Partial Reduction of Soy Protein Isolate Disulfide Bonds

Silvana Petruccelli and María Cristina Añón*

Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Consejo Nacional de Investigaciones Científicas y Técnicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calle 47 y 116, 1900 La Plata, Argentina

Partial reduction of disulfide bonds of soy protein isolates was followed electrophoretically. Isolates treated with Na_2SO_3 under different conditions showed disappearance of high molecular weight aggregates. Acidic and basic 11S polypeptides and some whey proteins that remain in the isolates were also affected; reduction of the AB-11S subunit was very limited. The sulfitolysis method was also studied. The addition of a catalyst (Cu) and oxygen showed a similar effect in the sulfitolysis of soy proteins with Na_2SO_3. To achieve complete sulfitolysis, the presence of a denaturing and an oxidizing agent were needed. Mainly AB subunits of glycinin were reduced when urea was used, while mostly components other than AB-11S subunits were reduced when Na_2SO_3 was used in the presence of Cu and/or oxygen.

Keywords: *Soybean isolates; reduction; soy proteins*

INTRODUCTION

Soy isolates contain 90% protein, the major components being glycinin (11S protein) and β-conglycinin (7S protein) (Nash and Wolf, 1967; Hermansson, 1978). These fractions constitute 61% of the isolate proteins (34 and 27%, respectively); the rest consists of whey proteins, such as τ-conglycinin, 7S basic globulin, lipoxygenase, β-amylase, agglutinins, and trypsin inhibitors (Iwabuchi and Yamauchi, 1987a,b).

Glycinin is a heterogeneous oligomeric protein, the molecular mass of which varies between 340 and 375 kDa (Utsumi et al., 1981). It is made up of six subunits, each consisting of a basic polypeptide (B polypeptide) and an acidic polypeptide (A polypeptide), which are connected by a single disulfide (SS) bond forming the AB subunits (Badley et al., 1975).

Disulfide bonds play an important role both in maintaining the structure of these and other proteins and in determining some of their physicochemical properties. Reduction of these bonds may improve the functional properties, leading—according to the protein species—to an increase in solubility; this, in turn affects properties such as gelation, foaming, and emulsification. The reduction can produce an increase of the molecular flexibility, too. This latter modification would improve the surface properties, affecting both the formation and stability (Kella et al., 1986, 1989; Kim and Kinsella, 1987; Klemaszewski and Kinsella, 1991).

Sodium sulfite is a reducing agent that is permitted in foods. Although its oxidation–reduction potential is lower than those of other reducing agents such as cysteine, 2-mercaptoethanol, and dithiotreitol, it is preferred because one of the protein sulfhydryls resulting from disulfide bond cleavage by Na_2SO_3 remains blocked as a sulfonate group, thus preventing its further reoxidation. Moreover, Na_2SO_3 increases the protein net charge, and this may lead to an improvement of functional properties.

The aim of the present study was to analyze the modifications arising from the reduction of SS bonds of soy proteins and to determine the process conditions that lead to isolates having different degrees of reduction.

MATERIALS AND METHODS

Preparation of Isolates. Protein isolates were prepared from defatted soy flour (Sanbra S. A. Brazil), which was extracted with water at pH 8, made alkaline with 2 N NaOH (flour/water ratio 1:10, w/v) for 2 h at room temperature; pH was periodically adjusted. The suspension thus obtained 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, suspended in water, and adjusted to pH 8. The suspension was then freeze-dried.

Protein Determination. Protein concentration was determined according to the biuret method, using bovine albumin as standard (Gornall et al., 1949).

Synthesis of 2-Nitro-5-thiosulfobenzoate (NTSB). Synthesis of NTSB was performed according to the method of Thannhauser et al. (1984), with minor modifications, as follows: 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB; 0.1 g) was dissolved in 10 mL of 1 M Na_2SO_3. The pH of the reaction mixture was adjusted to 7.5, and 50 μL of a 0.1 M ammoniacal solution of $CuSO_4$ was added. The reaction was started by shaking at a rate of 120 oscillations/min, at 38 °C; it was ended when more than 99% of the NTSB had been produced. The remaining 2-nitro-5-thiobenzoate (NTB) was determined by measuring the absorbance at 412 nm. The stock solution was stored at −80 °C.

The test NTSB solution was prepared by diluting the stock solution 1:100 in a fresh 0.2 M Tris base solution containing 0.1 M Na_2SO_3, 10 mM EDTA, and 3 M guanidinium thiocyanate. This solution was adjusted to pH 9.5 with 1 N HCl.

Determination of Disulfide and Sulfhydryl Content. Determination of disulfide and sulfhydryl content was carried out according to the method of Thannhauser et al. (1984) by mixing 70 μL of the solution of the protein isolate (10 mg/mL) with 1 mL of the NTSB test solution prepared just prior to use. Absorbance at 412 nm was determined at 20 min using the test NTSB solution as reference. The extinction coefficient used to transform absorbance values into concentration values was 13 600 M^{-1} cm^{-1}.

