Calorimetric Study of Soybean Protein Isolates: Effect of Calcium and Thermal Treatments

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The addition of calcium (1.23−9.73 mg/g of protein) during neutralization of isoelectric precipitate modified the thermal behavior of soybean protein isolates. In the samples that did not undergo thermal treatment, the calcium content increase caused an increase in thermal stability, especially in the 11S fraction, but no modifications were detected in the enthalpy values. Samples undergoing mild thermal treatments (5 min at 80 °C), after neutralization with different amounts of calcium, showed enthalpy values lower than those of the unheated samples. Isolates with no calcium aggregates or with values below 5 mg/g of protein and intense thermal treatment (15 min at 90 °C) showed no endotherms (denaturation enthalpies = 0). Calcium levels above 5 mg/g of protein protected the soybean proteins, partially preventing their denaturation by heating at 90 °C for 15 min. The activation energy of the thermal denaturation process of soybean protein isolates, calculated by the Ozawa method (1970), was modified in the presence of calcium. The E_a of the 11S fraction varied between 51.12 and 102.15 kcal/mol and that of the 7S between 42.69 and 58.05 kcal/mol. A minimal value for glycinin E_a was observed at a calcium concentration of 6.51 mg/g of protein. The E_a value of the fraction enriched in 11S, calculated by the Borchardt and Daniels method (1957), was higher than that obtained by the Ozawa method (1970) for a soybean isolate. This fraction would denature following second-order kinetics ($n = 2$).

Keywords: *Thermal properties; protein denaturation; interaction calcium; soybean proteins*

INTRODUCTION

Soybean isolates contain 90% protein, the major components being glycinin (11S) and β-conglycinin (7S) (Hermansson, 1978). These fractions constitute 61% of the isolate proteins, and the rest consists of whey proteins, such as γ-conglycinin, 7S basic globulin, lipoxygenase, β-amylase, agglutinins, and trypsin inhibitors (Iwabuchi and Yamauchi, 1987a,b). Soybean proteins are incorporated into many food systems. Most of these foods require some heat treatment. Heat is one of the most common physical treatments which often denatures proteins. The loss of native structure is critical to protein functionality such as gelling, emulsification, and foaming (Kinsella, 1976). Protein unfolding is accompanied by enthalpic changes (Privalov and Khechinashvili, 1974) which can be monitored by thermoanalytical techniques such as differential scanning calorimetry (DSC). The peak analysis enables the determination of the temperature of transition and enthalpy of denaturation from the maximum peak temperature and the area of the peak, respectively. The sharpness of the peak also indicates the cooperative nature of the transition from the native to a denatured state. If the transition occurs in a very narrow range of temperature, it is highly cooperative (Wright et al., 1977). DSC has been used to study thermal denaturation of many food proteins: muscle (Wright et al., 1977), egg albumin (Donovan et al., 1975), soybean proteins (Hermansson, 1978, 1979), whey proteins (Rüegg et al., 1977; De Wit and Klarenbeck, 1984), β-lactoglobulin (De Wit and Swinkels, 1980; Halwalkar, 1985), and oat globulin (Ma and Halwarkar, 1988).

Calcium is an essential mineral nutrient. It is known to be a coagulant of soybean protein (Watanabe and Saio, 1973; Torikata et al., 1987) as well as a thickening agent (Lee and Rha, 1977), where the regulation of the soy protein−calcium complex formation is necessary. Several investigators have studied the interactions between calcium ions and various soybean protein preparations by using different methods (Appu Rao and Narasinga Rao, 1975; Saio et al., 1968; Sakakibara and Noguchi, 1977; Kroll, 1984).

The functional properties of soybean protein isolates are affected by heat and the presence of calcium ions. The aim of this study was to analyze the thermal behavior of soybean protein isolates by DSC under various conditions. Heated and unheated samples with different contents of calcium ions were examined. We also studied the denaturation kinetics of some of the isolates.

