Adhesive Properties of Soy Proteins Modified by Urea and Guanidine Hydrochloride

Weining Huang and Xiuzhi Sun*

Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506

ABSTRACT: An investigation was conducted on the adhesive and water-resistance properties of soy protein isolates that were modified by varying solutions of urea (1, 3, 5, and 8 M) or guanidine hydrochloride (GH) (0.5, 1, and 3 M) and applied on walnut, cherry, and pine plywoods. Soy proteins modified by 1 and 3 M urea showed greater shear strengths than did unmodified protein. The 3 M urea modification gave soy protein the highest shear strength. Soy proteins modified with 0.5 and 1 M GH gave greater shear strengths than did the unmodified protein. The 1 M GH-modified soy protein gave the highest shear strength. Compared to the unmodified protein, the modified proteins also exhibited higher shear strengths after incubating with two cycles of alternating relative humidity, zero delamination, and higher remaining shear strengths after three cycles water soaking and drying. These results indicate that soy proteins modified with urea and GH enhance water resistance as well as adhesive strength. Secondary structures of globule proteins may enhance adhesion strength, and the exposure of hydrophobic amino acids may enhance water resistance. Proteins modified by 3 M urea or 1 M GH may have higher content of secondary structure and more exposed hydrophobic amino acids, compared with other modifications or unmodified proteins.

Paper no. J9255 in *JAOCS 77,* 101–104 (January 2000).

KEY WORDS: Adhesive, denaturation, differential scanning calorimetry, globular protein, plywood, protein modification, shear strength, soy protein, water resistance.

The various forms of wood utilization represent an extremely large and diverse market for adhesives (1). Soy-based adhesives were first developed in 1923 when a patent was granted for a soy meal-based glue (2). However, those soy protein adhesives had low gluing strength and water resistance. Adhesives produced from petroleum overcame those disadvantages, but the continuing emission of phenol/formaldehyde has caused environmental and toxicity problems during product manufacturing, distribution, and use (3,4). The greatly expanding markets for adhesives, the aggravating threat of limited world oil reserves, and the increasing concern over environmental pollution have forced the plywood industry to consider new types of wood adhesives from renewable resources. Soy protein adhesives are attractive because they are environmentally friendly.

*To whom correspondence should be addressed. E-mail: xss@ksu.edu

Soybeans are one of the most important crops grown in the United States today (5). Industrial uses of soy proteins are being promoted to increase their value (1,5). The use of soy proteins in industrial applications is based on their functional properties. Protein modification is designed to improve functional properties by altering protein molecular structure or conformation, through physical, chemical, or enzymatic agents at the secondary, tertiary, and quaternary levels. Research on functional properties of modified proteins has focused on food applications such as solubility, viscosity, gelation, and emulsion stability (6–8).

Little has been reported on modifications of soy protein to improve its adhesive properties on wood. Hettiarachchy *et al.* (9) prepared soy protein-based adhesives using alkali (NaOH)- and trypsin-modification methods. They found that adhesive strength and water resistance of both modified soy proteins were enhanced compared to those of unmodified proteins, but the alkali-modified soy protein adhesive was stronger and more water resistant. Previous research by our group compared urea-modified soy proteins with alkali- and heat-modified soy proteins in terms of their adhesive properties (10). The adhesive produced by urea modification was found to have stronger shear strength and water resistance, but the effects of using different concentrations for modification were not examined. Urea and guanidine hydrocholoride have been extensively reported to be protein denaturants (11–15). However, no reports were found on their effects on protein adhesive properties. Elucidating the chemistry, unfolding, modification, and denaturation of protein would undoubtedly lead to a better understanding of protein adhesion and may play a significant role in developing soy protein adhesives for industrial use. The objective of this research was to investigate the adhesive and water-resistance properties of soy protein isolates (SPI) modified by different concentrations of urea and guanidine hydrochloride and used on walnut, cherry, and pine plywoods.

