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Determination of Sulfhydryl Groups and Disulfide Bonds in Heat-Induced Gels of Soy Protein Isolate

Kazuko Shimada[1] and Jean Claude Cheftel*

Thermally induced gels were prepared from dispersions of soy protein isolate at varying protein concentrations, pH, and heating intensity. Increasing protein concentration (0.9–18.2%), pH (7–10), or temperature (80–120 °C) caused (1) a decrease in the content of free SH groups (from an initial 8 μmol/g of protein) and in the protein solubility of gels (in Tris–glycine buffer), (2) no change in the half-cystine content ($\sim$100 μmol/g of protein), and (3) an increase in gel firmness. Heating at 130 °C caused (1) an increase in the content of free SH groups and in the protein solubility of gels, (2) a decrease in the half-cystine content, and (3) a decrease in gel firmness. These data and the severely reduced firmness of gels formed in the presence of *N*-ethylmaleimide suggest that newly formed additional S–S bonds and/or SH-induced S–S interchange reactions contribute markedly to the strengthening of gels observed at alkaline pHs or at high temperatures (115–120 °C). At or above 130 °C, the partial breakdown of S–S bonds (with half-cystine losses) may contribute to gel softening.

Sulfhydryl (SH) groups and disulfide (S–S) bonds influence significantly the functional properties of food proteins and play an important role in the formation of relatively rigid structures such as protein gels or doughs. Heat-induced changes in SH group and S–S bond contents of food proteins have been reported by Saio et al. (1971), Hashizume and Watanabe (1979), and Yamagishi et al. (1984) for soy proteins, by Watanabe and Klostermeyer (1976) for β-lactoglobulin, by Patrick and Swaisgood (1976) for skim milk, by Li-Chan (1983) for whey protein concentrate, by Beveridge and Arntfield (1979) for egg white, and by Opstvedt et al. (1984) for fish protein. However, most of these studies have been carried out with dilute protein solutions or with protein powders, and little is still known concerning the changes occurring in SH groups and S–S bonds when heating is performed at concentrations leading to protein gelation. The firmness of tofu gel (Ca^{2+} coagulated after heating—a traditional Japanese food) made from soy 11S globulin increased with increasing SH group content before heating (Saio et al., 1971). Minced and washed fish muscle (surimi) with a high content of SH groups led to kamaboko gels of better quality (Jiang et al., 1986). The content of SH groups in protein gels has been determined with whey protein concentrate and egg white (Beveridge et al., 1984) and with ovalbumin (Hayakawa and Nakai, 1985). These authors have observed a corre-

lation between increased gel firmness and decreased SH group content as a result of gelation. These results suggest that S–S bonds form during heat gelation through oxidation of SH groups (and not only through SH, S–S interchange), and markedly influence the gel network structure and mechanical strength. Voutsinas et al. (1983) found that gel strength was significantly correlated with both the initial SH group content and the hydrophobicity of several globular proteins. In contrast, Hegg (1982) suggests that there is no obvious correlation between the S–S bond or SH group content and the gel-forming ability of conalbumin, serum albumin, and β-lactoglobulin. Such a contradiction may reflect differences in the reactivity of SH groups and in the respective roles of hydrogen bonds, electrostatic interactions, S–S bonds, and hydrophobic interactions for the gelation or different proteins (Shimada and Matsushita, 1980).

Various investigators have recently reported that S–S bonds play an important role in the gelation of soy proteins. Evidence for this role came from the effects of the presence or absence of *N*-ethylmaleimide (NEM) or of 2-mercaptoethanol (2-ME) on the formation of protein aggregates and from gel solubilization by 2-ME (Mori et al., 1982; Utsumi and Kinsella, 1985a,b; Mori et al., 1986). However, the changes in SH group and S–S bond contents were not directly determined.

In the present study, we have attempted to determine the changes in SH group and S–S bond contents in soy protein isolate (SPI) as a result of heat-induced gelation. These changes have been studied as a function of protein concentration, pH, and intensity of heat processing. Correlations have been sought between S–S bond formation and gel firmness. This necessitated a preliminary study of conditions leading to an extensive solubilization of protein gels into transparent solutions where SH groups

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could be determined with use of Ellman's colorimetric method. It was checked that solubilization conditions did not modify the SH/S–S balance through oxidation of SH groups.

MATERIALS AND METHODS

Materials. Soy protein isolate (SPI) (Purina Protein 500E) was purchased from SIO, Boulogne sur Seine (France). It contained 90% protein (N × 6.25, db) and 4.6% moisture. The nitrogen solubility index (NSI) estimated according to AOCS (1970) was 68.

Bovine serum albumin (globulin free, fatty acid free) was purchased from Sigma Chemical Co. Sephadex G-25 was obtained from Pharmacia Co., Ltd. The other chemicals used were of reagent grade.