MATERIALS AND METHODS

Preparation of Isolates. Protein isolates were prepared from defatted soy flour (Sanbra S.A., Brazil), with 50% protein as determined by the Kjeldahl method ($f = 5.7$). The flour was extracted with water at pH 8.0 and made alkaline with 2 N NaOH (flour/water ratio of 1:10, w/v) for 2 h at room temperature. The pH was periodically adjusted to 8.0. This suspension was filtered through gauze, and the filtered material was centrifuged at 10000*g* for 30 min at 4 °C. The supernatant was adjusted to pH 4.5 with 2 N HCl. The precipitate was separated by centrifugation at 5000*g* for 15 min at 4 °C and then suspended in water. Different treatments were made after this suspension:

Treatment 1. Isolates were adjusted to pH 8.0 with NaOH. No thermal treatment was applied.

Treatment 2. Isolates were adjusted to pH 8.0 with different amounts of $Ca(OH)_2$ (1.23−9.73 mg of calcium/g of protein) and NaOH. No thermal treatment was applied.

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Treatment 3. Isolates were heated for 5 min at 80 °C or for 15 min at 90 °C and then adjusted to pH 8.0 with different amounts of $Ca(OH)_2$ (1.23−9.73 mg of calcium/g of protein) and NaOH.

Treatment 4. Isolates were adjusted to pH 8.0 with different amounts of $Ca(OH)_2$ and NaOH. Then isolates were heated for 5 min at 80 °C or for 15 min at 90 °C.

The solutions used were 2 N NaOH and 5.5 mg/mL $Ca(OH)_2$. Thermal treatments were carried out in a constant water temperature bath. In some cases, the heating rate was controlled with a thin Cu-constantan thermocouple which was immersed in the protein suspension, and the times needed to reach these temperatures were calculated for all isolates. The soybean protein isolates were removed from the thermostatic bath and immediately cooled in an ice bath until they reached room temperature. The suspensions were freeze-dried and stored at 4 °C.

The protein concentration was measured by the Kjeldahl method using 5.7 as a conversion factor. The calcium content of the soybean protein isolates ranged from 1.02 to 9.73 mg/g of protein.

Preparation of 7S and 11S Globulin Rich Fractions. Soybean globulin rich fractions were prepared from defatted soybean flour (Sanbra S. A., Sao Paulo, Brazil) according to the method of Thanh and Shibasaki (1976), with some modifications (C. Puppo, personal communication). Soybean proteins, extracted with 2 N NaOH at pH 8.0, were adjusted to pH 6.4 with 2 N HCl, cooled to 4 °C overnight, and centrifuged at 13300*g* at 15 °C for 30 min. The precipitate was washed twice in distilled water (pH 6.4) and centrifuged at 13300*g* at 15 °C for 30 min. Then, it was resuspended at pH 8.0 with 2 N NaOH and freeze-dried to yield a crude glycinin fraction. The supernatant was adjusted to pH 4.8 with 2 N HCl and stirred for 2 h at 20 °C. The precipitate obtained by centrifugation at 3300*g* at 15 °C for 10 min was washed twice in distilled water and then resuspended at pH 8.0 with 2 N NaOH. The dispersion was freeze-dried to yield a crude β-conglycinin fraction.

Differential Scanning Calorimetry. Thermal analysis was performed in a DuPont 910 differential scanning calorimeter attached to a Hewlett-Packard 7046 B recorder (DuPont Co., Wilmington, DE). The cell constant and temperature calibrations were performed according to ASTM Norm E 698/79 and E 474/80 using indium thermograms, respectively. Hermetically sealed aluminum pans were prepared to contain 13−20 mg of soybean protein isolates suspended in water (20%, w/v). These samples were scanned at 10 °C/min from 30 to 130 °C. As a reference, an empty double capsule was used. After DSC analysis, the pans were punctured, and the dry matter weight was determined by drying at 105 °C overnight. The endothermic areas were measured with a Morphomat 34 Zeiss (error < 1%) image analyzer (Carl Zeiss, Ober Cochem, Germany). A minimum of triplicate samples were performed, and the denaturation temperature (T_d) and enthalpy of transition (ΔH) were obtained from the thermograms. A degree of cooperativity of the thermal transition was obtained from the width at half-peak-height ($\Delta T_{1/2}$).