Determination of Free Sulfhydryl Groups. Free SH (SH_F) groups were determined according to the procedure of Beveridge et al. (1974). Fifty milligrams of the protein isolate was dissolved in 5 mL of 0.086 M Tris buffer, 0.09 M glycine, 0.004 M EDTA, and 8 M urea, pH 8. Forty microliters of Ellman's reagent (4 mg/mL in methanol) was added to 1 mL aliquots, the absorbance being determined at 412 nm 15 min later. In the case of the isolates containing Na_2SO_3, they were

0021-8561/95/1443-2001$09.00/0

dissolved in water and then the Na_2SO_3 was eliminated using a Sephadex G-25 column. After this treatment, the determinations were done as we described before.

Bovine albumin dissolved in 8 M urea was used for the calibration curve. Determinations were performed at least in duplicate.

Treatment of Isolates with Sodium Sulfite. *Effect of the Concentration of the Reducing Agent.* Soy isoelectric precipitates (100 mg/mL) were treated with different amounts of Na_2SO_3 (0.01−1.0%) and were then adjusted to pH 8 with 2 N NaOH. A group of samples was kept at 50 °C for 60 min and at 100 °C for 90 min, whereas another group remained at room temperature.

Effect of pH. The isolate preparation was adjusted to different pH values (7, 8, 9, and 10) either in the presence or in the absence of 1% Na_2SO_3. Isolates were, in turn, treated either at 100 °C or at room temperature for 90 min.

Effect of Denaturing Agents. The isoelectric precipitate was carried out at pH 8, either with or without addition of 0.1% Na_2SO_3 and 8 M urea, followed by dialysis for 72 h at 4 °C against distilled water. All determinations were performed in duplicate.

Kinetics of the Sulfitolysis Reaction. Fifty milliliters of soy protein isolate solution (10 mg/mL) in 0.1 M phosphate buffer, pH 7, containing 0.1 M Na_2SO_3 was prepared in a 125 mL Erlenmeyer flask. The solution was incubated for 15 min at 42 °C with shaking. A 0.1 M ammoniacal solution of $CuSO_4$, pH 10, was added to obtain either 1.33 or 4.0 mM Cu^{2+} concentration in the reaction mixture. Sulfitolysis was started by shaking at a rate of 120 oscillations/min. It was assumed that under these conditions the solution is saturated with oxygen throughout the reaction time (Kella and Kinsella, 1985; Gonzalez and Damodaran, 1990). The course of the reaction was observed by stopping the reaction by adding 0.1 mL of 0.2 M EDTA, pH 7, to 0.4 mL of the reaction mixture. Concentration of the remaining SS bonds was determined as described above. Protein content was determined in a similar sample, with no EDTA addition, by the biuret method. When copper was not added, the determination of SS bonds was performed directly at each reaction time. When neither copper addition nor shaking took place, times of reaction were measured from the time at which Na_2SO_3 was added. To determine the effect of the concentration of the reducing agent on the reaction, different amounts of Na_2SO_3 were added to solutions of the soy protein isolate (8 mg/mL in 0.1 M phosphate buffer) at 42 °C and at room temperature. Samples were taken at different times after addition of the reducing agent, and total sulfhydryl groups were determined. Determinations were performed in duplicate.

Electrophoresis. Electrophoresis was performed using a continuous and dissociating buffer system: 0.375 M Tris-HCl, pH 8.8, and 0.1% SDS for the separating gel and 0.025 M Tris-HCl, 0.192 M glycine, and 0.1% SDS, pH 8.3, for the running buffer (Laemmli, 1970). A 5−15% polyacrylamide gradient was used. Densitometry corresponding to each gel was obtained by means of a TLC scanning CS-910 double-wavelength Shimadzu spectrodensitograph. Wavelengths used were 570 and 395 nm for the sample and reference, respectively. Determinations were performed in duplicate.

Differential Scanning Calorimetry. DSC studies were performed in a DuPont 910 system attached to a Hewlett-Packard 7046B recorder. The temperature calibrations were performed according to ASTM Norm E 474/80 using indium and ice melting thermograms.

The samples (13−20 mg wet weight) were placed in DSC hermetic aluminum pans. After DSC analysis, the capsules were punctured and the dry matter weight determined by drying at 105 °C overnight. As a reference an empty double capsule was used. The endotherm areas were measured with a Morphomat 34 Zeiss (1% error) image analyzer. Triplicate samples were evaluated by DSC.

RESULTS AND DISCUSSION

Chemical reduction of SS bonds in excess Na_2SO_3 was assessed by electrophoresis to avoid the interference of sulfite on the quantification of free sulfhydryl groups when DTNB is used. Figure 1 shows the densitometry scans corresponding to the soy protein isolates (100 mg/mL) reduced at 25 °C with different amounts of Na_2SO_3 (0.01−1.0%) and to nonreduced sample. The isolate that did not undergo a reducing treatment exhibits—in addition to the fractions corresponding to the α′, α, and β subunits of β-conglycinin—the AB-11S subunit, aggregates of molecular mass in the range of 100−200 kDa. In all analyzed cases, isolates treated with Na_2SO_3 show disappearance of high molecular mass aggregates (100−200 kDa), an increase of the proportion of the α′ and α-7S subunits, a gradual decrease of the AB-11S subunit, and a slight increase of A- and B-11S polypeptides.

