

# Alkali-Modified Soy Proteins: Effect of Salts and Disulfide Bond Cleavage on Adhesion and Viscosity

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**ABSTRACT:** Soy protein isolates were treated with NaCl, Na<sub>2</sub>SO<sub>4</sub>, or Na<sub>2</sub>SO<sub>3</sub> (disulfide bond-cleaving agent) at a pH of 10.0 and 50°C, and the effects of these salts on viscosity, adhesive strength on woods, and water resistance of the treated isolates were investigated. Viscosity and adhesive strength decreased with increasing concentrations of these salts. At a concentration of 0.1 M, these three salts reduced the viscosity of soy proteins with no significant adverse effects on adhesive strength and water resistance. Addition of 0.1 M NaCl, Na<sub>2</sub>SO<sub>4</sub>, or Na<sub>2</sub>SO<sub>3</sub> reduced adhesive strength insignificantly from 1230 N to 1120, 1060, or 1013 N, respectively. The viscosity of protein isolate modified at pH 10.0 and 50°C in the absence of salts was >30,000 cP. Treatment with NaCl or Na<sub>2</sub>SO<sub>4</sub> resulted in viscosity reductions to 6000 or 1050 cP, respectively. The Na<sub>2</sub>SO<sub>3</sub> treatment yielded an isolate with the lowest viscosity of 110 cP and which retained adhesive and water-resistive properties. The water resistance of modified soy proteins with and without 0.1 M Na<sub>2</sub>SO<sub>3</sub> treatment was not significantly different with 3.3 and 6.6% cumulative delaminations occurring after four water soaking cycles. Treatment with 0.1 M Na<sub>2</sub>SO<sub>3</sub> resulted in an isolate with a 28% decrease in disulfide linkages. *JAOCs* 73, 1063–1066 (1996).

**KEY WORDS:** Adhesive strength, alkaline modification, disulfide cleavage, soy proteins, viscosity, wood.

Soy proteins have long been used as ingredients in wood adhesives (1,2). However, petrochemical products have replaced the use of soy proteins in wood adhesives because they are comparatively inexpensive and have superior qualities. High adhesive strength, water resistance, and low viscosity are the basic requirements for a wood glue. Improved adhesive strength and water resistance have been observed for adhesives prepared from alkali-modified soy proteins (3). However, alkali-modified soy proteins had high viscosity (>30,000 cP at 14% solids concentration). The high viscosity of the modified soy proteins is a result of increased intermolecular interactions due to unfolded protein molecules. A possible approach to solving this problem is to minimize this intermolecular interaction. The major forces that facilitate such inter-

actions are electrostatic and covalent disulfide bondings. Hence, any physicochemical factors that weaken or disrupt these forces will minimize these interactions.

Ionic environments weaken the electrostatic interactions between protein molecules by electrostatic shielding. An ionic environment affects the solubility of proteins as a result of its effect on protein-protein and protein-solvent interactions. The effect of ionic strength on functional properties of soy proteins, such as emulsification, foaming, water binding, and gelation, has been well documented (4–6).

Reducing agents, such as sulfites, cleave the inter- and intradisulfide bonds in protein molecules. The presence of disulfide bonds in native protein molecules affects their flexibility and unfolding properties. Cleavage of disulfide bonds by reducing agents leads to an increase in surface hydrophobicity, foaming capacity, and foam stability (7). German *et al.* (8) showed that foaming and foam stability of soy 11S globulin was improved by reducing disulfide bonds. Klemazewski and Kinsela (9) observed improved emulsion properties of whey proteins as a result of disulfide bond cleavage. Hence, the effect of disulfide bonds on functional properties may depend on the type of proteins and thiol/disulfide interactions. Sulfites (7,10) and thiols (11,12) have been widely used to cleave disulfide bonds in proteins.

Circle *et al.* (13) reported the effects of ionic salts, lipids, polysaccharides, and disulfide-reducing agents on the viscosity of soy protein dispersions. A decrease in viscosity of soy protein dispersions was observed with the addition of ionic salts and disulfide-reducing agents.

The objectives of this work were to investigate the effect of treating soy protein isolate with NaCl and Na<sub>2</sub>SO<sub>4</sub>, and with a disulfide reducing agent, Na<sub>2</sub>SO<sub>3</sub>, on viscosity, water resistance, and adhesive strength of modified soy proteins. Chloride and sulfate were selected as ionic salts because of inherent differences in their effect on solubility profiles of soy proteins (6).

