Thermal Properties and Adhesion Strength of Modified Soybean Storage Proteins

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ABSTRACT: Soy proteins have shown great potential for adhesive and resin applications. This investigation characterized the thermal and adhesive properties of the major soy protein components conglycinin (7S) and glycinin (11S) after chemical modification. These globulins were extracted from defatted soy flour, then modified with either sodium hydroxide, sodium dodecyl sulfate (SDS), or urea. Modified 7S, 11S, and mixtures of 7S and 11S at varying ratios were evaluated for gluing strength with cherry veneer plywood and for thermal denaturation using DSC. Adhesive strength and water resistance were significantly improved for all proteins modified with sodium hydroxide. Gluing strength and water resistance were improved for SDS- and urea-modified proteins containing greater portions of 7S globulins. The opposite behavior was observed for proteins containing large amounts of 11S globulins. DSC results showed that the temperatures of denaturation (T_d) decreased for the proteins modified with sodium hydroxide or urea, whereas the T_d values of proteins modified with SDS were similar to the unmodified proteins. These results suggested that, at the concentrations studied, sodium hydroxide or urea could denature soybean protein more effectively than SDS, resulting in lower protein thermal stability. Soybean proteins with high ratios of 11S had more ordered structures, as evidenced by the high enthalpy values of protein denaturation observed in DSC measurements.

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KEY WORDS: 7S globulins, 11S globulins, adhesive, denaturation, protein modification, shear strength, soybean storage proteins, thermal property, water resistance.

Approximately 3.6 million metric tons of adhesives and resins are used annually in the United States in the construction, packaging, and furniture industries (1). These adhesives include urea formaldehyde, phenol formaldehyde, phenol resorcinol formaldehyde, and isocyanates. Concerns about limited petroleum resources and harm to the environment have been raised with the use of these adhesives. Therefore, the use of biobased adhesives as a substitute for petroleum-based adhesives is becoming increasingly important. Soybean proteins have been considered as a strong alternative because of their abundance and renewability. These proteins have shown great potential for adhesive and resin applications (2). They have been used to replace phenol resourcinol formaldehyde in finger-joint applications with fresh wood to improve performance and reduce cost (3), as well as in the fabrication of medium-density composite fiberboard (4) and low-density straw particleboard (5). However, research has shown that soybean protein-based adhesives have low water resistance (6).

The dominant storage proteins in soybean are globulins, which account for 50–90% of the total proteins (7). Storage globulins are grouped into 11S (glycinin) and 7S (conglycinin) globulins according to their sedimentation coefficients. The ratio of 11S to 7S globulins varies among cultivars within the range of 0.5–1.7 (7). The 11S globulin is a very heterogeneous oligomeric protein with a M.W. of 320–360 kDa. It has a quaternary structure and consists of six subunits: Six acidic (A) and six basic (B) polypeptides are joined by disulfide bonds, forming AB subunits. The 7S globulin contains a major fraction, β-conglycinin, which is a trimeric glycoprotein with a M.W. of 150–200 kDa, and also consists of three types of subunits: α′ (72 kDa), α (68 kDa), and β (52 kDa) (8). The structural differences between 7S and 11S globulins contribute to variations in their functional properties. Furthermore, the ratio of 11S to 7S globulins is known to affect the properties of soy products, such as the hardness of tofu and its sensory properties (9), the texture of extruded products (10), and the gelling, emulsifying, and foaming properties of the product (11).

Native soybean proteins have a highly ordered global structure, with hydrophilic groups exposed outside and hydrophobic groups buried inside. In gluing applications, a large number of reactive groups are unavailable for interaction with the wood substrate due to the internal bonds resulting from hydrogen bonding and because of hydrophobic interactions (6). Chemical modifications could break the internal bonds and unfold the protein molecules, making it possible for the entire protein complex and reactive structure to interact with cellulosic materials, thus increasing the adhesion potential. Protein denaturation using an alkali, detergent, or organic solvent could induce protein unfolding (12) and consequently increase bonding strength. Improved adhesive strength and water resistance have been observed for adhesives prepared from proteins modified with alkali (13), urea (14), and SDS (4,15).

Inherent differences in the structure and molecular properties of 11S and 7S globulins, as well as structural changes caused by chemical modification, would affect their adhesion performance. The objectives of this study were to characterize the thermal and adhesive properties of soybean 7S and 11S proteins modified

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with either sodium hydroxide, SDS, or urea and to examine the effects of chemical modification and the 11S-to-7S globulin ratios on adhesive strength and water resistance.