Heat Treatment of SPI and Determination of Gel Firmness. The aqueous solutions or dispersions of SPI (pH 6.7) were placed in glass bottles (5-cm i.d. × 6-cm height) with tightly closed caps, heated for 30 min in a water bath or in an autoclave (105–130 °C), and then cooled rapidly to room temperature in tap water. In some cases, the pHs of SPI dispersions were adjusted to the required values with 10 N NaOH before heating. In other cases, NEM or succinimide was added to the aqueous SPI dispersions at concentrations of 10 or 13 mM. After incubation at room temperature for 1 h, the pH was adjusted when necessary and the dispersions were then heated. The cooled samples were kept at 4 °C for 15–20 h before determination of SH group and half-cystine [SH + (2 × S–S)] contents and of gel firmness.

The firmness of gel was determined at 20 °C with a Stevens-LFRA texture analyzer using a cylindric plunger with a diameter of 4.0 mm. The instrument was operated at a crosshead speed of 1.0 mm/s. The penetration of the plunger into the gel was set at a depth of 10 mm. Gel firmness was expressed as the force in Newtons. Five determinations were carried out with each gel, and the average ± standard deviation were calculated.

Solubilization of SPI and SPI Gels. After preliminary trials with different types of denaturants at various concentrations, a buffer containing 6 M urea and 0.5% (17.3 mM) sodium dodecyl sulfate (SDS) was selected for solubilization of soy protein gels. About 90% of the protein present was solubilized, and the centrifuged solutions were transparent or slightly opaque (transmittance at 600 nm was always above 95%). Such solutions were adequate for SH group analysis as described in the next paragraph.

SPI and SPI gels were solubilized either in 0.086 M Tris–0.09 M glycine–4 mM ethylenediaminetetraacetic acid disodium salt (Na₂EDTA), pH 8.0 buffer (standard buffer) (free SH group analysis of proteins solubilized in this standard buffer) or in the same standard buffer containing also 6 M urea and 0.5% SDS (analysis of total SH groups) or 6 M urea, 0.5% SDS, and 10 mM dithiothreitol (DTT) [analysis of total SH + (2 × S–S)]. The protein solutions were adjusted to 0.2% (0.1 g of protein/50 mL of buffer), homogenized with an Ultra-Turrax below 25 °C for 3 min, and then centrifuged at 20 000g for 15 min. The supernatant fractions were analyzed for protein solubility and SH group content. Protein solubility was calculated as 100 × protein content of the supernatant/total protein content.

Determination of SH Groups. SH groups were determined with use of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) according to Ellman (1959) with some modifications. To a 3-mL aliquot of the protein supernatant in the standard buffer with or without denaturants was added 0.03 mL of Ellman's reagent solution (4 mg of DTNB/mL of standard buffer). After the solution was rapidly mixed and allowed to stand at room temperature for 15 min,

absorbance was read at 412 nm. The standard buffer was used instead of protein solutions as a reagent blank. The protein solutions in the standard buffer (with or without denaturants) showed a slight opalescence. To correct for this, a protein blank was used in which 0.03 mL of the standard buffer replaced Ellman's reagent solution. A molar extinction coefficient of 1.36×10^4 M⁻¹ cm⁻¹ was used for calculating micromoles of SH/gram of protein.

Removal of DTT and Determination of Half-Cystine [SH + (2 × S–S)]. Protein gels homogenized in the standard buffer with urea, SDS, and DTT were centrifuged. The supernatants were incubated at room temperature for 6–8 h and then subjected to gel filtration on a Sephadex G-25 column (2.0 cm (i.d.) × 8.0 cm) equilibrated with the standard buffer containing denaturants, in order to remove DTT. Elution was carried out with the same buffer. The absorbance of the eluate was monitored at 280 nm and at 412 nm after reaction with DTNB. Proteins and DTT were found to be completely separated. The protein fraction was collected, and the half-cystine content was determined with DTNB as described above.

The whole analytical procedure was checked with bovine serum albumin (BSA) as a control protein. A 0.8-mol portion of free SH groups and 35.1 mol of half-cystine/mol of BSA was found, in agreement with values reported in the literature. It was also checked with BSA that, after reduction of S–S bonds and removal of DTT, the protein fraction could be left exposed to air for 20 h at 20 °C without any decrease in the SH group content.

Determination of Half-Cystine by Amino Acid Analysis. Half-cystine was determined as cysteic acid according to Moore (1963). SPI (5 mg) was treated with 2 mL of performic acid (88% w/w formic acid–30% w/v hydrogen peroxide, 9:1, v/v) at 0 °C for 4 h. Then, 0.30 mL of hydrogen bromide (47%, w/w) was added to reduce the excess performic acid and prevent overoxidation. After removal of hydrogen bromide on a rotary evaporator, protein hydrolysis was carried out in 2 mL of 6 N HCl at 110 °C for 18 h. Amino acids were analyzed with a NCl Technicon autoanalyzer at 60 °C on a 75 × 0.6 cm ion-exchange column consisting of Chromobeads C₂ resin. The elution gradient was as described by Provansal et al. (1975). Norleucine was used as an internal standard. The half-cystine content is the mean of two independent determinations.