The thermal denaturation kinetics of soybean protein isolates were also studied by DSC using the dynamic Ozawa method (Ozawa, 1970). This method is based on the relationship between the heating rate and the maximum peak deflection temperature of the DSC thermograms. It was also assumed that the temperature dependence of the reaction rates followed the Arrhenius expression. From this relationship, it was possible to estimate kinetic parameters from the coefficients of a linear regression between $\ln(\beta/T_d^2)$ and $1/T_d$. Activation energy (E_a) and Arrhenius's pre-exponential factor (Z) were obtained from slope and interception of the linear regression, respectively. At least, triplicate analyses were carried out for six different heating rates: 2.5, 5, 10, 15, 20, and 25 °C/min.

The thermal denaturation kinetics of the 7S and 11S rich fractions were also studied. In these instances, the heat evolution method of Borchardt and Daniels (1957) was used. The method assumes that the reaction obeys the relationship $d\alpha/dt = k(1 - \alpha)^n$, where α is a fractional conversion, k is a rate constant, and n is a reaction order. In addition, the temperature dependence of the reaction rate follows the Arrhenius expression. Two parameters are measured from the DSC thermogram: the rate $d\alpha/dt$, obtained by dividing the peak height at temperature T by the total area, and the unreacted fraction $(1 - \alpha)$, obtained by measuring the ratio of the partial area at temperature T to the total peak area and subtracting this value from unity. Twenty equally spaced peak segments over the region from 10% to 50% were analyzed to calculate the parameters mentioned before. Duplicated samples were evaluated for these calculations. DSC measurements were performed in DSC Polymer Laboratories (Rheometric Scientific, Ltd., Epsom, England). The samples were heated from 25 to 140 °C at a rate of 10 °C/min. Calibration was carried out with indium, lauric acid, and stearic acid (pa) as standards, at a heating rate of 10 °C/min. The runs were analyzed with Software PLus V5.41 (Rheometric Scientific, Ltd., Epsom, England), which allows calculation of partial areas. Therefore, in the kinetic study of thermal denaturation of the 7S rich fraction, it was possible to subtract the remainder 11S corresponding partial area.

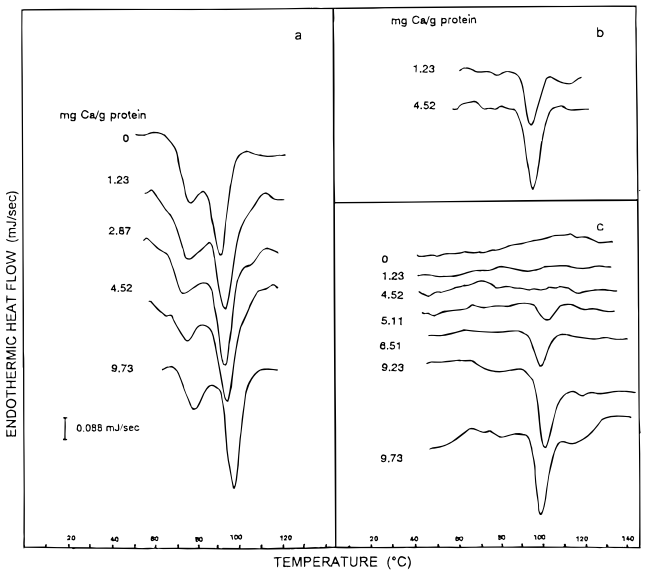

Figure 1. DSC thermograms of heated and unheated soybean protein isolates containing different amounts of calcium: (a) unheated samples, (b) samples heated for 5 min at 80 °C, and (c) samples heated for 15 min at 90 °C.

Statistical Analysis. The data obtained were statistically evaluated by variance analysis (ANOVA). The comparison of means was done by the least-significant difference (LSD) test at a level (α) of 0.05. Both were carried out using the statistical analysis package SYSTAT (Wilkinson, 1990).