MATERIALS AND METHODS

Materials. Defatted soy flour was obtained from Cargill (Cedar Rapids, IA) and used for the preparation of SPI. Urea (Mallinckrodt Chemical Works, St. Louis, MO) and guanidine hydrochloride (GH) (Sigma Chemical Co., St. Louis, MO) were both analytical-grade reagents. Unmodified SPI was used as control.

SPI preparation. Defatted soy flour (100 g) was mixed with 1500 mL distilled water and stirred for 30 min at room temperature. The pH of the mixture was then adjusted to 8.5 with 1 N NaOH and stirred for another 20 min. The slurry was centrifuged at 10,000 × *g* at 4°C for 20 min. The supernatant was recovered, its pH was adjusted to 4.2, and then it was kept at 4°C for 12 h. After another centrifugation at 6,500 × *g* at 4°C for 20 min, the precipitated SPI fraction was obtained. It was redissolved at pH 7.6, freeze-dried (model 6211-0495; The Virtis Co., Inc., Gardiner, NY), and then milled (Cyclone Sample Mill, model 3010-030; UDY Corp., Fort Collins, CO) into a powder with 90% passing through U.S. #100 mesh. The freeze-dried SPI powder samples had an average protein content of 88.26% (dry basis) (LECO, Leco Corp., St. Joseph, MI) and moisture content of 5%.

Protein modification. Solutions of urea (1, 3, 5, and 8 M) and GH (0.5, 1, and 3 M) were prepared at room temperature. SPI powder (10 g) was suspended in each urea and GH solution (100 mL), and stirred and reacted for 6 h.

Wood specimen preparation. Three wood varieties ranging from hard to soft (walnut, cherry, and pine, respectively) were used. The method described by Sun and Bian (10) was used to prepare the wood specimens for testing. Each wood piece was 3 × 20 × 50 mm (thickness, width, and length), and three pieces were glued to form a specimen. The modified protein adhesive slurry was brushed onto both sides of the middle piece and onto one side of the other two pieces. The applied area on each side was 2 × 2 cm and the protein concentration was 1.80 mg/cm^2 with a standard deviation of 0.04 mg/cm^2. The three wood pieces with the adhesive were allowed to rest at room temperature for about 5 min before they were assembled together by hand. Then they were hot-pressed (model 3890 Auto M; Carver Inc., Wabash, IN) at 115°C and 20 kg/cm^2 for about 7 min. The pressed specimens were cooled and then stored in polyethylene bags at ambient conditions for 4 d.

Adhesive strength. Shear strengths of wood specimens were determined by using an Instron testing machine (model 4466; Canton, MA) operated at a crosshead speed of 2.4 cm/min. The force (kg) required to break the glued wood specimen was recorded. All the adhesive strength data reported are means of eight replications.

Incubation aging. Water resistance (for interior application) of the adhesive was tested by following ASTM standard method D-1183 (16). For the first cycle, the glued specimens were incubated in a chamber at 90% relative humidity (RH) and 23°C for 60 h, and then conditioned at 25% RH and 48°C for 24 h. For the second cycle, the aging parameters were 90% RH and 23°C for 72 h and 25% RH and 48°C for 24 h. Ten specimens were used for each treatment.

Water-soaking. Water resistance (for exterior application) of the adhesive was tested according to the modified method described by Hettiarachchy *et al.* (9). The glued-wood specimens were placed in a container and soaked in tap water for 48 h at room temperature, and then were air-dried at room temperature for 48 h in a fume hood. Ten specimens were used for each treatment. After three cycles of soaking and drying, the dried-wood specimens were examined for delamination and shear strength.

Differential scanning calorimetry (DSC) measurement. Thermal transition properties of modified and unmodified soy protein samples were measured with a PerkinElmer DSC 7 instrument (PerkinElmer, Norwalk, CT). Each sample was analyzed in the presence of excess water (1:10). Large sample pans were used, and the DSC temperature range was from 30 to 200°C, and the heating rate was 10°C/min.