## EXPERIMENTAL PROCEDURES

**Materials.** Soy protein isolate (Ardex D, a general-purpose food-grade protein) was obtained from Archer Daniels Mid-

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land Co. (Decatur, IL). All chemicals were from Sigma Chemical Company (St. Louis, MO). Soft-maple wood blocks (5 × 2 × 0.3 cm) were purchased from White River Hardwoods, Woodworks, Inc. (Fayetteville, AR).

**Alkali-modified soy proteins.** Alkali-modified soy proteins were prepared by the method of Hettiarachchy *et al.* (3) with the following modification to incorporate ionic salts and disulfide-reducing agent. Ten-gram amounts of soy protein isolate (SPI) were dispersed in 140 mL of deionized water and stirred magnetically for 10 min to obtain uniform dispersions. Dispersions were then adjusted to 0.0, 0.1, 0.2, 0.5, or 1.0 M with NaCl, Na<sub>2</sub>SO<sub>4</sub>, or Na<sub>2</sub>SO<sub>3</sub>. Each resulting dispersion was adjusted to pH 10.0 with 1 N NaOH and incubated in a shaker at 180 rpm for 1 h at 50°C. These products were frozen at -5°C, freeze-dried, and stored at ambient temperature (23°C).

**Adhesive strength.** The procedures used for gluing of wood pieces and for determining adhesive strengths were as described by Kalapathy *et al.* (14). The reader is referred to this reference for details of the procedures. One hundred milligrams of 8.0% (w/w) protein solution was placed on opposite ends of a wood piece (5 × 2 × 0.3 cm) and spread on an area of 2 × 2 cm to give a protein concentration of 2.0 mg/cm<sup>2</sup>. Two other wood pieces of similar size were superimposed on these glued areas and pressed with a load of 5 kg for 2 h. A total of 5 blocks per treatment were prepared. The glued wood pieces were allowed to dry overnight at ambient conditions. The force (N) required to shear the glued wood pieces was measured with an Instron Model 1011 (Instron Corporation, Canton, MA) by pulling them apart from two edges at a loading rate of 20 mm/min, and was expressed as adhesive strength of protein glue. All values for the adhesive strength reported are means of five measurements.

**Water soaking test.** A total of 30 blocks per treatment were prepared. The glued wood pieces were placed in a 4-L container, submerged in 4 L of tap water (23°C) and allowed to soak for 24 h (a weight was used to keep the wood pieces submerged). The wood pieces were air-dried at ambient temperature (23°C) for 10 h under a fume hood with fan-forced air circulation. The wood pieces were subjected to four additional cycles of soaking and air-drying treatments. After each cycle, wood pieces were examined for delamination, and the delaminated wood pieces were removed. The percentage of the total number of glue joints that were delaminated was used as an indicator of lack of water resistance of the protein adhesive.

**Viscosity.** Viscosity of modified soy protein adhesives at 14% solid dispersions was determined by Brookfield viscometer (Stoughton, MA). Industrial formulation of adhesives requires a viscosity of <5000 cP at 20% solids (personal communication, National Casein). However, in the case of soy protein adhesives, 20% solids gave a paste rather than a dispersion. Hence, the highest possible concentration of 14% that gave uniform dispersions with all protein samples was selected for viscosity measurement. However, an 8% dispersion was used to glue wood pieces because it gave uniform spread on wood during the adhesion. The spindle speed was

20 rpm. All measurements were made in duplicate at ambient temperature (23°C).

**Sulfhydryl (SH) and disulfide (SS) content of proteins.** The SH and total SH/SS contents were determined with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) according to the method of Beveridge *et al.* (15). Modified soy protein samples (100 mg) were suspended in 10.0 mL of 0.08 M Tris-glycine buffer (pH 8.0), containing 8 M urea and 3 mM ethylene diaminetetraacetic acid (EDTA), and stirred for 1 h. Ten milliliters of 12% trichloroacetic acid (TCA) was added to this solution, vortexed, and incubated for 1 h at 23°C. The solution was then centrifuged at 12,000 rpm for 10 min. The precipitate was washed twice with 5 mL 12% TCA to remove Na<sub>2</sub>SO<sub>3</sub> and resuspended in 10 mL Tris-glycine buffer that contained urea and EDTA.

For SH determination, 4.5 mL of Tris-glycine buffer with urea and EDTA, and 0.05 mL DTNB reagent (4 mg/mL) were added to 0.5 mL of the above solution. Absorbances were measured at 412 nm, and the SH contents were calculated at an extinction coefficient of 13.6 mM<sup>-1</sup>.