RESULTS AND DISCUSSION

Thermal Analysis. The DSC thermograms of soybean protein isolates are shown in Figure 1. In all instances, when the samples were heated in the calorimeter, cooled at room temperature, and immediately rerun, there was no endothermic response indicating that the proteins had been extensively and irreversibly denatured. Thermal denaturation thermograms of the native isolates exhibited the two characteristic peaks corresponding to endothermic transitions of the 7S (first peak) and 11S (second peak) fractions. Temperatures of denaturation (T_d) for 7S and 11S were 75.53 ± 0.66 and 90.98 ± 0.73 °C, respectively, in the sodium proteinate control. The coefficient of variation for those samples ranged from 0.2% to 1.1% for T_d. These results are in agreement with those reported by other authors (Hermansson, 1978; Wagner and Añón, 1990; Arrese et al., 1989).

Table 1. Temperatures and Enthalpies of Denaturation

soybean isolates	mg of calcium/ g of protein	T_d (°C) 7S	T_d (°C) 11S	ΔH_T (J/g)	$\Delta T_{1/2}$ 11S (°C)
1	0	76.00	91.52	15.00	9.50
2	1.23	77.02	93.50	18.48	9.50
3	2.05	73.49	92.49	17.81	9.50
4	2.87	74.00	93.50	14.63	9.02
5	3.70	74.00	93.51	16.22	9.50
6	4.52	76.06	94.70	14.71	9.50
7	7.24	76.89	95.75	nd[a]	8.50
8	9.73	78.50	97.25	14.03	8.00

[a] nd, not determined.

In Figure 1a, the thermograms of unheated soybean protein isolates containing different amounts of calcium are shown. Thermal curves differ in T_d: There is a shift of T_d toward higher values with increasing calcium levels (Table 1). The shift is more pronounced for the 11S than the 7S protein fraction; these results clearly indicate that the calcium ion stabilizes the structure of 11S more than that of 7S. The effect of salts on the thermal stability of soybean 11S globulin was studied earlier by Bikbov et al. (1983) and Danilenko et al. (1986). They saw that at pH 7.6, the T_d increased with rises in salt concentration from 0 to 1 M. Ammonium sulfate was more efficient than sodium or potassium chloride in enhancing thermal stability. Damodaran (1988) showed that at higher salt concentrations, NaCl and NaBr progressively increased the thermostability of both 7S and 11S globulins, while $NaClO_4$ and NaSCN decreased their T_d values. The magnitude of the salt-induced effect on the stability of 7S and 11S globulins was almost the same (Damodaran, 1988). The improved stability associated with higher concentrations of stabilizing salts has also been noted for other plant protein isolates such as faba bean (Arntfield et al., 1986). An increase in salt concentration (NaCl, KCl, Na_2SO_4, $NaC_2H_3O_2$) resulted in increased thermal stability for both vicilin and legumin present in faba bean isolates, although the relative increase in T_d was not the same for the two (Arntfield et al., 1986).

The ΔH values of the soybean protein isolates without heat treatments were determined and are shown in Table 1. Determinations were at least performed in duplicate, and the coefficients of variation remained within the 2.8−9.0% range. No significant differences ($p \leq 0.05$) were observed in the ΔH values corresponding to these samples, except for numbers 2 and 3, which showed ΔH values higher than the rest.

Each $\Delta T_{1/2}$ value determination was done at least in duplicate; coefficients of variation for these data remained within the same range as obtained for T_d. The $\Delta T_{1/2}$ found for these isolates showed no significant differences ($p \leq 0.05$) between themselves, except for the higher levels of calcium (Table 1, samples 7 and 8); in these cases, a decrease in the $\Delta T_{1/2}$ values was observed, indicating an increase on the cooperativity of the process.

The samples which were heated for 5 min at 80 °C, before or after resuspension, exhibited endotherms which have a lower ΔH (Table 2) than the unheated samples ($p \leq 0.05$) due to the denaturation produced by thermal treatment (Figure 1b). The T_d of these samples showed no significant differences ($p \leq 0.05$) at the calcium levels studied (Table 2). When the samples were heated before resuspension for 15 min at 90 °C, no endotherms were recorded. In the isolates heated under the same condition, after calcium addition, an endotherm began to record calcium values above 5 mg/g