RESULTS AND DISCUSSION

Shear strength. The 3 M urea modification gave soy protein the highest shear strength in all wood types (Table 1). Modifications with 1 and 5M urea had lower shear strength, as compared to the 3 M urea modification, but were still higher than the unmodified proteins. However, for walnut and pine, the glue strengths for 1 and 5 M urea modification were not significantly decreased as compared to that for 3 M urea modification. The 8 M urea modification had the lowest shear strength among these modifications, and was even lower than the unmodified proteins.

The soy proteins modified by GH at 0.5 and 1 M concentrations exhibited greater shear strengths than the unmodified proteins (Table 1). Modification by 3 M GH had the least effect on adhesive strength. The soy protein modified with 1 M GH had the highest shear strength in all the wood samples.

Variations in adhesive strength with type of wood were observed (Table 1). Modified proteins had higher shear strengths with the hard wood (walnut) and intermediate-hard wood (cherry). At 1 M GH modification, for example, shear strengths were greater in walnut and cherry than in the soft pine wood. The same behavior was observed with 3 M urea-modified proteins (Table 1). These results are in agreement with the observation of Kalapathy *et al.* (17), who found that adhesive strength was much lower with pine than with walnut, cherry, maple, and poplar samples. The differences in physical properties and surface structures of the woods may account for the variations in adhesive strengths. Pine has the smoothest surface structure among these three wood types (10), and smooth surface structures often cause adhesive failure (18). However, smooth surface structures may not be as sensitive to adhesive molecular structures as rough surface

TABLE 1
Shear Strengths (kg/cm^2) of Wood Specimens Glued with Unmodified (UnM), Urea (U) (1, 3, 5, and 8 M)-Modified, and Guanidine Hydrochloride (GH) (0.5, 1, and 3 M)-Modified Soy Proteins[a]

	Urea (M)[b]				GH (M)			
Sample	1	3	5	8	0.5	1	3	UnM
Walnut	48^{b}	54^{a,b}	46^{b,c}	26^{d,e}	44^{b,c}	51^{b}	36^{c,d}	30^{d}
Cherry	42^{c}	59^{a}	37^{c}	33^{d}	49^{b}	60^{a}	36^{c,d}	41^{c}
Pine	41^{c}	42^{c}	40^{c}	36^{c,d}	48^{b}	47^{b}	41^{c}	31^{d}

[a]Means, based on n = 10, followed by different superscript roman letters are significantly different using least significant differences (LSD) and a probability level of α = 0.05.
[b]Molar concentration of solution for modification.

structures. Therefore, the glue strengths in the pine wood sample for 1 and 5 M urea or 0.5 or 3 M GH modification were not significantly decreased as compared to that for cherry. The exact reason for this phenomenon is unknown.

Water resistance. Water resistance is an important glue property that determines adhesive bond durability (1). After the incubation aging test, the shear strengths of the wood specimens glued with 1 and 3 M urea-modified soy proteins (Table 2) remained almost the same as the initial strengths (Table 1). Proteins modified by 3 M urea were found to have the best water resistance (zero delamination rate) in all wood types, as well as higher remaining shear strengths after three water-soaking cycles (Table 2). Shear strengths of wood specimens glued with proteins modified by 5 and 8 M urea significantly decreased, as did the shear strength of specimens glued with unmodified proteins.

Specimens glued with proteins modified by 0.5 and 1 M GH gave higher shear strengths after incubation aging, and zero delamination rate and higher remaining shear strengths after three water-soaking cycles compared with those glued with unmodified proteins and 3 M GH-modified proteins (Table 3). These results indicated that soy proteins modified by GH at 0.5 and 1 M concentrations had better water resistance. The GH modification at 1 M was found to be the best among the three concentrations selected.