Protein Determination. Protein concentration was determined by the method of Lowry et al. as modified by Bensadoun and Weinstein (1976).

RESULTS AND DISCUSSION

Influence of Protein Concentration. The firmness and protein solubility of dispersions or gels obtained by heating (80 °C, 30 min) diluted or concentrated solutions of SPI are shown in Figure 1. When unheated SPI was solubilized at a concentration of 0.2 g of protein/100 mL, the proportion of protein soluble in the standard pH 8.0 buffer was close to 40% and increased to 97 and 99% in 6 M urea plus 0.5% SDS without and with DTT, respectively. When a solution of SPI containing 0.9% protein was heated, protein solubility in the standard pH 8.0 buffer increased to 64%. However, the protein solubility in the standard buffer was lower with gels formed by heating dispersions of higher protein concentration, suggesting a higher prevalence in the gel matrix of hydrophobic interactions and S–S bonds (both resistant to the standard buffer). Protein solubility in the standard buffer containing denaturants decreased from 97 to about 90% after heat treatment, whatever the protein concentration of the SPI dispersions. Protein solubility in the standard buffer
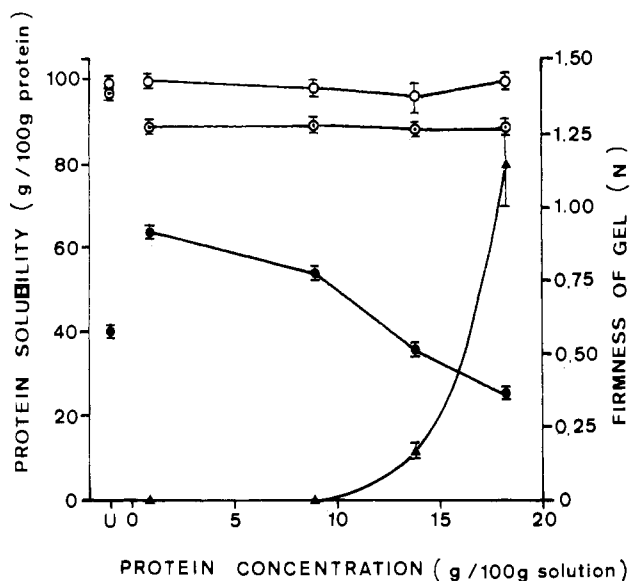
Figure 1. Protein solubility and firmness of soy protein isolate (SPI) gels as a function of protein concentration during heating. Heat processing at 80 °C for 30 min (pH 6.7). Concentration for all solubilization assays: 0.2 g of protein/100 mL. Buffers used for protein solubilization: (●) standard buffer; (⊙) standard buffer containing 6 M urea and 0.5% SDS; (○) standard buffer containing 6 M urea, 0.5% SDS, and 10 mM DTT. Gel firmness: ▲. The bars for protein solubility show the range of duplicate determinations except for three replications of unheated SPI (U). The bars for gel firmness show the standard deviation of five replicate determinations.
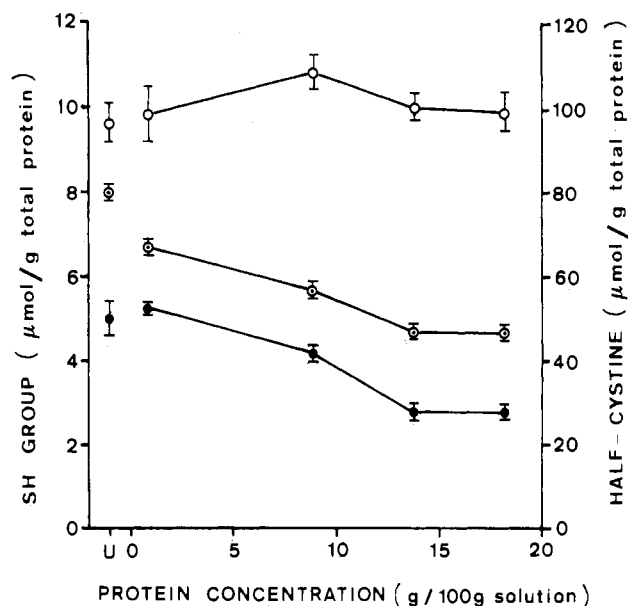

Figure 2. Contents of free SH groups and of half-cystine in heated SPI as a function of protein concentration during heating. Heat processing at 80 °C for 30 min. Key: (●) free SH in the standard buffer solubilized proteins; (⊙) total SH; (○) half-cystine. The bars show the range of duplicate determinations except for three replications of unheated SPI (U).

containing DTT in addition to denaturing agents was close to 100% in all cases. This appears to indicate that about 10% of soy protein constituents are insolubilized during heating due to the formation of intra- or intermolecular S-S bonds. Utsumi and Kinsella (1985b) have reported protein solubilities of SPI gels in Tris-HCl buffer (pH 8.0) and in 8 M urea of, respectively, 49 and 100% (12% SPI gel) or 15 and 96% (15% SPI gel). The slight discrepancy in solubility values between these data and the present results may reflect differences in the unheated SPI and in the condition of solubilization.

