Food Chemistry **40** (1991) 101–107

Thermal Behaviour of Soya 7S Globulin: Effect of Moisture Content and Added Hydrocolloid on the Denaturational Change in Heat Capacity

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(Received 2 February 1990; revised version received and accepted 22 May 1990)

ABSTRACT

This paper describes an unusual and previously unreported phenomenon relating to the change in specific heat function of soya 7S globulin on denaturation in the presence of alginate, rich in mannuronic acid. Measurements of the relative change in the specific heat of soya 7S globulin on denaturation were determined at a range of moisture contents (5 to 50%) by differential scanning calorimetry. The specific heat function associated with denaturation of the 7S globulin was found to increase in most of the soya and soya + hydrocolloid systems examined, the magnitude of the increase was dependent on initial moisture content. In the presence of 2% of a mannuronic acid-rich alginate, soya 7S globulin unusually displayed a lower specific heat function following denaturation.

INTRODUCTION

Relatively little information is available with regard to the physical and chemical changes that occur when proteins are heated at the low moisture

Food Chemistry 0308-8146/91/$03·50

contents found in extrusion cooking, although it is established that the denaturation characteristics of proteins are dependent on the moisture content. The temperature of denaturation invariably increases with decreasing water content whereas the enthalpy of the transition generally decreases (Finch & Ledward, 1972; Fujita & Noda, 1981; Sheard *et al.*, 1986). Unfortunately, little is known of the changes occurring when heterogeneous protein mixes are heated, this may reflect the problems associated with obtaining accurate thermodynamic estimates of such systems. Proteins are present in food systems in combination with other protein or non-protein components; the state of these proteins will play a vital role in their functional properties which will be reflected in the product. For such reasons it is important that knowledge of the behaviour of proteins in the presence of other components be known. Heat changes and subsequent thermal events in these systems can be influenced by many factors, the most important being component interactions which result in aggregation (Sheard *et al.*, 1987). The extents of these interactions are not fully known for soya proteins; German *et al.* (1982) suggest that only limited interaction is taking place between the major subunits of soya.

In a previous publication we reported on the influence of the presence of a small quantity of alginate, rich in mannuronic acid, on the denaturation characteristics of soya protein (Oates *et al.*, 1987*a*). It was shown that, in the presence of alginate, the denaturation temperature was reduced and the transition enthalpy was significantly affected. Such effects were evident over a wide range of moisture contents.

Further investigation of these systems has revealed an unusual and unexpected phenomenon brought about by the addition of alginates, rich in mannuronic acid residues, on the denaturation characteristics of soya 7S protein as indicated by the change in denaturational specific heat capacity. This paper reports on these changes and, in particular, how they may be influenced by moisture content and addition of low levels of hydrocolloid.

MATERIALS AND METHODS

Materials

Soya isolate (pH 7·0) was prepared under mild conditions as outlined by Hermansson (1978) from a 70 PDI flour (McCauley Edwards, Ltd).

Alginates used throughout this work were supplied by Kelco International Ltd; all had different mannuronic:guluronic acid ratios, which were given as: Manucol DM (1·9), Manugel GMB (0·7) and Keltone (1·56).

Xanthan gum, gum arabic, guar gum and a partially methylated pectin

were obtained from Sigma Chemical Co. A sample of carboxy methyl cellulose, medium viscosity, was obtained from BDH Ltd.

Methods

Soy isolates or soya isolates plus hydrocolloid mixed in various proportions were:

(1) Stored over distilled water and removed at various times, up to 3 days; or
(2) Equilibrated over silica gel or solutions or known water activity at 25°C.

Aliquots of approximately 10 mg of hydrated sample were sealed in weighed volatile aluminium pans (sample pans used for volatile materials which exert a significant vapour pressure) and subjected to calorimetric analysis. The denaturation profiles were determined at a scan rate of 5°C per min from 75 to 140°C in a Perkin Elmer DSC-2 scanning calorimeter at a sensitivity of 0·2 mcal/s. Data were captured and analysed by micro-computer.

The moisture contents of the samples were determined following denaturation by puncturing the lids of the sample pans and drying *in vacua* at 70°C for 48 h. All sample pans were checked for weight loss during DSC analysis by weighing before and after determination of the thermogram.

The change in specific heat function ΔCp was determined from the base line at the start and end of the transition. The line of best fit, determined with the aid of a computer, for each base line, was extrapolated to the point of maximum heat change and a measure of the difference in heat flow taken, this was assumed to be proportional to heat capacity. The specific heat function of soya 7S globulin was calculated assuming that the 7S globulin comprises 18% of the total protein content (Wolf & Sly, 1967), which was determined as 92% (by Kjeldahl) and this protein was in the native form (Sheard *et al.*, 1986).

RESULTS AND DISCUSSION

Two endothermic peaks were evident on the thermograms of the soya isolate (Fig. 1) corresponding to denaturation of the 7S and 11S globulins (Hermansson, 1978).