For total SH/SS, 50 μL mercaptoethanol was added to SPI suspended in Tris-glycine/urea/EDTA buffer and stirred for 1 h at 23°C. The total SH/SS was determined from this solution as described above for alkali-modified soy proteins.

**Statistical analysis.** The general linear models procedure (SAS Institute, Cary, NC) was used for data analysis. The differences between means were tested with Tukey's studentized range test at the 5% level.

## RESULTS AND DISCUSSION

**Effect of ionic environment on adhesive strength and viscosity.** Figures 1 and 2 show the effect of various concentrations of NaCl and Na<sub>2</sub>SO<sub>4</sub> on adhesive strength and viscosity of modified soy proteins. A 14% concentration of solids was used for viscosity measurement because higher concentrations gave thick pastes. Adhesive strength decreased gradually as the concentration of salts increased from 0 to 1.0 M. However, the adhesive strength values for the control alkali-

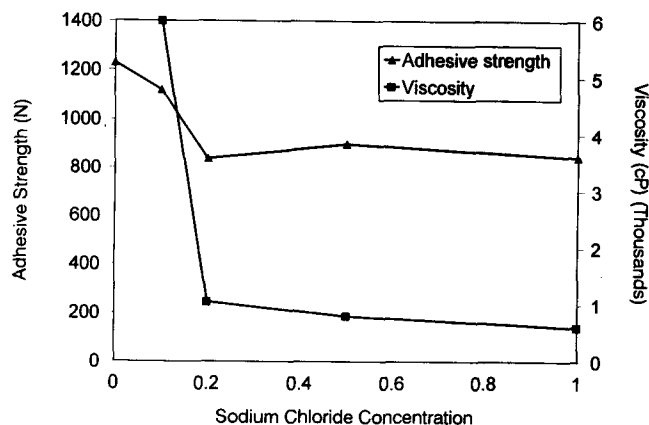


FIG. 1. Effect of various concentrations of NaCl on adhesive strength and viscosity of modified soy proteins.

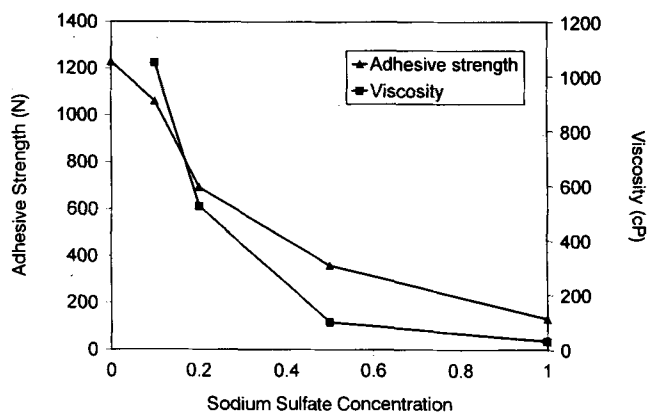


FIG. 2. Effect of various concentrations of  $\text{Na}_2\text{SO}_4$  on adhesive strength and viscosity of modified soy proteins.

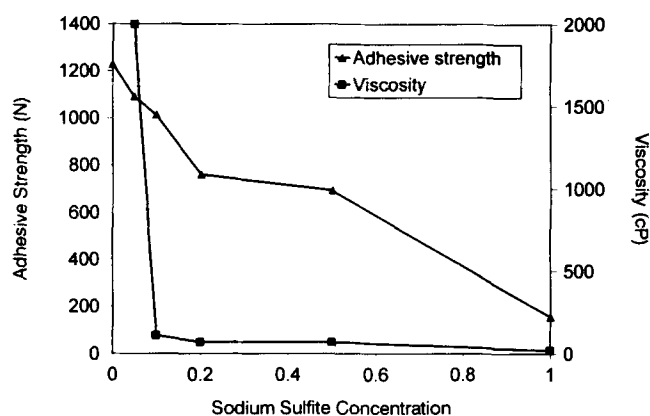


FIG. 3. Effect of various concentrations of  $\text{Na}_2\text{SO}_3$  on adhesive strength and viscosity of modified soy proteins.

modified proteins (no additives) vs. the modified soy proteins treated with 0.1 M salts were not significantly different ( $P < 0.05$ ). Further, increases in salt concentration from 0.1 to 0.2 M resulted in a significant decrease in adhesive strength, from 1120 to 837 N for  $\text{NaCl}$  and from 1060 to 693 N for  $\text{Na}_2\text{SO}_4$ . The decrease in adhesive strength at higher salt concentrations may be a result of weakening of the interaction of the polar groups of the proteins with the polar groups in woods. When the  $\text{NaCl}$  concentration increased further, up to 1.0 M, no significant change in adhesive strength was observed, but with  $\text{Na}_2\text{SO}_4$ , adhesive strength decreased significantly. These observed differences could be due to the differences in effective wood-adhesive interfacial area in the presence of  $\text{NaCl}$  and  $\text{Na}_2\text{SO}_4$ .