The free SH group and half-cystine contents of dispersions or gels obtained by the heat processing (80 °C, 30 min) of diluted or concentrated SPI solutions are indicated in Figure 2. In unheated SPI, the contents of total SH groups and of free SH groups from protein soluble in the standard buffer were 8.0 and 5.0 μmol/g of total protein, respectively. The major fraction of soy proteins, 11S globulin, contains approximately 2 mol of free SH groups/mol of protein (6.3 μmol of free SH group/g of protein, assuming MW 320 000) (Draper and Catsimpoolas, 1978; Nakamura et al., 1984b), although higher value of 5.6 mol/mol of protein (15.7 μmol of SH/g of protein, assuming MW 356 000) has also been found (Simard and Boulet, 1978). The 11S globulin is generally considered to contain 18-20 S-S bonds/mol (Draper and Catsimpoolas, 1978; Kim and Kinsella, 1986). The former investigators have determined the content of SH groups both at the surface and inside the 11S globulin molecule using solutions of the protein in either phosphate buffer (pH 7.6) or denaturant-containing buffer (Simard and Boulet, 1978; Nakamura et al., 1984b). Values were in the range of 0.4-1.4 mol/mol of protein for surface SH groups (1.1-3.9 μmol of SH/g of protein, MW 356 000) and of 1.6-5.6 mol/mol of protein for total SH groups (4.5-15.7 μmol of SH/g of protein, MW 356 000). Such variations may be attributed to differences between soy cultivars, kind of

denaturants, conditions of solubilization, etc. Even though the present results are obtained from SPI, it is obvious that the contents of total SH groups and of free SH groups of protein soluble in the standard buffer are not too different from those obtained with the 11S globulin.

Heating a 0.9% protein solution at 80 °C for 30 min reduced the content of total SH groups but had no significant effect on the content of free SH groups from protein soluble in the standard buffer, as compared with the data of unheated SPI. Hashizume and Watanabe (1979) have shown that the SH group contents of diluted solutions of soy proteins (phosphate buffer, pH 7.6) were 6.2 and 5.5 μmol/g of protein before and after heat processing at 80 °C for 10 min. These values are consistent with the results obtained in the present study. The contents of free and total SH groups were lower when concentrated SPI dispersions were heated (Figure 2).

These data do not give any indication concerning the possible occurrence of SH, S-S interchange reactions as a result of heating. They do suggest however that the observed 40% maximum decrease in total SH groups is due to oxidation into additional S-S bonds (since the total half-cystine content remains constant). Some of these additional bonds may be responsible for the 10% decrease in protein solubility in the denaturant-containing buffer (Figure 1). The decrease in total SH groups markedly depends on the protein concentration during heating, while the decrease in protein solubility (in the presence of denaturants) is clearly independent of protein concentration. It is therefore difficult to speculate wheter S-S bond formation occurs mainly as intramolecular or as intermolecular reactions. Additional information may be derived from the free SH content of the proteins soluble in the standard buffer. This content decreases (by 50% at maximum) as a function of the protein concentration during heating, due to a parallel decrease (by 60% at maximum) in protein solubility. Oxidation of SH groups into S-S bonds therefore mainly takes place in protein constituents that become insoluble in the standard buffer but remain soluble in the presence of urea and SDS. From a comparison of Figures 1 and 2, no obvious correlation
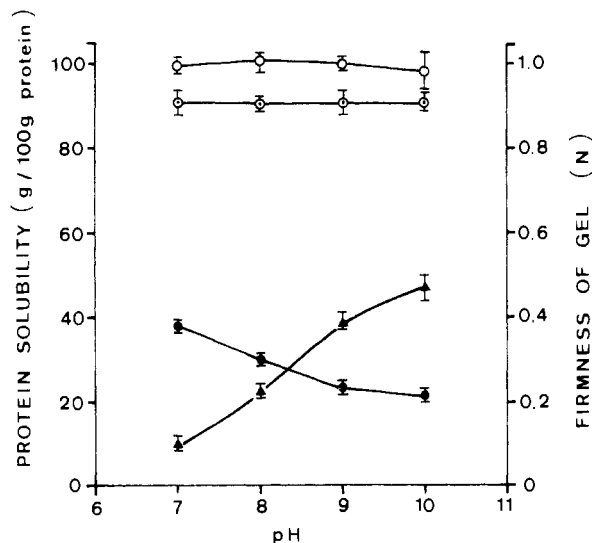
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Figure 3. Protein solubility and firmness of SPI gels as a function of pH during heating. SPI dispersions of 13% protein concentration (w/w) were heated at 80 °C for 30 min. Symbols are the same as in Figure 1. The bars for protein solubility show the range of duplicate determinations. The bars for gel firmness show the standard deviation of five replicate determinations.