MATERIALS AND METHODS

Materials. Defatted soy flour with a protein dispersion index of 90 was obtained from Cargill (Cedar Rapids, IA) and used for the preparation of soy protein isolate. Plywood [50 (width) × 127 (length) × 3 mm (thickness)] was produced from cherry veneer supplied by Veneer One (Oceanside, NY). Urea, SDS, and sodium hydroxide (NaOH) were obtained from Sigma Chemical Co. (St. Louis, MO).

Isolation of 7S and 11S globulins. 7S and 11S globulins were separated from the soy flour by the method of Thanh and Shibasaki (16). Soy flour was extracted with 30 mM Tris buffer (pH 8.0) containing 10 mM mercaptoethanol. The pH of the extract was adjusted to 6.4, and the precipitate collected was 11S globulins. 7S globulins were separated from the whey proteins by isoelectric precipitation at pH 4.8. The precipitated 7S and 11S globulins were redissolved in distilled water with pH adjusted to 7.8, freeze-dried (Labconco FreezeZone 6 Liter; Kansas City, MO), and then milled (Cyclone Sample Mill; UDY Corp., Fort Collins, CO) into a powder with a particle size of less than 1 mm.

Protein modification and specimen preparation. The optimal concentrations of each denaturant that provided maximum gluing strength were chosen based on previous studies (13–15). The proteins were added to solutions of either 1% SDS, 3 M urea, or 0.4% NaOH at a weight ratio of 12:100, stirred, and allowed to react for 2 h at room temperature. Proteins with no modification were used as the control. Protein slurry (600 mg) was placed on each side of a piece of wood and spread uniformly with a brush on a marked area of 127 × 20 mm (Fig. 1). Two wood pieces were allowed to rest at room temperature for 15 min and were then assembled and pressed together using a Hot Press (Model 3890 Auto "M"; Carver Inc., Wabash, IN) at a molding pressure of 3.57 MPa at 130°C for 5 min.

DSC measurements. Soybean protein adhesives usually require thermal curing to effectively develop bond strength. Chemical modification of soybean proteins also will denature proteins and change their thermal transition properties, including denaturation temperatures and the enthalpy of denaturation. The loss of a certain degree of the native structure could be critical to the protein's adhesive performance. The thermal properties of modified protein adhesives were studied by using a differential scanning calorimeter (DSC 7; PerkinElmer, Norwalk, CT), which was calibrated with indium and zinc. A large DSC pan was used with about 50 mg of modified proteins. All samples were held at 30°C for 1 min and then scanned from 30 to 140°C at a heating rate of 10°C/min. The denaturation enthalpy was calculated as the sum of the 7S and 11S denaturation enthalpies. The reported values are the average of two replicates.

Shear strength. Wood specimens (80 × 20 mm, with a glue area of 20 × 20 mm) for shear strength testing were prepared and tested according to ASTM Standard Method D 2339-98 (17) using an Instron Model 4465 (Canton, MA). Wood specimens were preconditioned at 23°C and 50% RH for 7 d before testing. The crosshead speed was 1.6 mm/min, and the stress at maximum load was recorded. The reported results are the average of five samples.

Water resistance. Water resistance was measured following ASTM Standard Methods D 1183-96 (18) and D 1151-00 (19). Preconditioned specimens were soaked in tap water at 23°C for 48 h. Wet strength was measured immediately after soaking, and soaked strength was obtained by testing the specimens after they were dried and conditioned at 23°C and 50% RH for 7 d. Shear strength was tested as described in the preceding section.