Table 2. Treatment Conditions, Temperature, and Enthalpies of Denaturation

soybean isolates	thermal treatment	before re-suspension	after re-suspension	T_d (°C) 7S	T_d (°C) 11S	ΔH (J/g)
Samples with 1.23 mg of Calcium/g of Protein						
2	no	−	−	77.02	93.50	18.48
9	5 min, 80 °C	+	−	74.00[a]	93.20	5.10
10	5 min, 80 °C	−	+	77.50[a]	94.50	4.00
11	15 min, 90 °C	+	−			0.00
12	15 min, 90 °C	−	+			0.00
Samples with 4.52 mg of Calcium/g of Protein						
6	no	−	−	76.06	94.70	14.70
13	5 min, 80 °C	+	−	73.50[a]	94.50	4.80
14	5 min, 80 °C	−	+	75.02[a]	95.50	4.30
15	15 min, 90 °C	+	−			0.00
16	15 min, 90 °C	−	+			0.00

[a] In these endotherms the 7S fraction is very small.

Table 3. Temperatures and Enthalpies of Denaturation: Samples with Intensive Thermal Treatment (15 min, 90 °C)

mg of calcium/ g of protein	T_d (°C) 7S	T_d (°C) 11S	ΔH_T (J/g)	$\Delta T_{1/2}$ 11S (°C)
0				
1.02, 1.23, 1.85, 2.77, 3.70, 4.52				
5.11		95.70	1.93	8.75
6.51		97.02	3.80	7.77
7.24	79.12[a]	98.33	5.28	7.83
9.23	78.02[a]	99.75	9.85	7.50
9.73	79.25[a]	98.97	13.14	7.00

[a] In these endotherms the 7S fraction is very small.

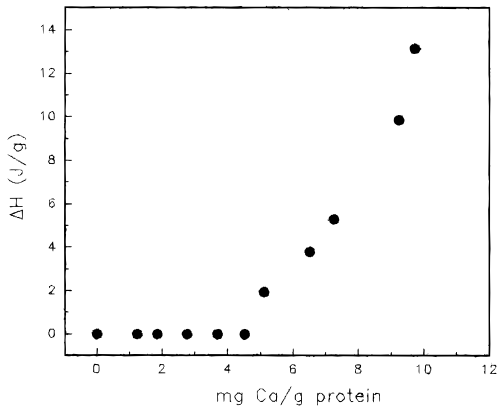

Figure 2. Denaturation enthalpy changes for soybean protein isolates heated for 15 min at 90 °C as a function of calcium content.

of protein (Figure 1c). In these cases, the ΔH values increased when the calcium content increased, reaching values of approximately 15 J/g for protein isolates with the greatest calcium content studied (Figure 2, Table 3). These results corroborate the hypothesis that the calcium ion exerts a protective effect on soybean proteins, especially on the 11S fraction. It was observed that the thermal treatment (15 min at 90 °C), with either no or low calcium levels, resulted in denaturation by heat ($\Delta H = 0$). At this time, only partial denaturation occurred at calcium levels above 5 mg/g protein. The T_d values for the 11S fraction (Table 3) also showed a shift toward higher values with the calcium increase ($p \leq 0.05$), as was shown with the unheated samples (Table 1). The endotherm for the 7S fraction was greatly decreased; for that reason, the T_d determination became quite difficult (Table 3). In the cases when endotherms were recorded, the $\Delta T_{1/2}$ values for the 11S were measured. The samples that underwent intense