can be found between gel firmness and the content of total SH groups. This indicates that gel firmness, which is strongly related to protein concentration (a minimum concentration of 12–13% protein is required for gelation under heating conditions of 80 °C for 30 min), is less dependent on the formation of additional S–S bonds than on other reactions (possibly SH, S–S interchange, hydrophobic interaction, hydrogen bond, etc.).

The content of total half-cystine [total SH + (2 × S–S)] was measured in unheated and heated SPI by the Ellman method and found to remain close to 100 μmol/g of protein (Figure 2). A similar value (104.0 ± 6.6 μmol/g of unheated soy protein) was obtained by amino acid analysis after performic acid oxidation. The ratio of total SH/half-cystine in unheated soy protein is therefore close to 8% (Figure 2). Such low values have also been found for 11S globulin by other investigators (Draper and Catsimpoolas, 1978; Simard and Boulet, 1978; Kella and Kinsella, 1985). They may explain why gel firmness does not appear to be correlated with the formation of additional S–S bonds as a result of heating.

Influence of pH. The firmness and protein solubility of gels obtained by heating (80 °C, 30 min) SPI dispersions (13% protein, w/w) of different pHs (7–10) are shown in Figure 3. Gel texture increased from soft to firm with increasing pH, while protein solubility in the pH 8.0 standard buffer decreased when heat processing was carried out at increasing pH. Protein solubilities in the standard buffer containing denaturants without or with DTT were constant whatever the pH during heat processing and equal to 90 or 100%, respectively. The relationship between gel firmness and protein solubility in the three different buffers is quite similar to that previously observed in Figure 1.

The free SH group and half-cystine contents of the same gels are given in Figure 4. Both the total SH groups and the free SH groups from protein soluble in the standard buffer decreased with increasing pH during heat processing. The half-cystine content remained constant in the range pH 7–10. These results indicate that additional S–S bonds are formed through oxidation of SH groups at alkaline pHs. Cystine and cysteine residues do not appear to be converted to degradation products such as H_2S and
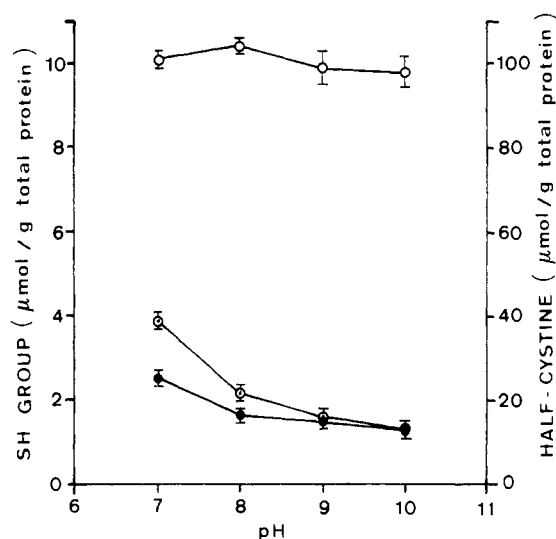

Figure 4. Contents of free SH groups and of half-cystine in SPI gels as a function of pH during heating. Heat processing at 80 °C for 30 min; protein concentration 13%. Symbols are the same as in Figure 2. The bars show the range of duplicate determinations.
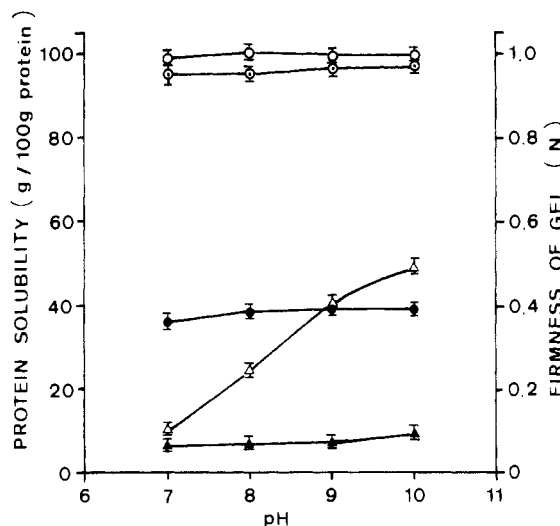

Figure 5. Effect of NEM on protein solubility and firmness of SPI gels as a function of pH during heating. Aqueous dispersions of SPI (13% protein) were incubated for 1 h in the presence of 10 mM NEM or 10 mM succinimide at room temperature, adjusted to the required pH, and then heated at 80 °C for 30 min. Symbols are the same as in Figure 1 except for the following: (▲) firmness of gels formed in the presence of 10 mM NEM; (△) firmness of gels formed in the presence of 10 mM succinimide. The bars for protein solubility show the range of duplicate determinations. The bars for gel firmness show the standard deviation of five replicate determinations.

dehydroalanine (Asquith and Carthew, 1972) under the present processing conditions. The increase in gel firmness may be partly due to the formation of additional S–S bonds, although other mechanisms (chain unfolding, SH, S–S interchange, etc.) may be predominant.