Experimental design and data analysis. A 5 × 4 full-factorial design was used to study the effects of protein adhesives and chemical treatment and their interactions on adhesive strength. The 11S-to-7S ratios tested were 0:1, 1:3, 1:1, 3:1,

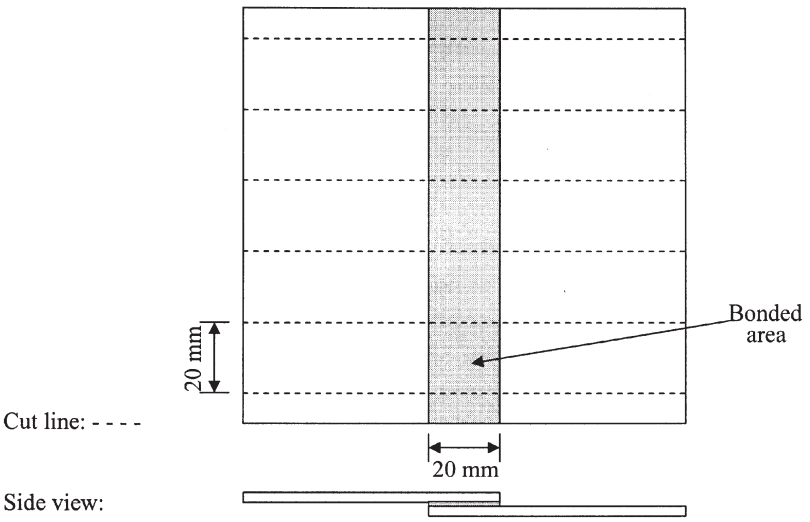

FIG. 1. Fiberboard assembly for the shear strength test.

and 1:0, whereas the four modifications were with urea, SDS, NaOH, and unmodified protein. ANOVA and LSD tests were performed using SAS (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Thermal properties. The thermal denaturation of protein involves the disruption of intramolecular bonding and the unfolding and aggregation of protein molecules. The unfolding step is usually a highly cooperative transition accompanied by a significant uptake of heat, which is revealed as endothermic peaks in the DSC thermogram. The denaturation temperature (T_d) and enthalpy of denaturation (ΔH_d) were determined from the maximal peak temperature and the area of the peak, respectively. The thermogram of 11S exhibited one peak with a T_d of 87.8°C, corresponding to the endothermic transitions (denaturation) of 11S globulins. The thermogram of 7S exhibited two peaks—one major peak with a T_d of 77.2°C, corresponding to the thermal denaturation of 7S, and a small shoulder, corresponding to the endothermic denaturation of 11S globulin, which apparently contaminated the 7S fraction. The thermogram of protein with equal amounts of 11S and 7S fractions showed two peaks, corresponding to the denaturation of 11S and 7S, respectively (Fig. 2). The T_d values of soy protein with different 11S-to-7S ratios after modification were similar to those of soy protein with equal amounts of 11S and 7S (data not shown). SDS-modified soy protein had T_d values similar to that of unmodified protein (Table 1). However, only one peak (T_d value = 68°C), for 11S globulin, was observed for the NaOH-modified soy protein, indicating that NaOH modification could completely denature 7S globulins and greatly destabilize 11S globulins. The T_d values of urea-modified soy protein were lower than that of the control (Table 1), which means urea modification could decrease the stability of soy protein, especially the 7S globulin.

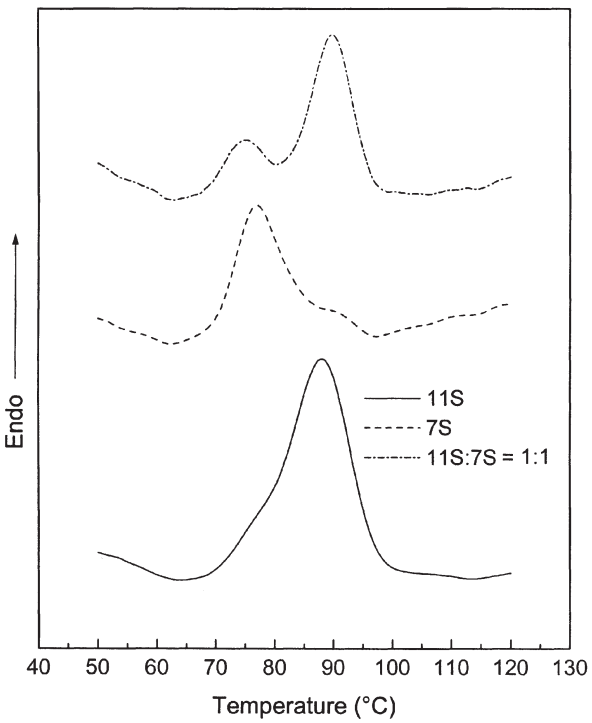

FIG. 2. DSC thermogram of soy protein components. 11S: 11S-rich fraction; 7S: 7S-rich fraction; 11S:7S = 1:1, equal amount of 11S and 7S fractions.