The endothermic peaks of both the 7S and 11S globulins are characterised by a change in the specific heat function following denaturation (Hermansson, 1978). With most samples a large exotherm was seen

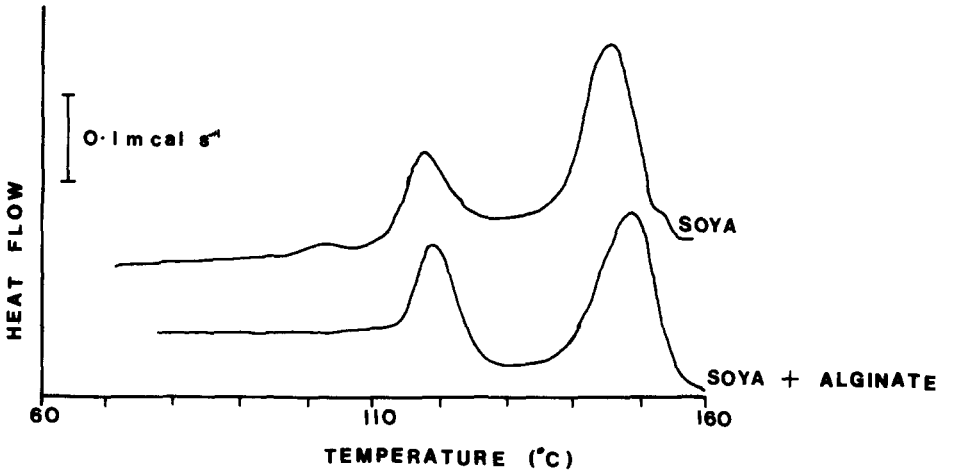

Fig. 1. Thermograms of soya alone (12·1 mg soya, 2·5 mg water) and soya + 2% mannuronic acid-rich alginate (10·1 mg soya, 2·0 mg water).

following denaturation of the 11S globulin (Sheard *et al.*, 1987), the presence of which made accurate determinations of many of the parameters associated with the 11S globulin difficult. Sufficient peak separation, which varied little between treatments and establishment of a reasonably stable baseline following denaturation, facilitated measurement of the change in specific heat associated with denaturation of the 7S globulin. Measurement of the change in heat flow occurring after denaturation for a purified protein can be defined in terms of specific heat capacity (ΔC_p). For all pure proteins studied ΔC_p on denaturation is positive (Finch & Ledward, 1972; Privalov, 1982) and this was found to be so for the 7S fraction in the absence of alginate (Fig. 1). In this work, we are only comparing the relative changes in heat flow, described by the quasi specific heat function, $\Delta C'_p$. The use of this function was thought necessary as it allowed us to describe the effect of different processing conditions within the same sample of soya grits without isolating the 7S globulin or precisely measuring the quantity of this protein present. The values reported should not be used to compare different soya samples as they were obtained using an ill-defined heterogeneous mixture. Further, the precise quantity of 7S globulin is not known—an average value based on work reported by other authors was used; this value will be influenced by genetic, environmental, processing and extraction conditions.

Specific heat functions were found to be dependent upon the moisture content of soya (Fig. 2). These values increased with increasing water content (between 5 and 50%). Changes in the heat flow after denaturation have been associated, in pure protein systems, to increased interaction of polar groups with the aqueous environment (Kauzman, 1959). However, the system

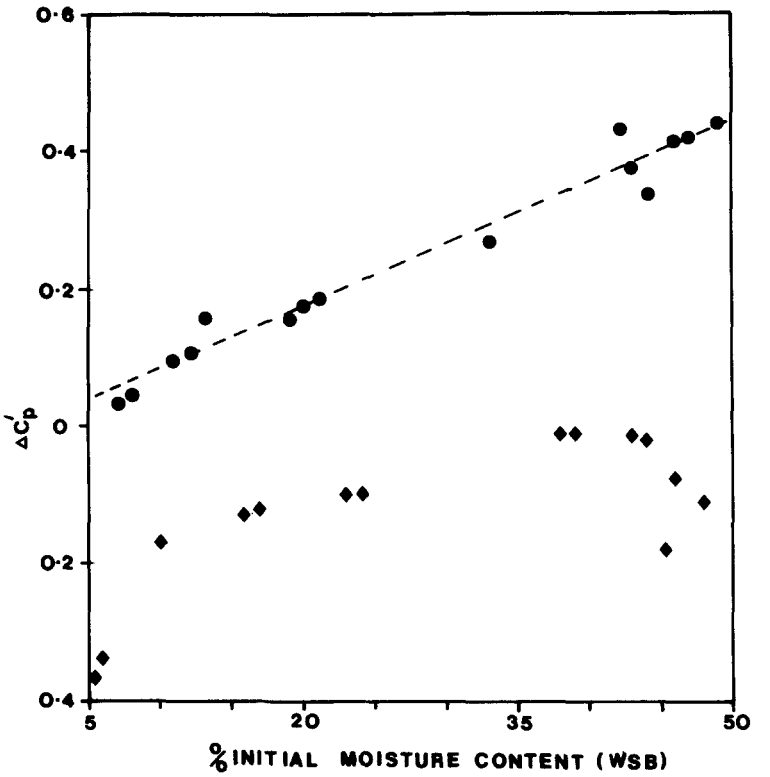

Fig. 2. Dependence of the change in specific heat function following denaturation on initial moisture content for soya 7S globulin, alone (●) and in the presence of 2% mannuronic acid-rich alginate (◆).

investigated was far from pure and associated component interactions must be considered.