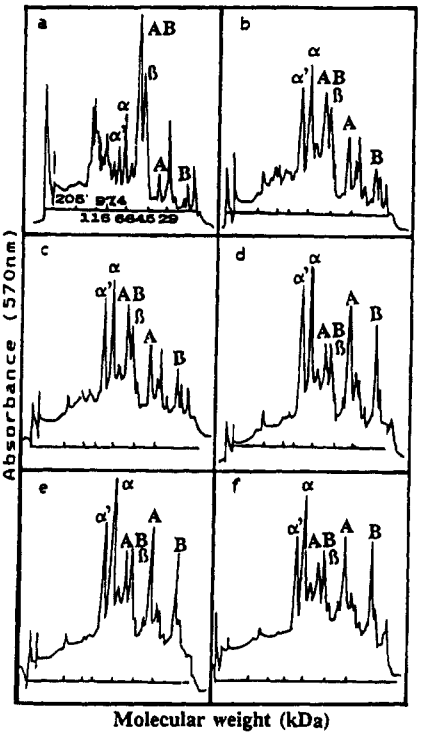

Figure 1. SDS−PAGE densitometry in a 5−15% linear gradient of soy protein isolates (100 mg/mL) treated for 90 min at 25 °C with different concentrations of Na_2SO_3: nonreduced isolate (a), 0.01% (b), 0.05% (c), 0.25% (d), 0.5% (e), and 1.0% (f), respectively.

DSC studies show that the treatment of soy proteins with increasing amounts of Na_2SO_3 (0.05−10%) leads to an increase of their thermal stability, as indicated by the shift of the maximum temperature peak toward higher values, for the endotherms corresponding to both the 7S protein (first endotherm) and the 11S protein (second endotherm). This shift can be attributed to a salt effect (Hermansson, 1978; Damodaran and Kinsella, 1982). A slight decrease of the enthalpy of denaturation of glycinin is also observed (ΔH = 7.69 ± 0.84 and 9.42 ± 0.58 J/g for samples treated with 10 and 0.05% Na_2SO_3, respectively) (Figure 2). This effect could be attributed to an exothermic aggregation.

A decrease of the protein concentration, as well as an increase of the temperature at which the reducing treatment is performed, leads to an increase of the extent of the reduction attained (Figures 3 and 4). At protein concentrations of 50 and 100 mg/mL, an increase of the Na_2SO_3 concentration from 0.1 to 1% (Figure 3c,a; d,b, respectively) increases the extent of reduction of the AB-11S subunit; in no case is the presence of high molecular mass aggregates observed. On the other hand, at a protein concentration of 10 mg/mL (Figure 3e,f), an even higher degree of reduction is achieved,

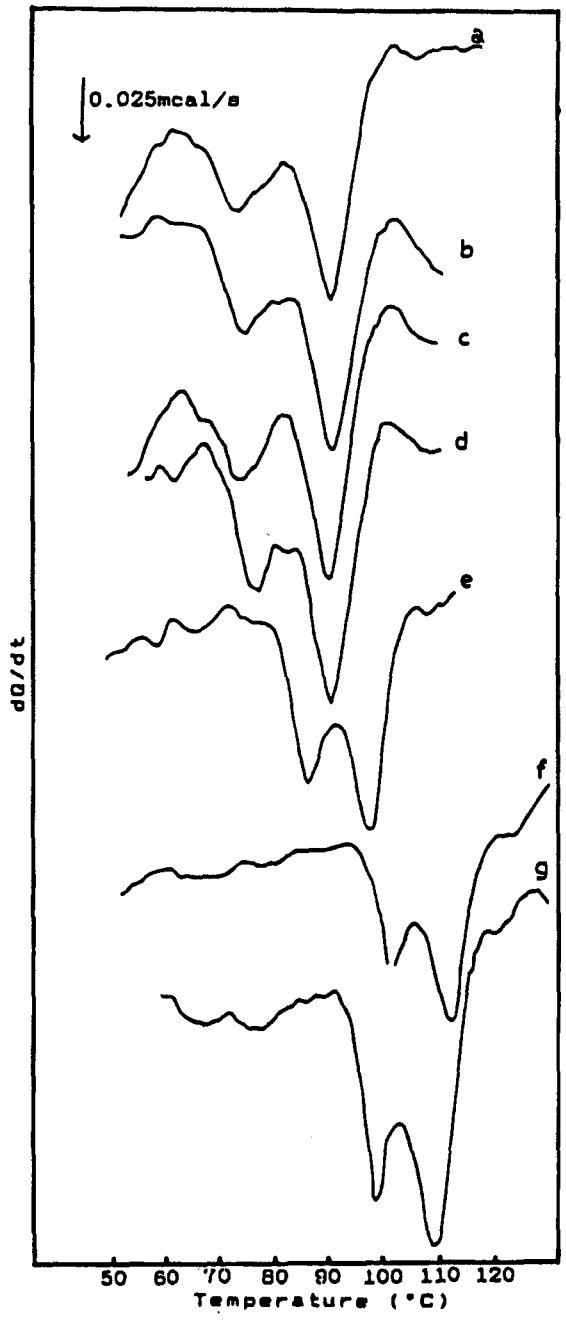

Figure 2. DSC thermograms of soy protein isolates treated with different concentrations of Na_2SO_3: control sample (a), 0.05% (b), 0.2% (c), 0.5% (d), 2% (e), 5% (f), and 10% (g), respectively.

both Na_2SO_3 concentrations used being saturating. An increase of the reaction temperature from 20 to 50 °C has no effect on the degree of reduction, but an increase in reduction occurs when samples are treated at 100 °C. At a Na_2SO_3 concentration of 0.01%, heating at 100 °C produces a slight increase of the reduction level, while with higher sulfite concentration a greater effect is obtained. It should be noticed that the heights of the peaks corresponding to A- and B-11S polypeptides are greater than the height of the peak corresponding to the β-7S subunit (Figure 4). Addition of denaturing agents, such as 8 M urea, which unfold the protein molecules and lead to a greater exposure of SS bonds, also favors the reduction reaction; complete disappearance of the AB-11S subunit is then observed, together with the simultaneous increase of A and B polypeptides (Figure 5).