Sharp decreases in soy protein dispersion viscosity were observed with increasing salt concentrations up to 0.2 M. The viscosity decreased from >30,000 cP to 6000 cP for  $\text{NaCl}$  and to 1050 cP for  $\text{Na}_2\text{SO}_4$  as the salt concentration was increased from 0 to 0.1 M. The viscosity decreased progressively with further increases in salt concentrations. However, the maximum concentration of  $\text{NaCl}$  or  $\text{Na}_2\text{SO}_4$  that could be used for viscosity reduction with no significant decrease in adhesive strength under the conditions tested was 0.1 M.

**Effect of reducing agent on adhesive strength and viscosity.** The effect of reducing agent  $\text{Na}_2\text{SO}_3$ , at concentrations ranging from 0 to 1.0 M on adhesive strength and viscosity, is shown in Figure 3. Again, adhesive strength decreased gradually with an increasing concentration of  $\text{Na}_2\text{SO}_3$ . However, there was no significant decrease ( $P < 0.05$ ) in adhesive strength of modified soy proteins when the concentration of  $\text{Na}_2\text{SO}_3$  increased from 0 to 0.1 M. Adhesive strength decreased significantly from 1013 to 759 N when the concentration of  $\text{Na}_2\text{SO}_3$  was increased from 0.1 to 0.2 M. As observed with  $\text{Na}_2\text{SO}_4$ , a further increase in  $\text{Na}_2\text{SO}_3$  concentration also resulted in a significant decrease in adhesive strength. Although SH groups were converted to more polar  $-\text{SSO}_3$  groups due to the reaction with  $\text{Na}_2\text{SO}_3$ , the adhesive strength gradually decreased with increasing concentration of  $\text{Na}_2\text{SO}_3$ . This suggests that the increase in interaction result-

ing from the conversion of SH to  $-\text{SSO}_3$  is insufficient to compensate for the decrease in adhesive strength resulting from a decrease in effective wood-protein interfacial area due to the presence of ions.

The viscosity sharply decreased from >30,000 to 110 cP when the  $\text{Na}_2\text{SO}_3$  concentration increased from 0 to 0.1 M. Hence, treatment with 0.1 M concentration of  $\text{Na}_2\text{SO}_3$  at an alkaline pH of 10.0 is desirable to obtain modified soy proteins with low viscosity without causing a significant effect on adhesive strength on wood.

Free SH contents of modified soy proteins, treated with various concentrations of  $\text{Na}_2\text{SO}_3$ , are given in Table 1. Free SH contents of soy protein isolate at pH 10.0 and modified soy protein treated with 0.1 M  $\text{Na}_2\text{SO}_3$  were 1.35 and 10  $\mu\text{M/g}$  protein, respectively. Total SH content of reduced soy protein isolate was 70.35  $\mu\text{M/g}$  protein. Hence, the percentage of disulfide bonds cleaved in modified soy proteins due to the treatment with 0.1 M  $\text{Na}_2\text{SO}_3$  was 28%.

**Water resistance.** Water resistance is an important property that determines glue durability (3). The water resistive properties of unmodified SPI, SPI at pH 10.0, and modified soy protein treated with 0.1 M  $\text{Na}_2\text{SO}_3$ , as measured by the water soaking test, are shown in Table 2. A total of 30 blocks (three replicates with 10 blocks in each) were tested for each sample. The total number of blocks delaminated after the fourth cycle of the soaking test for unmodified SPI, control alkali-modified soy protein, and modified soy protein treated

TABLE 1  
Amounts of Free Sulfhydryl (SH) Groups Present in Modified Soy Proteins Treated with Various Concentrations of  $\text{Na}_2\text{SO}_3$

$\text{Na}_2\text{SO}_3$ concentration (M)	Free SH group ( $\mu\text{M/g}$ of protein)	Percentage of SS groups cleaved
0.0	1.35	—
0.05	8.24	20
0.1	10.97	28
0.2	13.89	36
0.5	16.49	44
2-Mercaptoethanol	70.35 <sup>a</sup>	100

<sup>a</sup>Total disulfide (SS) and SH as micromoles of SH/gram of protein.