Influence of NEM. In order to investigate the role of free SH groups in the increase in gel firmness at alkaline pHs, NEM was added to the SPI dispersion (13% protein) at a concentration of 10 mM. This corresponds to about 10 times the free SH concentration. Incubation was carried out for 1 h at room temperature. The dispersion was then adjusted to the required pH and heated at 80 °C for 30 min. It was checked that the remaining contents of total SH groups were below 1 μmol/g of protein.
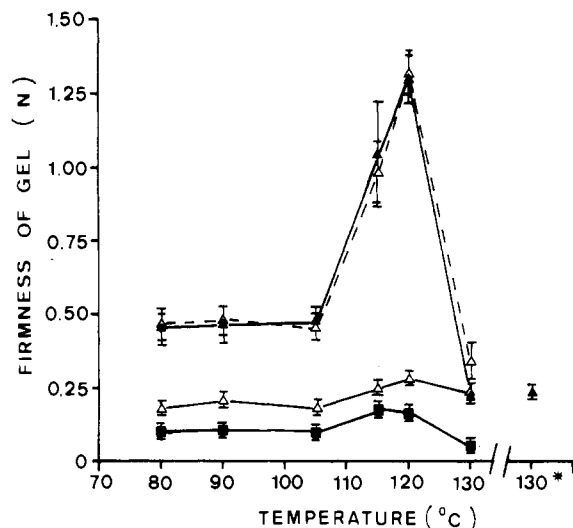
Figure 6. Firmness of SPI gels as a function of processing temperature. The aqueous dispersions of SPI (pH 6.7) were heated in a water bath or in an autoclave for 30 min (* or for 1 h). Key: (-▲-) 16% protein; (-△-) 16% protein + 13 mM NEM; (--△--) 16% protein + 13 mM succinimide; (-■-) 13% protein. The bars show the standard deviation of five replicate determinations.

The presence of NEM decreased the firmness of all gels in the pH 7–10 range (Figure 5). When NEM was replaced by 10 mM succinimide, a fully saturated structural analogue of NEM, the firmness of all gels was identical with that previously observed in the absence of NEM. This suggests that the detrimental effect of NEM on gel firmness is due to the masking of SH groups and not to modifications in hydrogen, electrostatic, or hydrophobic interactions. It is therefore likely that the increase in gel firmness observed at alkaline pHs (Figure 3) results from an enhancement of S–S bond formation and/or SH, S–S interchange reactions catalyzed by sulfhydryl anions (P–S$^-$). In the presence of NEM, the protein solubility in the standard buffer of pH 7–10 gels remained close to 40%. Protein solubility in the standard buffer containing denaturants was slightly higher with than without NEM (≥95% vs 91;, respectively).

These results support the hypothesis that the network structure of SPI gels is strengthened as a result of P–S$^-$-induced S–S bond formation and/or S–S interchange reactions taking place at alkaline pHs. The contribution of hydrogen, electrostatic, and/or hydrophobic interactions to the strength of SPI gels is qualitatively indicated by the difference in protein solubility in standard buffer with or without denaturants (Figures 1, 3, and 5).

Nakamura et al. (1984a) have suggested from transmission electron microscopy observations of heated 11S globulin solutions that linear strands (soluble aggregates) are first formed by association of glycinin molecules through hydrophobic interactions. Branched strands and gel network are formed at a later stage and appear to depend on S–S interchange reactions.

Influence of the Intensity of Heat Processing. The relationship between gel firmness and processing temperature is shown in Figure 6. No change in gel firmness takes place when the temperature of heat treatment (30 min) is increased from 80 to 105 °C. A marked increase in gel firmness is however apparent when the SPI dispersion (13 or 16% protein) is heated at 115 or 120 °C. The difference in gel firmness between the 13% and the 16% protein dispersions is also notable. When heating is carried out at 130 °C (for 30 min or for 1 h), drastic gel softening occurs. The addition of 13 mM succinimide to the 16% protein dispersion did not influence gel firmness.
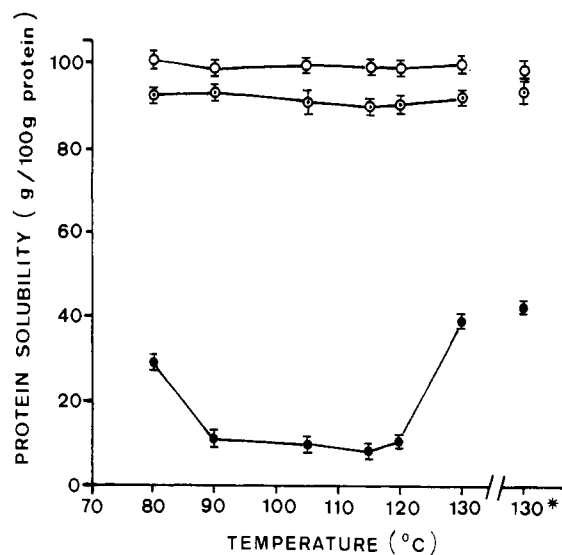