Table 1 shows values of the free SH groups detected under different reaction conditions. It can be noticed

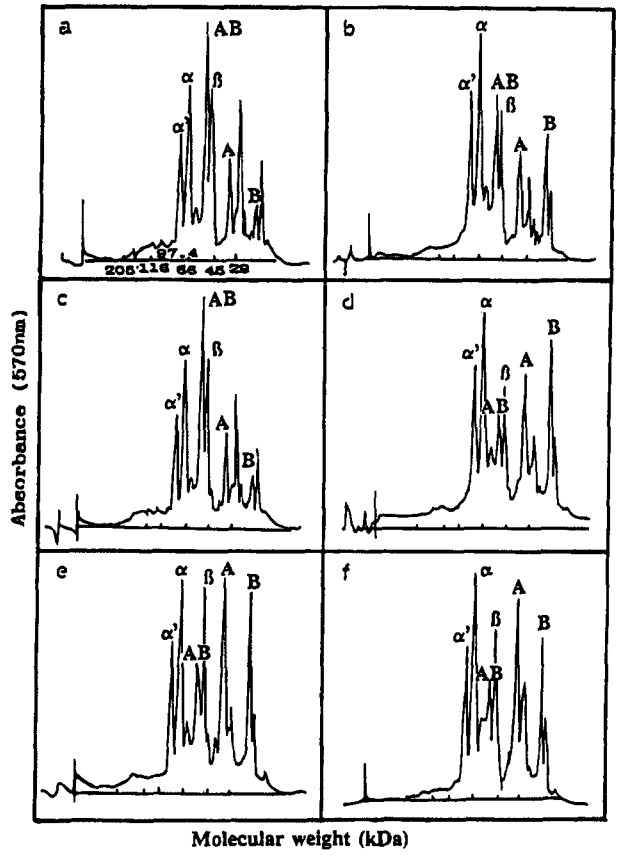

Figure 3. SDS–PAGE densitometry scans in a 5–15% linear gradient of soy isolates treated for 90 min at 25 °C with 0.1% (a, c, and e) and 1.0% Na_2SO_3 (b, d, and f) at different protein concentrations: 100 (a, b), 50 (c, d), and 10 mg/mL (e, f), respectively.

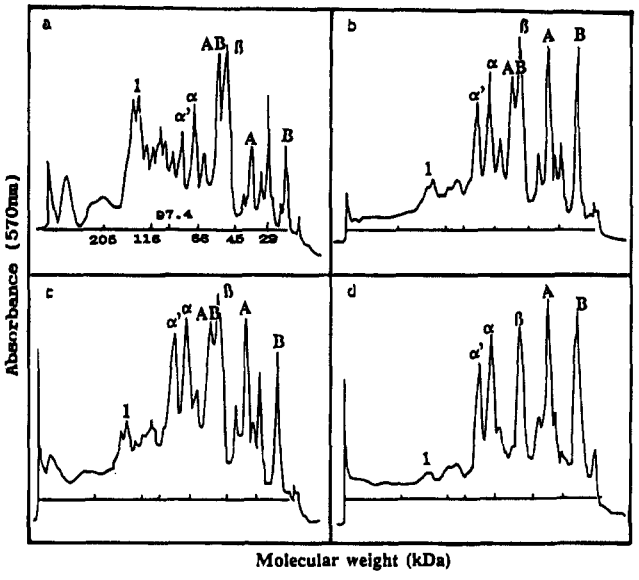

Figure 4. SDS–PAGE densitometry scans in a 5–15% linear gradient of soy protein isolates (80 mg/mL) treated for 90 min at different temperatures: 20 (b), 50 (c), and 100 °C (d) in the presence of 0.1% Na_2SO_3 and of a nonreduced isolate treated for 90 min at 20 °C (a).

that in samples treated with 8 M urea, the content of free SH groups (4.97 ± 0.09 μmol/g of isolate) is lower than that corresponding to the complete reduction of the 11S protein. According to the results obtained by Wolf (1993), the fully reduced glycinin contains 41 SH groups/mol, which is equivalent to 118 μmol/g of 11S, assuming a molecular mass of 350 kDa (Badley et al., 1975). If it is also considered that the soy protein isolate used

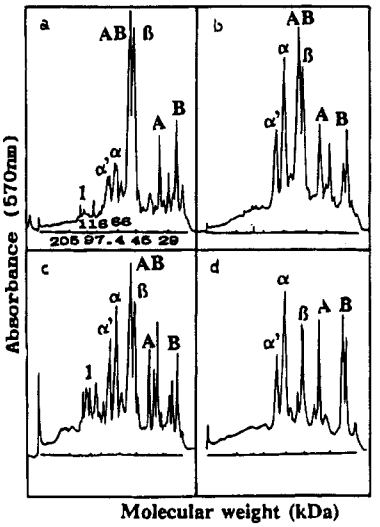

Figure 5. SDS–PAGE densitometry scans in a 5–15% linear gradient of soy protein isolates (10 mg/mL) treated with Na_2SO_3 in the presence of urea: control without urea and Na_2SO_3 (a), without urea, with 1% Na_2SO_3 (b); with 8 M urea, without Na_2SO_3 (c), and with 8 M urea and 1% Na_2SO_3 (d).