TABLE 1
Denaturation Temperatures of Soy Protein with Equal Amounts of 11S and 7S Globulins[a]

Modifier	T_{d1}[b] (°C)	T_{d2}[b] (°C)
None (control)	73.4[a]	89.7[a]
NaOH	—	68.0[c]
SDS	74.1[a]	89.4[a]
Urea	68.8[b]	87.4[b]

[a]Means with the same superscript letters in the same column are not significantly different at $\alpha = 0.05$.
[b]T_{d1} and T_{d2} represent denaturation temperatures for 7S and 11S globulins, respectively.

The ΔH_d of soy protein increased as the amount of 11S fraction increased for both unmodified and modified soy protein (Table 2). Because ΔH_d is an estimation of the thermal energy required to denature the protein, the data indicated that the 11S fraction was more heat stable than that of the 7S globulins. The ΔH_d values were in the order of NaOH < urea < SDS < control when compared within a fixed ratio of 11S to 7S, indicating that the denaturation power of modifiers at the concentrations tested was in the order of NaOH > urea > SDS. The ΔH_d values for soy protein containing larger proportions of 7S globulins modified with NaOH or urea were much lower than those modified with SDS, indicating that both NaOH and urea could denature 7S globulins more effectively.

NaOH, urea, and SDS are all commonly used as denaturing reagents. However, their mechanisms of denaturation are different, and the resulting denatured protein structures might also differ (20). For example, NaOH could break the internal hydrogen bonds of compacted molecules and extensively unfold the protein, resulting in the exposure of buried sulfhydryl and hydrophobic residues. The protein molecules also could hydrolyze in the alkaline condition, with little residual structure remaining. The anionic detergent SDS can bind the protein molecules through hydrophobic interactions and dissociate the protein by disrupting the hydrophobic and electrostatic bonds that maintain the stability of protein conformation. The final product of denaturation is still remarkably compact, consisting of globular regions in close contact with each other. The partially unfolded proteins also could interact with the hydrophobic moieties of

TABLE 2
Denaturation Enthalpies (J/g) of Modified Soy Protein with Different 11S-to-7S Ratios[a]

	11S-to-7S ratio				
Modifier	0:1	1:3	1:1	3:1	1:0
None (control)	7.25[a]	7.92[a]	10.04[a]	10.94[a]	12.07[a]
NaOH	0.42[c]	0.48[d]	1.28[d]	2.50[d]	2.59[d]
SDS	5.43[b]	5.37[b]	6.43[b]	7.56[b]	9.03[b]
Urea	0.46[c]	1.01[c]	3.41[c]	3.25[c]	3.06[c]

[a]Means with the same superscript letters in the same column are not significantly different at $\alpha = 0.05$.

the SDS molecules to form micelle-like regions. The relatively high ΔH_d values of SDS-modified soy protein obtained in DSC thermoanalysis confirmed that the compact structures still remained after modification. Urea can destabilize globular protein by forming strong hydrogen bonds with the water molecules that surround the protein while disrupting the protein's hydrogen bonds, resulting in partially unfolded protein structures. The protein denatured by urea probably exists as random coils. The transition to the denatured state is often incomplete when the native conformation is stabilized by disulfide bonds (20). The thermoanalysis in this study indicated that the ΔH_d values for urea-modified protein with a higher portion of 11S globulins were much higher than those with a higher portion of 7S globulins (Table 1). This might be because more disulfide bonds are present in 11S globulins, allowing for a more ordered structure after denaturation.

Adhesive strength. Shear strength increased as the 11S globulin content increased for the unmodified soy proteins (Fig. 3). The adhesive strength is largely dependent on interactions among the chemical groups of the protein and the wood material. 11S globulin has a higher M.W. than 7S globulin, and the native 11S globulin could have more active chemical groups available for bonding, resulting in high shear strength. The effects of the denaturant on shear strength of the modified proteins varied (Figs. 4–6). For NaOH-modified soy protein, the maximum shear strength was observed at an 11S-to-7S ratio of 1:3, and the lowest shear strength was at an 11S-to-7S ratio of 3:1 (Fig. 4). For the SDS-modified soy protein, the maximum shear strength was observed with equal amounts of 11S and 7S globulins, whereas the lowest shear strength was observed at an 11S-to-7S ratio of 3:1 (Fig. 5). For urea-modified soy proteins, the shear strength decreased as the amount of 11S in the soy proteins increased (Fig. 6). These results indicate that the adhesive strengths of modified soybean proteins differed greatly and were affected by the ratio of 11S to 7S and by the chemical modification methods applied. Chemical modifications could either improve the adhesive strength at certain ratios of 11S to 7S or be detrimental to bonding strength, usually with protein rich in 11S globulins. An optimal 11S-to-7S ratio was essential for each type of chemical modification of soybean protein to achieve the best adhesion performance.