DSC analysis. Modifications that change secondary, tertiary, or quaternary structure of a protein molecule have been referred to as denaturation (9). Urea has oxygen and hydrogen atoms that would interact with hydroxyl groups of the soy proteins, which could break down the hydrogen bonding in the protein body and, consequently, unfold the protein complex. Previous studies have suggested that complete unfolding of a protein could happen at higher urea concentrations, such as 8, 9, or 10 M (11,12,15,19). The DSC data for soy proteins treated with urea at varying concentrations showed that as urea concentration increased, the peak temperatures for soy protein subunits conglycinin (7S) and globulin (11S), as well as the total enthalpy, decreased (Table 4). This indicated that the higher the urea concentration, the greater the degree of denaturation, i.e., the greater the extent of protein unfolding. The lower shear strength of soy proteins at higher urea concentrations (5 and 8 M, Table 1) may have resulted from the higher extent of unfolding. Urea could unfold the secondary structure of a protein at concentrations greater than 5 M (11,12,15). The secondary structure might be desirable for protein adhesion. Proteins modified at relatively lower urea concentrations (1 and 3 M) may have been partly unfolded and had a certain amount of secondary structure, resulting in better shear strengths (Table 1). As protein molecules disperse and unfold in solution, the partly unfolded mol-

TABLE 2
Shear Strengths and Delamination of Wood Specimens Glued with UnM and U-Modified Soy Proteins After Incubation Aging and Water Soaking Tests[a]

	U-1 M[b]	U-3 M	U-5 M	U-8 M	UnM
Shear strength after incubation (kg/cm^2)[c]					
Walnut	49^{a}	45^{a,b}	33^{b,c}	21^{d}	25^{c,d}
Cherry	42^{b}	49^{a}	29^{c}	25^{c,d}	38^{b}
Pine	41^{b}	39^{b}	31^{c}	21^{d}	21^{d}
Delamination after water soaking (%)[d]					
Walnut	10	0	20	90	100
Cherry	0	0	30	100	100
Pine	0	0	0	0	90
Shear strength after water soaking (kg/cm^2)[d]					
Walnut	8^{e}	10^{e}	5^{f}	4^{f}	—
Cherry	12^{e}	14^{d,e}	7^{e,f}	—	—
Pine	17^{d}	25^{c,d}	12^{e}	5^{f}	6^{e,f}

[a]Means, based on n = 10, followed by different superscript roman letters are significantly different using LSD and a probability level of α = 0.05. See Table 1 for abbreviations.
[b]Molar concentration of solution for modification.
[c]Shear strengths of wood specimens were determined with an Instron testing machine (model 4466; Canton, MA) operated at a crosshead speed of 2.4 cm/min.
[d]Conditions of modified method described in Reference 9.

TABLE 3
Shear Strengths and Delamination of Wood Specimens Glued with UnM and GH-Modified Soy Proteins After Incubation Aging and Water Soaking Tests[a]

	GH-0.5 M[b]	GH-1 M	GH-3 M	UnM
Shear strength after incubation (kg/cm^2)				
Walnut	41^{b}	38^{b}	32^{c}	25^{d}
Cherry	38^{b}	49^{a}	32^{c}	38^{b}
Pine	40^{b}	37^{b}	42^{b}	21^{d}
Delamination after water soaking (%)				
Walnut	0	0	100	100
Cherry	0	0	100	100
Pine	0	0	0	90
Shear strength after water soaking (kg/cm^2)				
Walnut	11^{e}	7^{f}	—	—
Cherry	9^{e,f}	13^{e}	—	—
Pine	20^{d}	37^{b}	9^{e,f}	6^{f}

[a]Means, based on n = 10, followed by different superscript roman letters are significantly different using LSD and a probability level of α = 0.05. See Table 1 for abbreviations.
[b]Molar concentration of solution for modification. Conditions and measurement methods are the same as describe in footnotes *c* and *d* of Table 2.