Table 1. Free SH Groups of Soy Isolates Detected under Different Reaction Conditions

	μmol of SH/g of protein	
sample	without urea	with 8 M urea
without Na_2SO_3	1.48 ± 0.09	1.34 ± 0.05
1.26% Na_2SO_3	3.31 ± 0.07	4.97 ± 0.09

	μmol of SH/g of protein		
sample	not heated	50 °C, 60 min	100 °C, 90 min
without Na_2SO_3	1.54 ± 0.02	1.27 ± 0.01	0.04 ± 0.01
0.1% Na_2SO_3	4.54 ± 0.03	3.57 ± 0.21	7.06 ± 0.27

contains approximately 34% 11S fraction and 90% protein, the total SH groups reached a value of 40 μmol/g isolate. In turn, an increase of the temperature of treatment provokes a significant increase of the number of free SH groups.

The above results show clearly that the reduction of soy protein isolates with Na_2SO_3 under different conditions affects mainly those aggregates having molecular masses in the range of 100–200 kDa, which form again when the reducing agent is removed. Low molecular mass components, such as A- and B-11S polypeptides, and some whey proteins that remain in the isolate are also affected, reduction of the AB-11S subunit being very limited under the conditions used in the present work.

To increase the extent of reduction achieved, the sulfitolysis method based on reduction—by means of Na_2SO_3—of the SS bonds exposed to the solution and the further oxidation of the free SH groups thus formed, was studied next.

This method has been used to produce complete reduction of proteins such as bovine serum albumin, trypsin, and chymotrypsin, which, in the absence of these oxidizing agents, are completely sulfonated only in the presence of denaturing agents (Kella and Kinsella, 1985).

The degree of reduction of the isolates was monitored through the number of disulfide bonds and sulfhydryl groups according to the Thannhauser method. These groups originate both by reduction of SS bonds with Na_2SO_3 (which produces a free sulfhydryl group and a sulfonate group) and from the sulfhydryl groups already present in the protein under study.

Figure 6 shows the effect of the addition of an ammoniacal solution of $CuSO_4$ on the reduction with

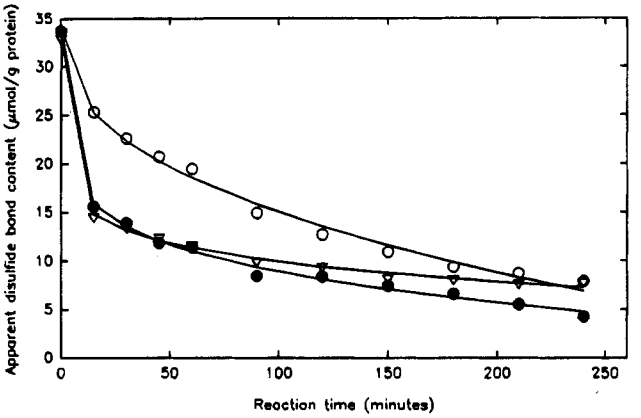

Figure 6. Effect of the addition of ammoniacal $CuSO_4$ on the reduction of soy protein isolates (8 mg/mL) treated at 42 °C for different times with 0.1 M Na_2SO_3, with shaking at 120 oscillations/min. Concentrations of $CuSO_4$ used were 0 (○), 1.33 (▽), and 4.00 (●) mM.

0.1 M Na_2SO_3 (1.26%) at 42 °C of a solution of a soy protein isolate (8 mg/mL) in 0.1 M phosphate buffer, pH 7.0. Initial values of disulfide bonds plus free sulfhydryl groups and free SH groups (SH_F) of the sample were 33.75 ± 0.45 and 1.51 ± 0.30 μmol/g, respectively. Since the free sulfhydryl content is negligible as compared to values determined by the Thannhauser method, it is possible to assume that the majority of color in the NTSB method is due to protein disulfide bonds. For this reason the values determined by the Thannhauser method are expressed as apparent disulfide bond content (SS_{app}). If Cu is absent, a gradual decrease of the number of apparent disulfide bonds (SS_{app}) is detected, reaching, at 240 min of treatment, values similar to those attained with 1.33 or 4.00 mM $CuSO_4$ (7.8 ± 0.15 μmol of SH/g of protein). The effect of the catalyst is very fast; over 50% of the SS_{app} present are reduced at 15 min of reaction. This is followed by a gradual decrease, reaching reduction values greater than 75%. Differences in the reduction process occurring as a consequence of the different Cu concentrations used in this study are negligible, thus suggesting that those concentrations are saturating.