Interestingly, the addition of 2% alginate, rich in mannuronic acid, caused a dramatic deviation in the expected heat flow following denaturation. In such mixes, post denaturational heat flow was lower than that prior to processing (Figs 1 and 2); consequently, negative specific heat functions were estimated. Such negative functions have never previously been observed in protein systems and cannot be due to interference by the 11S denaturation peak since this would cause ΔC_p to be even more positive. This effect was evident over the whole range of moisture contents investigated. Further, this effect was specific to addition of alginates, especially those rich in mannuronic acid (Table 1). Addition of 2% manucol DM was required before the specific heat function was affected (Table 2), after which further addition had little effect.

This unusual decrease in the post transition heat flow due to hydrocolloid addition is difficult to explain. The interaction is not only specific to alginates but expresses itself most strongly after the addition of alginates

TABLE 1
Specific Heat Function (cal $°C^{-1} g^{-1}$) for Soya 7S as a Result of Denaturation at Two Different Moisture Contents ($10 \pm 0{\cdot}5$ & $50 \pm 1\%$) in the Presence of 2% Added Hydrocolloids. Values are Means $\pm$ Standard Error of Five Determinations

Hydrocolloid	*Water content*	
	10%	*50%*
Soya alone	$0{\cdot}16 \pm 0{\cdot}01$	$0{\cdot}41 \pm 0{\cdot}02$
Manucol DM	$-0{\cdot}17 \pm 0{\cdot}04$	$-0{\cdot}25 \pm 0{\cdot}08$
Keltone	$-0{\cdot}08 \pm 0{\cdot}01$	$-0{\cdot}17 \pm 0{\cdot}04$
Manugel GMB	$0{\cdot}02 \pm 0{\cdot}01$	$0{\cdot}04 \pm 0{\cdot}01$
Xanthan	$0{\cdot}21 \pm 0{\cdot}01$	$0{\cdot}30 \pm 0{\cdot}07$
Guar gum	$0{\cdot}19 \pm 0{\cdot}01$	$0{\cdot}35 \pm 0{\cdot}07$
Xanthan gum	$0{\cdot}21 \pm 0{\cdot}01$	$0{\cdot}30 \pm 0{\cdot}07$
Gum arabic	$0{\cdot}17 \pm 0{\cdot}02$	$0{\cdot}46 \pm 0{\cdot}01$
Pectin	$0{\cdot}18 \pm 0{\cdot}01$	$0{\cdot}34 \pm 0{\cdot}07$

containing a high proportion of mannuronic acid residues. As a consequence of such specificity it seems unlikely that electrostatic interactions are important. Previously, we have suggested that covalent bonds are formed between alginate and certain amino acid residues of soya protein (Oates *et al.*, 1987*b*). Such interactions could well reduce specific heat flow by either lowering side group mobility in the partially unfolded protein or by a direct effect due to aggregation taking place immediately after denaturation.

The results presented in this paper support our previous assumption (Oates *et al.*, 1987*b*) that specific interaction between alginate rich in

TABLE 2
Effect of Manucol DM Concentration on the Denaturation Characteristics of the Soya 7S Globulin, Initial Moisture $50 \pm 1\%$. Values are Means $\pm$ Standard Error of Twelve Determinations

Manucol DM content (%)	*Specific heat function* ($cal°C^{-1}g^{-1}$)
1	$0{\cdot}14 \pm 0{\cdot}01$
2	$-0{\cdot}25 \pm 0{\cdot}08$
3	$-0{\cdot}20 \pm 0{\cdot}01$
4	$-0{\cdot}16 \pm 0{\cdot}06$
Soya alone	$0{\cdot}41 \pm 0{\cdot}02$

mannuronic acid residues and soya take place on heating. Such chemical interactions can be expected to be stable and thus be of importance in texturisation of proteins during extrusion processing. The formation of crosslinks may be envisaged as primarily aligning and holding together the protein strands prior to the formation of the less stable but texturally important interactions, i.e. hydrophobic, electrostatic and disulphide bonds (Sheard, 1985).

ACKNOWLEDGEMENTS

We thank Drs J. R. Mitchell, I. Hodgson and I. Imeson for valuable discussion. We also acknowledge the financial support of the agriculture and food research council.

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