For dry strength, the NaOH modification significantly improved adhesive strength regardless of the 11S-to-7S ratio, and the percentage increase in strength was higher for soy protein with a higher 7S content (Table 3). The proteins modified with SDS showed improved adhesive strength for samples with 50% or more of 7S. Urea modification improved the adhesive strength of protein samples with high proportions of 7S.

Water resistance. Both wet and soaked adhesive strengths are important properties that determine adhesive bond durability for exterior applications. During soaking, water molecules can penetrate into the glue areas, interact with protein molecules, and weaken the interface between the proteins and the wood. Soaked strength decreased by less than 10% compared with dry strength, but wet strength greatly decreased after 48 h of soaking (Figs. 3–6). The adhesive bonding strength was mostly recovered after removing the water.

The wet adhesive strength of the protein modified with NaOH improved by 16.2% to 80% (Table 3). Similar to the

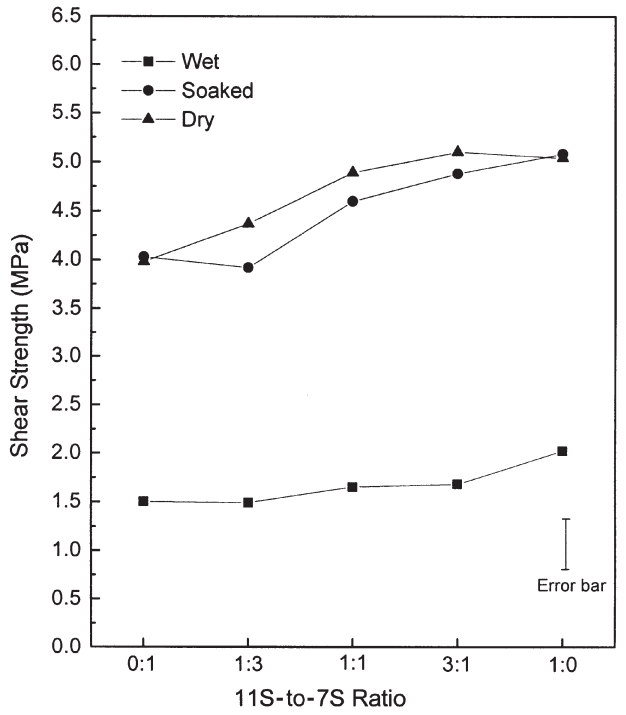

FIG. 3. Shear strength of soy protein adhesives as affected by the 11S-to-7S ratio. Dry: dry strength; soaked: soaked strength; wet: wet strength.

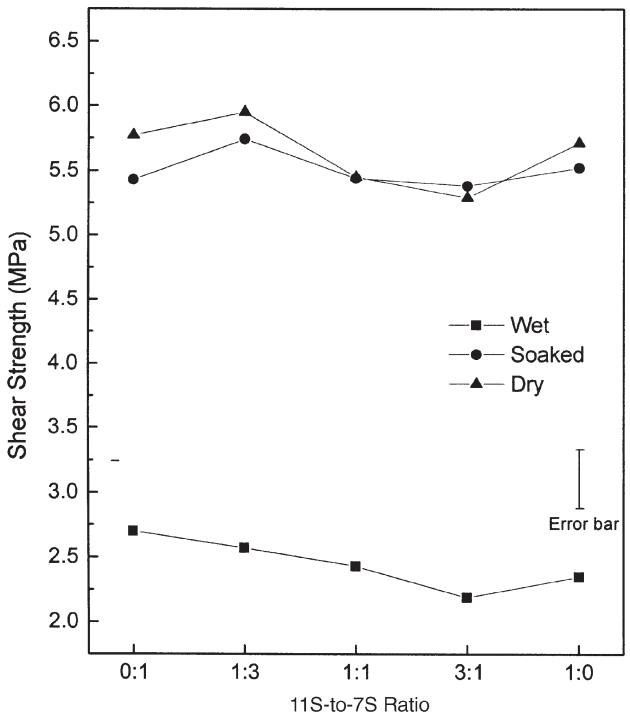

FIG. 4. Shear strength of soy protein adhesives as affected by the 11S-to-7S ratio and NaOH modification. See Figure 3 for abbreviations.