Figure 7 shows the effect of shaking on the reducing process. Shaking alone (Figure 7B) leads to a gradual decrease of the apparent disulfide bonds (SS_{app}), reaching values similar to those observed in the presence of Cu. This gradual decrease is related to the rate of oxygen uptake due to shaking. At 1 min of reaction in the presence of 1.33 mM Cu, it can be observed that the amount of apparent disulfide bonds (SS_{app}) decreased to 15.00 ± 0.25 μmol/g; at longer reaction times the rate decreases, reaching at 1 h values of about 9.00 ± 0.16 and 12.00 ± 0.88 μmol/g of protein, depending on whether the isolate had been shaken or not.

Reduction of SS_{app} is somewhat faster at higher temperatures, but differences are unimportant. Figure 8 shows the effect of temperature corresponding to two Na_2SO_3 concentrations, in the absence of Cu and with no shaking. Reduction is practically independent of temperature at 1% Na_2SO_3 and 3 h of treatment. At longer times it becomes somewhat faster at 42 °C. Reduction is faster at 25 °C than at 42 °C at a Na_2SO_3 concentration 100 times lower. It is likely that the increase of temperature favors both the reduction reaction and further reoxidation, modifying the amount of oxygen dissolved in the medium as well.

When the lower concentration of Na_2SO_3 (0.01%) is employed, the extent of reduction achieved is very low

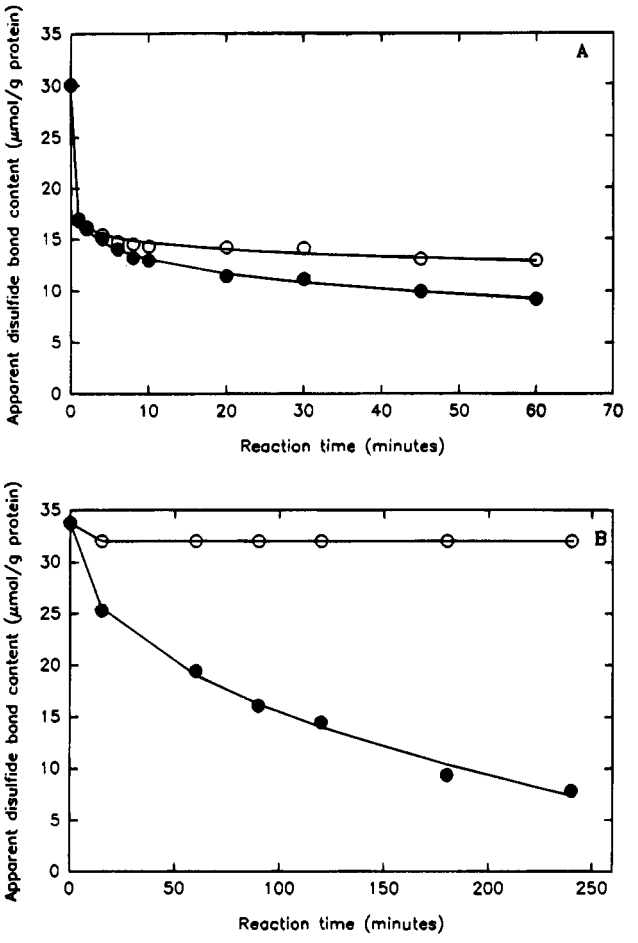

Figure 7. Effect of shaking on the reduction of soy protein aggregates (8 mg/mL) treated with 0.1 M Na_2SO_3, either in the presence of 1.33 mM ammoniacal $CuSO_4$ (A) or in its absence (B), at 42 °C for different lengths of time with no shaking (○) and with shaking at 120 oscillations/min (●).

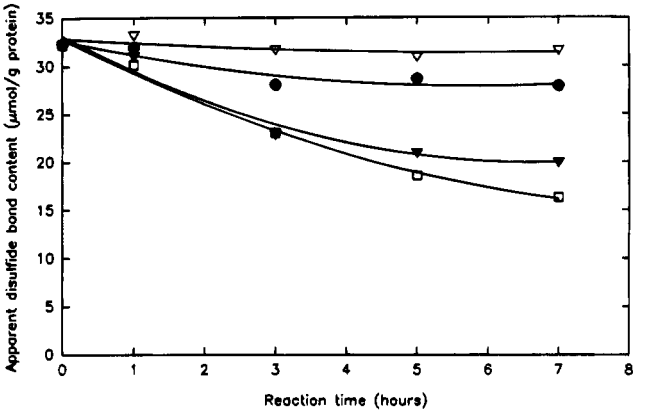

Figure 8. Effect of temperature and Na_2SO_3 concentration on the reduction of soy protein isolates (8 mg/mL). The treatment was carried out in the absence of ammoniacal $CuSO_4$ and with no shaking. Samples were treated with 0.01% Na_2SO_3 at 25 °C (●) and 42 °C (▽) and with 1.0% Na_2SO_3 at 25 °C (▼) and 42 °C (□).

(12% at 25 °C and 6% at 42 °C); this probably occurs because this is the concentration stoichiometrically needed to reduce all SS bonds present in the isolate. This results in the balance of the reduction equation being only a little shifted and would also explain why the reaction is faster at 25 °C.

The reduction reached when 1.0% concentrations of Na_2SO_3 are used is approximately 77% when Cu is used or when O_2 is introduced by shaking; this value decreases to 40% in their absence.