**TABLE 2**  
**Water Resistance of Modified and Unmodified Soy Protein Glues**

Sample <sup>a</sup>	Cycle <sup>b</sup>				Total
	1	2	3	4	
Unmodified SPI	3.3	6.7	16.7	46.7	72.3
Control soy proteins at pH 10.0	0	0	0	3.3	3.3
Soy proteins treated with 0.1 M Na <sub>2</sub> SO <sub>3</sub>	3.3	0	0	3.3	6.6

<sup>a</sup>A total of 30 blocks of wood were used for each treatment.

<sup>b</sup>Values are percentages of wood blocks delaminated during each cycle of water soaking test.

with 0.1 M Na<sub>2</sub>SO<sub>3</sub> was 22, 1, and 2, respectively. These results show that the water resistance of modified soy proteins was not significantly affected by the incorporation of 0.1 M Na<sub>2</sub>SO<sub>3</sub>.

In conclusion, the data show that cleavage of 28% of the disulfide bonds in alkali-modified soy proteins resulted in a product with low viscosity without adversely affecting the adhesive and water-resistive properties. Low-viscosity adhesives allow easy handling and are useful for gluing highly absorbing materials, such as dry woods in particle board.

#### ACKNOWLEDGMENT

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#### REFERENCES

- Lambuth, A.L., Soybean Glues, in *Handbook of Adhesives*, 2nd edn., edited by I.S. Keist, Van Nostrand Reinhold, New York, 1977, pp. 172–180.
- Myers, D.J., Industrial Applications for Soy Protein and Potential for Increased Utilization, *Cereal Foods World* 38:355–360 (1993).
- Hettiarachchy, N.S., U. Kalapathy, and D. Myers, Alkali Modified Soy Proteins with Improved Adhesive and Hydrophobic Properties, *J. Am. Oil Chem. Soc.* 72:1461–1467 (1995).
- Shen, J.L., Solubility Profile, Intrinsic Viscosity, and Optical Rotational Studies of Acid Precipitated Soy Protein and Commercial Soy Isolate, *J. Agric. Food Chem.* 24:784–788 (1976).
- Kinsella, J.E., Functional Properties of Soy Proteins, *J. Am. Oil Chem. Soc.* 56:242–258 (1979).
- Kinsella, J.E., S. Damodaran, and B. German, Physicochemical and Functional Properties of Oilseed Proteins with Emphasis on Soy Proteins, in *New Protein Foods*, edited by A.M. Altschul and H.L. Wiclke, Academic Press, Inc., 1985, pp. 113–161.
- Kella, N.K.D., S.T. Yang, and J.E. Kinsella, Effect of Disulfide Bond Cleavage on Structural and Interfacial Properties of Whey Proteins. *J. Agric. Food Chem.* 37:1203–1210 (1989).
- German, J.B., T.E. O'Neil, and J.E. Kinsella, Film Forming and Foaming Behavior of Food Proteins, *J. Am. Oil Chem. Soc.* 62:1358–1366 (1985).
- Klemazewski, J.L., and J.E. Kinsella, Sulfitolysis of Whey Proteins: Effects on Emulsion Properties, *J. Agric. Food Chem.* 39:1033–1036 (1991).
- Kella, N.K.D., Y.J. Kang, and J.E. Kinsella, Effect of Oxidative Sulfitolysis of Disulfide Bonds of Bovine Serum Albumin on Its Structural Properties: A Physicochemical Study, *J. Protein Chem.* 7:535–548 (1988).
- Kim, S.H., and J.E. Kinsella, Effects of Reduction with Dithiothreitol on Some Molecular Properties of Soy Glycinin, *J. Agric. Food Chem.* 34:623–627 (1986).
- Kawamura, Y., Y. Matsumura, T. Matoba, D. Yonezawa, and M. Kito, Selective Reduction of Interpolypeptide and Intrapolypeptide Disulfide Bonds of Wheat Gluten from Defatted Flour, *Cereal Chem.* 62:279–283 (1985).
- Circle, S.J., E.W. Meyer, and R.W. Whitney, Rheology of Soy Protein Dispersions. Effect of Heat and Other Factors on Gelation, *Ibid.* 41:157–172 (1964).
- Kalapathy, U., N.S. Hettiarachchy, D. Myers, and M.A. Hanna, Modification of Soy Proteins and Their Adhesive Properties on Woods, *J. Am. Oil Chem. Soc.* 72:507–510 (1995).
- Beveridge, T., S.J. Toma, and S. Nakai, Determination of SH and SS Groups in Some Food Proteins Using Elman's Reagent, *J. Food Sci.* 39:49–51 (1974).

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