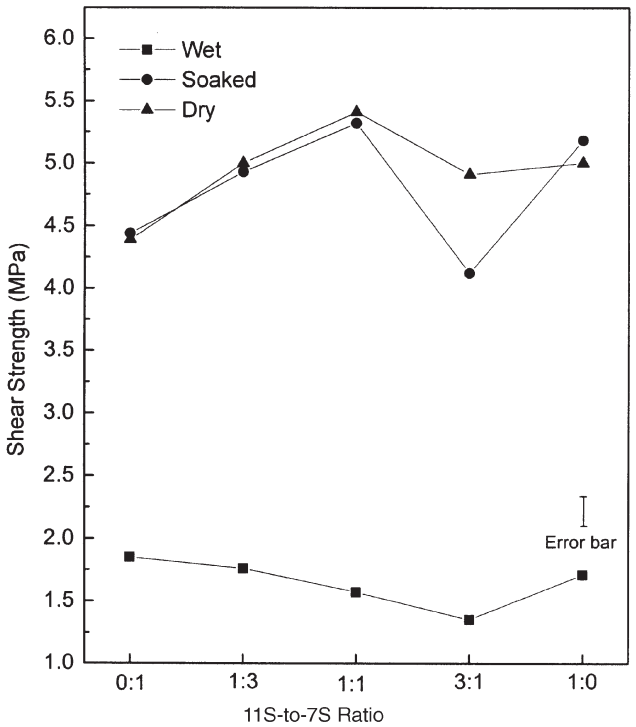

FIG. 5. Shear strength of soy protein adhesives as affected by the 11S-to-7S ratio and SDS modification. See Figure 3 for abbreviations.

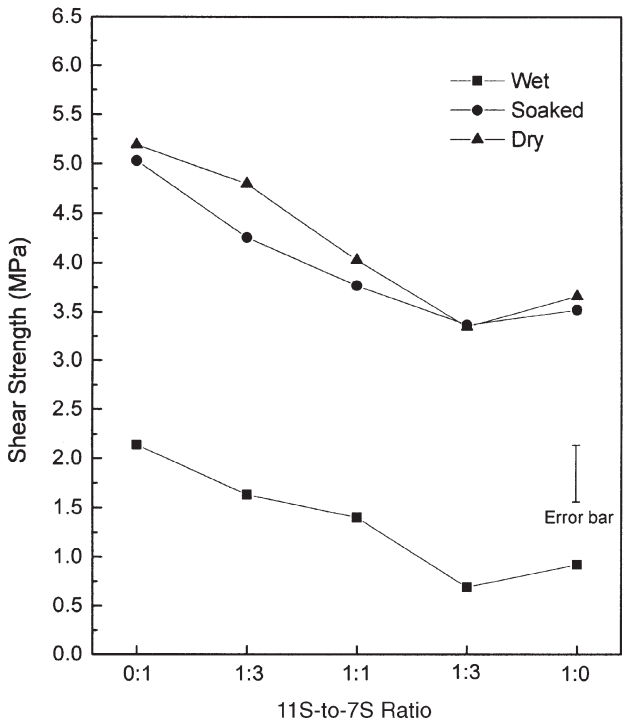

FIG. 6. Shear strength of soy protein adhesives as affected by the 11S-to-7S ratio and urea modification. See Figure 3 for abbreviations.

trend observed for dry strength, the greatest improvement was observed for the protein composed mainly of 7S globulin, and the degree of improvement decreased as the proportion of 11S globulins increased. SDS modification increased the wet adhesive strength by about 20% for the protein containing a high proportion of 7S globulins. Wet adhesive strength improved most for the urea-modified protein containing only 7S globulins. The urea-modified soy protein containing high proportions of 11S globulins showed significantly lower shear strengths than the corresponding unmodified soy proteins, as indicated by the negative values (Table 3).