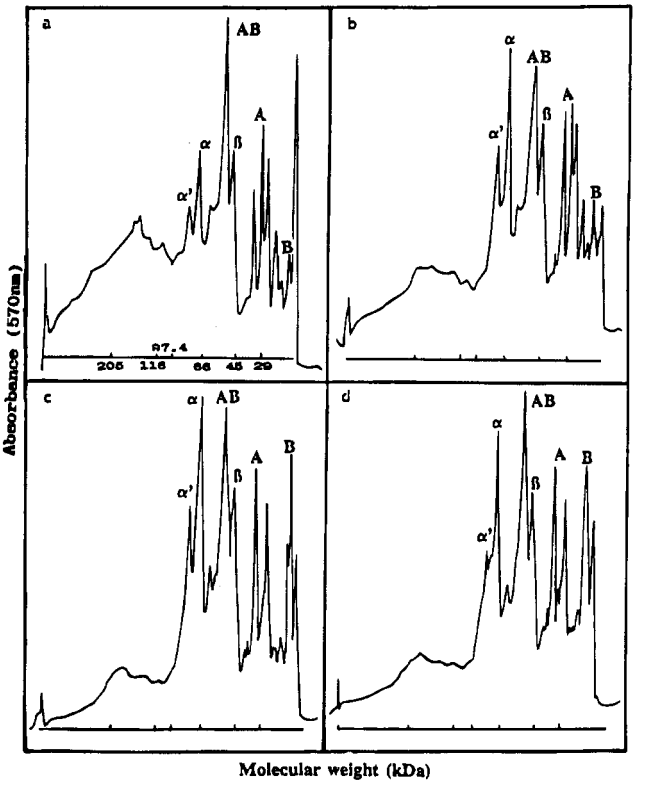

Figure 9. SDS–PAGE densitographies in 5–15% linear gradient of soy protein isolates (8 mg/mL) treated at 42 °C with Na_2SO_3 under different conditions: control isolate (a), isolate treated with Na_2SO_3 in the absence of ammoniacal $CuSO_4$ (b), isolate treated with 0.1 M Na_2SO_3 and 1.33 mM ammoniacal $CuSO_4$ at zero time (c), isolate treated with 0.1 M Na_2SO_3 and 1.33 mM ammoniacal $CuSO_4$ for 60 min (d).

Electrophoresis was performed on samples treated with the reducing agent, at both 25 and 42 °C in the presence of 1.33 and 4.0 mM Cu, either with or without shaking. Densitometry scans obtained are shown in Figure 9. Three types of profiles can be seen, as follow:

(1) Isolates that did not undergo a reducing treatment exhibit the α, α′ and β subunits of β-conglycinin, the AB-11S subunit, and aggregates of high molecular weight.

(2) Isolates treated with reducing agents in the absence of copper show disappearance of high molecular mass aggregates, an increase of the proportion of the α′ and α-7S subunits, a decrease of the AB-11S subunit, and a slight increase of A-11S polypeptide.

(3) Isolates treated with the reducing agent in the presence of copper show disappearance of both the high molecular mass aggregates and a mild increase of A- and B-11S polypeptides.

No changes were observed in the protein profile when Cu concentration or reaction time (1–60 min) was increased or by changes in shaking.

Isolates treated with Na_2SO_3 also show a decrease of the ratio between peak height of the AB-11S subunit and that of the β-7S subunit ($AB/\beta = 1.30$) when compared to the ratio corresponding to untreated isolates ($AB/\beta = 1.65$). In spite of the fact that 70% of the SS bonds present in the isolate are being reduced, the AB-11S subunit is only 20% reduced. The rest of the reduced SS bonds correspond to protein species of molecular mass lower than 35 kDa, leading to breaking of the intra- or interchain bonds. Rupture of the first type of bond gives rise to a molecule having a lower

electrophoretic mobility, either because the structure unfolds when that bond is lost—leading to an increase of the Stokes radius—or because of a better contact to SDS, since the interaction with the protein had been impaired by the protein itself.

The extent of reduction obtained under these conditions is similar to that achieved with long heating times (15 h) with Na_2SO_3.

In the presence of a catalyst (Cu) and/or an oxidizing agent (O_2), the AB-11S subunit is not completely reduced, probably because the reducing agent is unable to reach all SS bonds. This inability could result from the fact that the structure does not unfold completely as the SS bonds are being reduced or because rupture of the SS bonds leads to exposure of hydrophobic groups, followed by aggregation of the protein.

In the absence of denaturing agents, 75% of the apparent disulfide bonds are reduced (Figure 6), only 20% of the AB dimer being reduced (Figure 9). All SS groups of the AB-11S dimers are reduced in the presence of 8 M urea (Figure 5), whereas 50% of the total SH groups remain unreduced. Then, reduction of soy protein isolates with sodium sulfite affects different subunits according to the reaction conditions employed; namely, mostly AB dimers are reduced if urea is used, whereas if Cu or O_2 is employed, mainly components other than AB-11S undergo the change. To obtain complete sulfitolysis, both urea and Cu or O_2 are required.

CONCLUSIONS

The addition of a catalyst (copper) and an oxidizing agent (oxygen) affects similarly the sulfitolysis of soy proteins with Na_2SO_3; the simultaneous presence of both agents is not required. They are unable to improve the access of sulfite to produce a complete reduction of the AB-11S subunit, as occurs in simpler proteins.