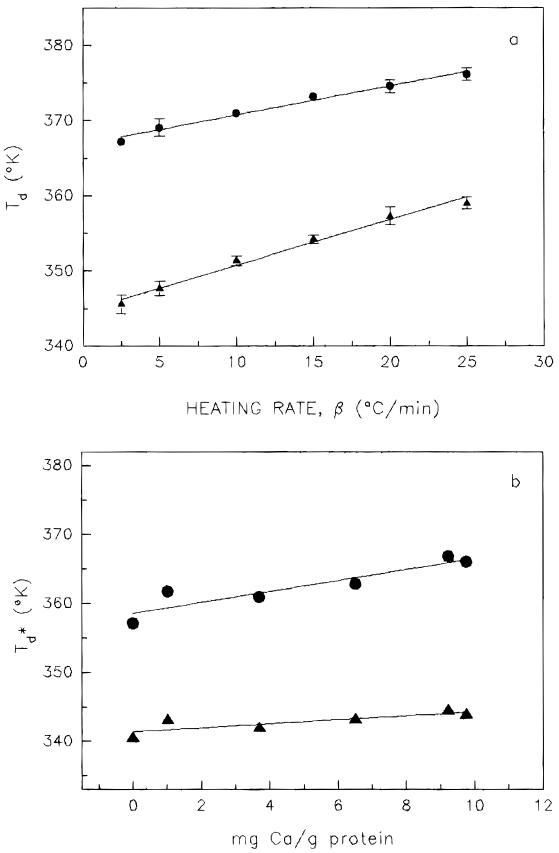

Figure 3. Temperature of denaturation (T_d) (a) as a function of heating rate and (b) extrapolated at $\beta = 0$ °C/min (T_d*) as a function of the calcium content: (▲) 7S fraction and (●) 11S fraction.

thermal treatment (Table 3) showed an increase in the process cooperativity as the calcium content was increased and also with regard to the unheated samples (Table 1). The decrease in $\Delta T_{1/2}$ suggested that glycinin had, in these conditions (intensive heat treatment and calcium), a more compact and ordered structure than the native conformation.

Thermal Denaturation Kinetics of Soybean Protein Isolates. It is known that the heating rate (β) modifies the T_d obtained (De Wit and Swinkels, 1980). In order to become β independent, and with the aim of studying the effect of calcium content on the denaturation temperatures, the T_d* as a function of calcium content was graphed (Figure 3b). For that purpose, the T_d obtained at different heating rates for the two fractions of all the isolates studied was also graphed (Figure 3a). It shows, as an example, the straight lines obtained for the isolate containing 9.23 mg of calcium/g of protein; the correlation coefficients found for these regressions were 0.9934 and 0.9918 for 7S and 11S, respectively. The T_d*, T_d extrapolated to $\beta = 0$ °C/min, was obtained from the ordinate to the origin. Figure 3b illustrates the T_d* found for the 7S and 11S fractions of the protein isolates studied; each data point was obtained from the mean of at least three endotherms. The coefficients of variation for the determination of T_d were, in all cases, within the 0.49−1% range. It was observed that at higher calcium levels, the T_d* underwent a shift toward higher values, this effect being more pronounced for the 11S and rather low for the 7S, as was observed for $\beta = 10$ °C/min. These results agree with those reported by Appu Rao and Narasinga Rao (1976), who observed that the 11S soybean protein binds

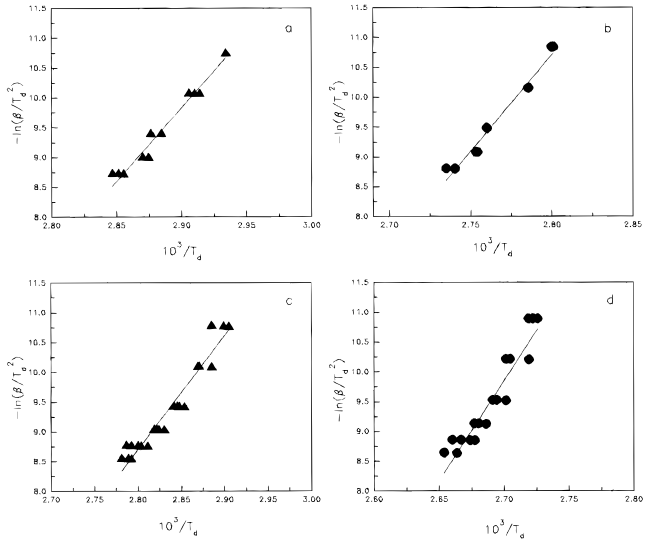

Figure 4. Values and linear regression for $\ln(\beta/T_d^2)$ as a function of $1/T_d$ for soybean protein isolates neutralized with NaOH (a and b) and soybean protein isolates neutralized with $Ca(OH)_2$ and NaOH (c and d). The amount of calcium in this isolate was 9.23 mg/g of protein. Regression was applied to data corresponding to the 7S fraction (▲) and 11S fraction (●). Regression coefficients (r^2) were 0.9845, 0.9889, 0.9676, and 0.9636 for a−d, respectively.