The gluing strength of a protein adhesive depends on its structure and components, on its ability to disperse in water, and on the interaction of the hydrophobic and hydrophilic groups of the protein with the wood material (2). During curing, the protein adhesives flow into the rough wood surface, harden, and become physically stuck. Soy proteins have a large number of reactive groups, which have the potential to form covalent bonds with the hydrophilic groups of the wood. Chemical modification could unfold the soy protein globular structure and expose residues that are buried inside the native protein. In addition, the combination of modification and globulin ratio could affect disulfide bonding, noncovalent bonds, and interactions between the subunits of 11S and 7S, which could, in turn, determine the extent of protein structural changes and the subsequent interactions of proteins with the wood. The increased interactions of the soy protein residues with the wood could contribute to improved bonding strength.

An alkali-modified soy protein adhesive was reported to be stronger and more water-resistant than one with unmodified soy protein (13). As indicated by the thermal properties (Table 2), the highest degree of denaturation was observed for the NaOH-modified soy proteins. Racemization of L-amino acid to D-isomers has been reported to occur after exposing soy proteins to strongly alkaline conditions (21). The NaOH could extensively unfold and hydrolyze the protein, resulting in a mixture of oligomeric polypeptides with a good molecu-

TABLE 3
Percentage Increase (%) in the Shear Strength of Plywood Bonded with Modified Soybean Storage Proteins[a,b]

	11S-to-7S ratio				
	0:1	1:3	1:1	3:1	1:0
Dry strength					
NaOH	45.0[a]	36.2[a]	11.4[a]	3.7[a]	13.3[a]
SDS	10.3[c]	14.4[b]	10.6[a]	–3.7[b]	–0.8[b]
Urea	30.4[b]	9.8[c]	–17.6[b]	–34.3[c]	–27.4[c]
Wet strength					
NaOH	80.0[a]	72.5[a]	47.3[a]	30.7[a]	16.2[a]
SDS	23.3[c]	18.1[b]	–4.8[b]	–19.4[b]	–15.3[b]
Urea	42.7[b]	9.4[c]	–15.2[c]	–58.8[c]	–54.4[c]
Soaked strength					
NaOH	34.7[a]	46.4[a]	18.3[a]	10.2[a]	8.7[a]
SDS	10.2[c]	25.8[b]	15.6[b]	–15.6[b]	1.9[b]
Urea	24.8[b]	8.67[c]	–18.0[c]	–30.9[c]	–30.7[c]

[a]Calculated as (average shear strength of unmodified soybean storage proteins – average shear strength of modified soybean storage proteins)·100/average shear strength of unmodified soybean storage proteins.
[b]Means with the same superscript letters within the same group of adhesive strength test in the same column are not significantly different at $\alpha = 0.05$.

lar size distribution. The NaOH-modified soy protein might also have a molecular structure that exposes a large number of reactive groups for bonding with the wood, leading to an improvement in adhesive strength and water resistance.

Soy protein modified with urea or SDS had a higher ΔH_d compared with that modified by NaOH (Table 2), indicating that a certain ordered structure still remained and left a limited number of chemical groups available for bonding with the wood. Both urea and SDS modification denatured 7S globulins more effectively than 11S globulins (Table 2). As a result, the shear strength of modified soy protein rich in 7S globulins showed greater improvement than that rich in 11S. As the amount of 11S in the soy protein increased with chemical modification, the degree of protein denaturation decreased. This resulted in a denatured protein structure that might not be favorable for bonding with the wood; thus, shear strength decreased or showed limited improvement. The 7S globulin has a quaternary structure that is stabilized by hydrophobic and hydrogen bonding. The extent of disulfide cross-linking is limited because it has only two to three cysteine groups per mole of protein (22). On the other hand, the 11S globulin is tightly folded and linked *via* disulfide bonds. It contains approximately 48 moles of cysteine, and disulfide groups account for about 37 moles per mole of protein (23). The high content of disulfide bonds greatly helps maintain the stability of the 11S structure. As a result, proteins with a high 11S content have a more ordered structure than proteins with a high 7S content; they also show a lower degree of denaturation and give relatively low improvements in adhesive strength after modification with urea or SDS.

The adhesion performance of soybean protein adhesives is greatly determined by their structure. A high adhesive strength usually can be achieved for modified soybean protein with high a degree of denaturation. The adhesive strength and water resistance of soybean protein rich in 7S globulins can be improved considerably by chemical modification with denaturants, particularly NaOH.

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