To achieve complete sulfitolysis with Na_2SO_3, not only is the presence of a denaturing agent needed, but it is also necessary to favor shifting of the equilibrium state of the reaction by means of an oxidizing agent. The use of O_2 has some advantages over that of Cu, since, even though its action is slower, Cu is kept within the protein structure; this situation is dangerous both from the functional and from the nutritional point of view (Gonzalez and Damodaran, 1990a,b).

When 0.1 M Na_2SO_3 is used, soy protein isolates reach 75% of reduced apparent disulfide bonds groups in 15 h if Cu^{+2} is not employed, in 3 h if O_2 is added by shaking, and in a few minutes if copper is added to the system. This isolate, in spite of having a high percentage of reduced SS bonds, only has 20% reduction of the AB subunit of glycinin. Treatments with 0.01% Na_2SO_3 lead to only 10% reduction, whereas a concentration 100 times higher increases that percentage to 40% (at 7 h and in the absence of either denaturing or oxidizing agents).

From the above-mentioned results it can be stated that reduction of soy protein isolates with Na_2SO_3 affects different subunits, depending on the conditions under which reduction is carried out. Mainly AB subunits of glycinin are reduced when urea is used, while mostly components other than AB-11S are reduced if Na_2SO_3 is used either alone or in the presence of Cu and/or oxygen.

ACKNOWLEDGMENT

This investigation was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. S.P. is a Research Fellow of the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC) and M.C.A. is Member of the Researcher Career of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

LITERATURE CITED

Badley, R. A.; Atkinson, D.; Hauser, H.; Oldani, D.; Green, J. P.; Stubbs, J. M. The structure, physical and chemical properties of the soybean protein glycinin. *Biochim. Biophys. Acta* **1975**, *412*, 214–228.

Beveridge, T.; Toma, S. J.; Nakai, S. Determination of SH and SS-groups in some food proteins using Ellman's reagent. *J. Food Sci.* **1974**, *39*, 49–51.

Damodaram, S.; Kinsella, J. E. Effects of ions on protein conformation and functionality. In *Food Protein Deterioration Mechanisms and Functionality*; ACS Symposium Series 206; American Chemical Society: Washington, DC, 1982; pp 327–357.

Gonzalez, J. M.; Damodaran, S. Recovery of proteins from raw sweet whey using a solid state sulfitolysis. *J. Food Sci.* **1990a**, *55*, 1559–1563.

Gonzalez, J. M.; Damodaran, S. Sulfitolysis of disulfide bonds in proteins using a solid-state copper carbonate catalyst. *J. Agric. Food Chem.* **1990b**, *38*, 149–153.

Gornall, A. C.; Bardawill, C. J.; David, N. M. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **1949**, *177*, 751–766.

Hermansson, A. M. Physico-chemical aspects of soy proteins structure formation. *J. Texture Stud.* **1978**, *9*, 33–58.

Iwabuchi, S.; Yamauchi, F. Electrophoretic analysis of whey proteins present in soybean globulin fractions. *J. Agric. Food Chem.* **1987a**, *35*, 205–209.

Iwabuchi, S.; Yamauchi, F. Determination of glycinin and β-conglycinin in soybean proteins by immunological methods. *J. Agric. Food Chem.* **1987b**, *35*, 200–205.

Kella, N. K.; Kinsella, J. E. A method of controlled cleavage of disulfide bonds in proteins in the absence of denaturants. *J. Biochem. Biophys. Methods* **1985**, *11*, 251–263.

Kella, N. K.; Barbeau, W. E.; Kinsella, J. E. Effect of oxidative. sulfitolysis of disulfide bonds of glycinin on solubility, surface hydrophobicity and in vitro digestibility. *J. Agric. Food Chem.* **1986**, *34*, 251–256.

Kella, N. K.; Yang, S. T.; Kinsella, J. E. Effect on disulfide bond cleavage on structural and interfacial properties of whey proteins. *J. Agric. Food Chem.* **1989**, *39*, 1033–1036.

Kim, S. H.; Kinsella, J. E. Surface active properties of food proteins. Effect of reduction of disulfide bonds on film properties and foam stability of glycinin. *J. Food Sci.* **1987**, *52*, 128–130.

Klemaszewski, J. L.; Kinsella, J. E. Sulfitolysis of whey proteins. Effect on emulsion properties. *J. Agric. Food Chem.* **1991**, *39*, 1033–1036.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680–685.

Nash, A. M.; Wolf, W. J. Solubility and ultracentrifugal studies on soybean globulin. *Cereal Chem.* **1967**, *44*, 183–192.

Tannhauser, T. W.; Konishi, Y.; Sheraga, H. A. Sensitive quantitative analysis of disulfide bonds in polypeptides and proteins. *Anal. Biochem.* **1984**, *138*, 181–188.

Utsumi, S.; Inaba, H.; Mori, T. Heterogeneity of soybean glycinin. *Phytochemistry* **1981**, *20*, 585–589.

Wolf, W. J. Sulfhydryl content of glycinin: effect of reducing agents. *J. Agric. Food Chem.* **1993**, *41*, 168–176.

Received for review August 15, 1994. Revised manuscript received January 3, 1995. Accepted May 26, 1995.[⊗]

JF930336A

[⊗] Abstract published in *Advance ACS Abstracts,* July 1, 1995.