TABLE 4
Differential Scanning Calorimetry Data Presenting Thermal Behavior for UnM, UM, and GHM Soy Protein Isolates[a]

Sample	T1 (°C)[b]	T2 (°C)[c]	Enthalpy (J/g)[d]
UnM	74.94	88.79	9.973
UM[e]			
1	71.80	85.48	9.767
3	63.13	83.68	1.540
5	—	81.67	0.622
8	—	—	—
GHM[e]			
0.5	79.49	98.52	10.280
1	79.33	96.52	10.735
3	70.81	85.85	2.550

[a]See Table 1 for abbreviations.
[b]Peak temperature for 7S soy protein fraction.
[c]Peak temperature for 11S soy protein fraction.
[d]Sum of the enthalpy for both 7S and 11S peaks.
[e]Molar concentration of solution for modification.

ecules with a certain amount of secondary structure increase the contact area and adhesion force onto other surfaces, such as wood materials, and they interact with each other during the curing process to achieve bonding strength. Protein modification also could expose to the surface some hydrophobic amino acids that are buried inside, increasing hydrophobicity and thus increasing water resistance. This was supported by the experimental data at 3 M urea modification (Table 2).

GH is more effective than urea in denaturing proteins (11–15,19,20). However, the DSC data (Table 4) showed that soy proteins modified by 0.5 and 1 M GH had higher thermal transition temperatures and enthalpies than the unmodified protein. The 1 M GH modification resulted in a denatured protein with the highest enthalpy (10.735 J/g) (Table 4). Numerous studies have been done on the denatured state of globular protein molecules under different GH concentrations (11,12,14,15,19–21). At 0.3–2 M GH, the specific tertiary structure of a protein, for example, human α-lactalbumin, was destroyed as monitored by near-ultraviolet circular dichroism (UV CD) spectra, whereas at 2–6 M GH, in the secondary structure, as monitored by far-UV CD spectra, the compactness of molecules was destroyed (21). The molten-globule state of some globular proteins denatured by GH at smaller concentrations has been reported extensively since Tanford (11) summarized experimental evidence that protein could have structures intermediate between native and highly unordered states (19–24). A globular protein in this state is nearly as compact as native proteins and has a high content of a secondary structure with fluctuating tertiary structure (19,22–24). Heat capacity of the molten globule state is much higher than that of the native state (24). The soy proteins modified by 0.5 and 1 M GH may have been in the molten globule state, resulting in the higher DSC peak temperature and enthalpy (Table 4).

Although the molten-globule state is rather compact, more hydrophobic groups can be expanded in water than in the native state, which was confirmed by the fact that proteins in the molten globule state were usually less soluble in water than in the native state (19). Proteins in the molten-globule state had a more labile surface, which might facilitate their ability to penetrate membranes more easily than native proteins (19). This relationship implies that 1 M GH-modified protein in the molten-globule state could easily penetrate the wood surface and generate more adhesion force, resulting in strong bonding strength and water resistance (Table 3).

ACKNOWLEDGMENT

This work was supported in part by research grants from the Kansas Soybean Commission and the Kansas Agricultural Experiment Station. We thank Xiaoquan Mo for her assistance in DSC analysis.