Figure 7. Protein solubility of SPI gels as a function of processing temperature. SPI dispersions (16% protein, pH 6.7) were heated in a water bath or in an autoclave for 30 min (* or for 1 h). Symbols are the same as in Figure 1. The bars show the range of duplicate determinations.

Figure 8. Contents of free SH groups and of half-cystine in SPI gels as a function of processing temperature. SPI dispersions (16% protein, pH 6.7) were heated in a water bath or in an autoclave for 30 min (* or for 1 h). Symbols are the same as in Figure 2. The bars show the range of duplicate determinations.
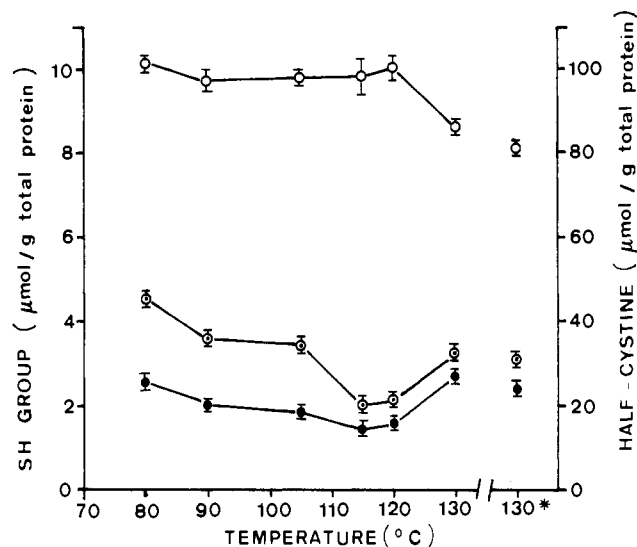
The addition of 13 mM NEM, however, greatly decreased the firmness of gels prepared at all temperatures, especially at 115 and 120 °C (but not at 130 °C). This clearly shows that free SH groups are necessary for the increase in firmness observed at 115–120 °C.

Protein solubility and contents of free SH group and half-cystine from 16% protein gels are shown in Figures 7 and 8. Solubility in the standard buffer decreased with temperature but increased at 130 °C. Solubility in the standard buffer containing denaturants without or with DTT was close to 91 or 100%, respectively. The contents of total SH groups and of free SH groups from proteins soluble in the standard buffer first decreased with increasing temperature up to 115 °C and then increased at 130 °C (Figure 8). The contents of half-cystine remained close to 100 μmol/g of total protein when heating was carried out for 30 min at 80–120 °C but decreased sharply at 130 °C (losses were about 15% after 30 min and 20% after 1 h).

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Nakamura et al. (1984a) observed that when a 5% solution of 11S globulin was heated at 100 °C in a pH 7.6 phosphate buffer (ionic strength 0.5); the strands that formed in the gel network had a thickness (10–12 nm) similar to the diameter of the native 11S molecule. Hermansson (1985) found similar results with a 12% solution of 11S globulin heated in water at 85 or 95 °C. Strands appear to form directly through association of 11S molecules, but prior and transient dissociation into subunits is not ruled out. Although a commercial SPI (partly heat denatured) has been used in the present study rather than 11S globulin, it is likely that gel formation also occurred through association reactions. Some evidence is presented that association takes place through noncovalent interactions and through formation and/or interchange of S–S bonds. These reactions are enhanced both at alkaline pHs and at higher temperature (up to 120 °C), possibly because of increased protein unfolding and surface exposure of SH groups, S–S bonds, hydrophobic residues, etc.