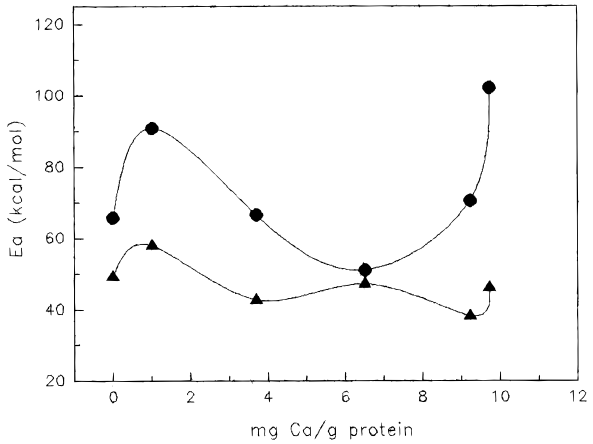

Figure 5. Effect of calcium content on the energy of activation for soybean protein isolates: E_a corresponding to the 7S fraction (▲) and 11S fraction (●).

approximately 2 times as much calcium as the 7S fraction.

The kinetic study of the thermal denaturation process of the soybean isolate proteins was carried out by using the Ozawa method (1970). Figure 4 shows the straight lines obtained for the two fractions, 7S and 11S, $\ln(\beta/T_d^2)$ vs $1/T_d$ for a sodium proteinate and another one of sodium and calcium. The coefficients of such regressions were acceptable in all cases.

Application of this method allowed the determination of the E_a, the pre-exponential factor of the Arrhenius equation, and other kinetic parameters. The activation energies were calculated for the soybean isolates that did not undergo a prior thermal treatment, as from the slope or the straight lines of the linear regression obtained (Figure 4), and they were graphed as a function of the calcium content (Figure 5). A significant variation of the thermal denaturation E_a of the 11S fraction (51.12−102.15 kcal/mol) was found, while the same process E_a for the 7S fraction remained in the smallest range, between 42.69 and 58.05 kcal/mol. These E_a

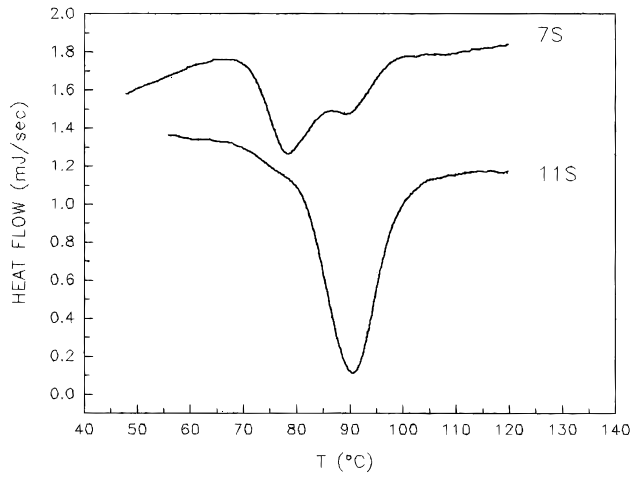

Figure 6. Thermal curves of the 7S and 11S soybean globulin rich fractions.

values were of the same order as those for other proteins in the literature: between 100 and 120 kcal/mol for oat globulin (Ma and Halwarkar, 1988) and β-lactoglobulin (Park and Lund, 1984) and 98 kcal/mol for wheat germ proteins (Lupano and Añón, 1985). The minimal value in the 11S fraction E_a was observed for the isolate containing 6.51 mg of calcium/g of protein increasing when the level of calcium is changed. Therefore, it is the lowest energy barrier to be overcome by the macromolecules to reach the activated state and denature by the effect of temperature. As calcium was added (between 1 and 5 mg/g of protein), the ion binds to the protein, mainly the 11S fraction (Appu Rao and Narasinga Rao, 1975; Kroll, 1984). This binding would produce an increase in the protein internal charge, thus increasing the repulsive electrostatic forces and weakening the noncovalent forces. In this way, the protein thermal denaturation is easier to obtain. For calcium contents above 6.51 mg/g of protein, the ions would start making saline bridges that would stabilize a new structure, reinforced by noncovalent bindings which would make the unfolding by the heat effect difficult. In our laboratory, we have detected, for higher calcium levels, an important water solubility decrease in isolates that were not submitted to a thermal treatment (results have not been shown). This value is also similar to the calcium content that produces protection against intense thermal treatment in the studied samples. Appu Rao and Narasinga Rao (1975) observed that the addition of calcium to the 11S fraction treated with EDTA changed its sedimentation rate which suggested protein aggregation. These glycinin conformational changes caused by calcium aggregation were not observed for β-conglycinin.