REFERENCES

1. Lambuth, A.L., Protein Adhesives for Wood, in *Advanced Wood Adhesive Technology*, edited by A. Pizzi and K.L. Mittal, Marcel Dekker, Inc., New York, 1994, pp. 259–281.
2. Johnson, L.A., D.J. Myers, and D.J. Burden, Early Uses of Soy Protein in Far East, *INFORM 3*:282–284 (1984).
3. O'Brien, D.J., and J.A. Olofsson, Phenol Leachability from Phenolic Resin Materials, Abstract in *Proceedings of Industrial Waste Conference*, Engineering Information, Inc., Hoboken, 1980, pp. 155–159.
4. Henderson, J.T., Volatile Emissions from the Curing of Phenolic Resins, *Tappi J. 62*:93–96 (1979).
5. Myers, D.J., Industrial Applications for Soy Protein and Potential for Increased Utilization, *Cereal Foods World 38*:355–358 (1993).
6. Wolf, W.J., Soybean Proteins: Their Functional, Chemical, and Physical Properties, *J. Agric. Food Chem. 18*:969–976 (1970).
7. Kinsella, J.E., Functional Properties of Soy Proteins for Food Applications, *J. Am. Oil Chem. Soc. 56*:242–258 (1979).
8. Wu, Y.V., and G.E. Inglet, Denaturation of Plant Proteins Related to Functionality and Food Applications. A Review, *J. Food Sci. 39*:218–225 (1974).
9. Hettiarachchy, N.S., U. Kalapathy, and D.J. Myers, Alkali-Modified Soy Protein with Improved Adhesive and Hydrophobic Properties, *J. Am. Oil Chem. Soc. 72*:1461–1464 (1995).
10. Sun, X., and K. Bian, Shear Strength and Water Resistance of Modified Soy Protein Adhesives, *Ibid. 76*:977–980 (1999).
11. Tanford, C., Protein Denaturation, in *Advanced in Protein Chemistry*, edited by C.B. Anfinsen, M.L. Anson, J.T. Edsall, and F.M. Richards, Academic Press, Inc., New York, 1968, pp. 121–283.
12. Chandra, B.R.S., A.G.A. Rao, and M.S.N. Rao, Effect of Temperature on the Conformation of Soybean Glycinin in 8 M Urea and 6 M Guanidine Hydrochloride Solution, *J. Agric. Food Chem. 32*:1402–1405 (1984).
13. Tanford, C., K. Kawahara, and S. Lapanie, Proteins as Random Coils, *J. Am. Chem. Soc. 89*:729–734 (1967).
14. Tanford, C., K. Kawahara, S. Lapanie, T. Hooker, and M. Zarlengo, Protein as Random Coils. III. Optical Rotatory Dispersion in 6 M Guanidine Hydrochloride, *Ibid. 89*:5023–5028 (1967).
15. Pace, C.N., The Stability of Globular Proteins, *CRC Crit. Rev. Biochem. 3*:1–43 (1975).
16. *ASTM Standards for Wood and Adhesives*, American Society of Testing and Materials, Philadelphia, 1981, pp. 407–409, Methods D-1183.
17. 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).
18. Mackay, C.D., Good Adhesive Bonding Starts with Surface Preparation, *Adhesive Age 41*:30–32 (1998).
19. Ptitsyn, O.B., Protein Folding: Hypotheses and Experiments, *J. Protein Chem. 6*:273–293 (1987).
20. Wang, K.P., and C.D. Tanford, Denaturation of Bovine Carbonic Anhydrase B by Guanidine Hydrochloride, *J. Biol. Chem. 248*:8518–8523 (1973).
21. Nazaka, M., K. Kuwajima, K. Nitta, and S. Sugai, Detection and Characterization of the Intermediate on the Folding Pathway of Human Alpha-Lactalbuman, *Biochem. 17*:3752–3758 (1978).
22. Dolgikh, D.A., R.I. Gilmanshin, E.V. Brazhnikov, V.E. Bychkova, G.V. Semisotnov, S.Y. Venyaminov, and O.B. Ptitsyn, Alpha-Lactalbumin: Compact State with Fluctuating Tertiary Structure? *FEBS Lett. 136*:311–315 (1981).
23. Dolgikh, D.A., A.R. Kolomietz, I.A. Bolotina, and O.B. Ptitsyn, 'Molten-Globule' State Accumulates in Carbonic Anhydrase Folding, *Ibid. 165*:88–92 (1984).
24. Pfeil, W., V.E. Bychkova, and O.B. Ptitsyn, Physical Nature of the Phase Transition in Globular Proteins, *FEBS Lett. 198*:287–290 (1986).

[Received May 24, 1999; accepted September 8, 1999]