At higher temperatures ($\sim$130 °C) however, other reactions may take place, such as breakdown of S–S bonds with release of H_2S, release of NH_3 from amide groups, possibly dissociation of subunits, and/or breakdown of these subunits into compounds of small molecular weights. Such reactions may be responsible for the marked decrease in gel firmness (Figure 6), the enhanced protein solubility in the standard buffer (Figure 7), the decrease in half-cystine contents, and the increase in free SH groups (Figure 8). Such degradation reactions have been noted by various investigators. Watanabe and Klostermeyer (1976) showed that when a 1% solution of β-lactoglobulin A (pH 7.0) was heated for 2 min, the loss of half-cystine amounted to 3% at 125 °C and to 10% at 145 °C. Heating of ground fish meat at 115 °C caused a half-cystine loss of about 10% (Opstvedt et al., 1984). Hamm and Hofmann (1965) found that the half-cystine loss in heated meat was accompanied by the formation of H_2S. The amide groups of crude 11S globulin decreased rapidly with increasing temperature above 105 °C, while those of crude 7S globulin decreased only above 140 °C (Saio et al., 1975b). Catsimpoolas and Meyer (1970) speculated that the conversion of asparagine and glutamine residues to carboxyl groups by release of ammonia not only introduced repulsive electrostatic forces but also inhibited the formation of hydrogen bonds between amide groups. Saio et al. (1975a,b) also found that above 150 °C the subunits of 7S and 11S globulins were degraded to small molecular weight compounds.

It can be concluded from the data presented in this study that the free SH groups present in the unheated SPI are involved in the formation of a firm gel structure (upon heating of 13–18% protein dispersions at pH 6.7 and 80 °C). This involvement appears to be enhanced at alkaline pHs or at higher temperatures (up to 120 °C). The proportion of half-cystine present as free SH groups in the unheated SPI is low (8%) and further decreases upon heat gelation. It remains to be determined whether the increase in gel firmness (and the decrease in protein solubility) is influenced mainly by SH oxidation into additional S–S bonds or by SH-induced S–S interchange reactions. Heating SPI dispersions at 130 °C causes marked gel softening. Disulfide bonds appear to be also involved in this phenomenon since the contents of total half-cystine decreases while the content of total SH groups slightly increases.

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Registry No. *N*-Ethylmaleimide, 128-53-0.

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Production Study of a Low-Gossypol Protein Product from Cottonseed Meal[1]

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A process was developed to reduce the level of free gossypol in cottonseed meal from either an oil extraction or an expelling process. The level of free gossypol reached was below the maximum allowable in edible meal according to federal regulations (0.045%). The process included extrusion, drying, fine grinding, and air classification. The final coarse product, with particle size larger than 84 μm, and a yield ranging from 67 to 80% by weight of the original raw material, had a protein content of 36.6–45.2%.

Historically, man's interest in cottonseed was as a source of oil. However, problems of malnutrition have been the driving force for research and industry to develop additional protein sources for human consumption. Worldwide, about 5.3×10^7 metric tons of meal is available yearly from glanded cottonseed, i.e. about 1.7×10^7 metric tons of protein that can be potentially used for human consumption (FAO, 1984).

Cottonseed proteins are rarely utilized as a source of edible food by monogastric animals, because of the presence of pigment glands that contain toxic gossypol. Gossypol is a highly reactive yellow polyphenolic binaphtaldehyde. In the metabolically active or free form, it has adverse physiological effects when ingested by monogastric animals (Berardi et al., 1969). Food and Drug Administration (FDA) regulations specifically require that a protein food product made from cottonseed have a content of less than 0.045% free gossypol to be considered edible (*Fed. Reg.*, 1974).

Cottonseed kernels contain about 7% moisture, 30% crude protein, 30% oil, 24% nitrogen-free extract, 4.8 crude fiber, and 4.4% ash (Altschul et al., 1958). Geddes (1951) notes that cottonseed flour is high in vitamins, such as thiamin, riboflavin, and niacin. The protein of cottonseed has been shown to consist of globulin, a pentose-containing protein, and glutelin. Its amino acid composition was compiled by Johns and Jones (1916).

Different approaches were taken in the past in processing the defatted cottonseed meal to a highly concentrated, edible, protein product. Smith (1970) describes the preparation of a glandless cottonseed flour by direct solvent extraction. Air classification can also be used to produce a protein concentrate, as described by Martinez et al. (1967). In this process, the protein bodies are separated from clusters of unruptured cells and cell wall fragments with adhering residual cytoplasm. The process provides two products: one high in nitrogen, for food; and one low in nitrogen but high in cell wall particles, for food or feed.

Kadan et al. (1980) describe a process comprised of five steps by which it is possible to produce from glanded cottonseed a high-protein flour essentially without free gossypol and suitable for human consumption: (a) flaking dehulled glanded cottonseed having moisture contents from 5 to 12%; (b) solvent extracting the cottonseed flakes to reduce fat content; (c) desolventizing the solvent-extracted flakes; (d) milling the desolvenized flakes into a flour; (e) air classifying the flour to produce a coarse fraction and a fine fraction.

All the above procedures are concerned merely with raw materials produced by oil extraction. The objective of this study was to develop a production procedure of a high-protein product low in free gossypol from a cottonseed meal. The cottonseed meal used could come from an oil-extraction or an oil-expelling process.

MATERIALS AND METHODS

A series of experiments were performed using cottonseed meal that originated from three different oil-removing processes. One batch of cottonseed meal was obtained

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