater for alginates with a high content of mannuronate residues (Berrington *et al.*, 1984). It is well established that the viscosity at these low moisture levels is strongly dependent on water content. The strong dependence on a_w makes us consider that the production of volatiles is due to browning/condensation reactions. The association with the content of mannuronate residues suggests that these

reactions are chemically very specific. In this respect it is interesting that it has been recently reported that it is probably the esterified mannuronate rich regions of propylene glycol alginate that react with the $C-NH_2$ groups of gelatin (McKay *et al.*, 1985). We are currently attempting to define the reactions responsible for the development of extra volatiles and determine whether they are unique to soya.

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