Thermal Denaturation Kinetics of 7S and 11S Rich Fractions. Figure 6 shows the thermograms of soybean globulin rich fractions 7S and 11S. The T_d for these preparations were 78.54 ± 0.42 and 90.15 ± 0.26 °C, and the denaturation enthalpies were 9.721 ± 0.809 and 23.451 ± 0.674 J/g, respectively. In the ΔH calculation for the 7S fraction, the area used excluded the portion corresponding to the presence of a small amount of 11S as protein as described in the Materials and Methods section.

The Borchartd and Daniels method (1957) was applied to the data. In order to determine the apparent reaction order of the thermal denaturation process of the 7S and 11S fractions, diverse straight lines, ln k vs $1/T_d$, varying the reaction order were carried out. Using a statistical calculation program, SYSTAT (Wilkinson, 1990), the adjustment to the model by means of residual analysis vs independent variable was studied. It was corroborated that the n allowing the best adjustment of the data to the model was the same as that showing the best linear correlation coefficient. The apparent reaction orders thus determined were $n = 3.5$ and 2 for the 7S and 11S fractions, respectively. Other authors have demonstrated the reaction order of 7S globulins (phaseolin) of *Phaseolus vulgaris* (Hohlberg and Stanley, 1987) and of oat globulin (Ma and Halwakar, 1988); both found that n for thermal denaturation was about 2.5. Iwabuchi et al. (1991) have studied the kinetics of this process for the β-conglycinin by spectrophotometric methods and concluded in their work that the apparent reaction order was greater than 2. A reaction order higher than unity may be attributed to polymolecular denaturation reactions, presence of intermediate products, or dissociation of polymeric proteins before denaturation (Hohlberg and Stanley, 1987; Ma and Halwarkar, 1991). Also, it can be attributed to the existence of multiple domains in proteins, which may posses regions with different thermal stability (Iwabuchi et al., 1991).

Furthermore, the E_a values of the soybean globulin rich fractions were calculated by the Borchardt and Daniels method (1957), from the slopes of the previously mentioned straight lines. The E_a value obtained for 11S globulin (79.96 ± 1.46 kcal/mol, $r = 0.999$) was similar to that obtained by Watanabe (1988) and somewhat above that calculated with the Ozawa method (1970) from a protein soybean isolate. The E_a of the 7S fraction was higher than that given by other authors (Iwabuchi et al., 1991) (67 kcal/mol) and also higher than that calculated in this work from a protein isolate of soybean globulins with the Ozawa method (1970). This result could be explained considering that an important error is introduced when assigning the area corresponding to 7S due to this protein as a not-purified fraction.

CONCLUSION

The results obtained through differential scanning calorimetry indicate that calcium interacts preferably with the 11S fraction, confirming previous results (Appu Rao and Narasinga Rao, 1975; Kroll, 1984). This interaction is reflected in the changes in thermal properties evidenced by a greater thermal stability of protein as calcium content is increased (1.23−9.73 mg of calcium/g of protein). Furthermore, variations in the activation energy corresponding to the denaturation process by heating were detected. The glycinin E_a reached a minimal value (51.12 kcal/mol) for a calcium content of 6.51 mg/g of protein; this E_a value increases when increasing or decreasing the calcium ion levels. These variations would be the consequence of the conformational changes produced in the protein by the presence of calcium.

The glycinin E_a values determined by the methods of Ozawa and Borchardt and Daniels were similar to those reported for other proteins. According to the apparent reaction orders found for the thermal denaturation process of 11S and 7S fractions, in both cases, intermediate products or products arising from the dissociation of the native structure prior to the unfolding were formed.

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