Thermal Properties and Adhesiveness of Soy Protein Modified with Cationic Detergent

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ABSTRACT: This research studied the effects of cationic detergents on the adhesiveness and thermal properties of soy protein isolate (SPI). Three cationic detergents, hexadecyltrimethyl ammonium bromide, ethylhexadecyldimethyl ammonium bromide (EDAB), and benzyldimethylhexadecyl ammonium chloride, each at concentrations of 1.3, 2.6, 5.2, and 7.8 mM, were used to modify SPI. The effect of pH at selected EDAB concentrations was also studied. Results showed that both detergent concentration and pH had significant effects on the adhesiveness of modified SPI. SPI modified with detergent at a concentration of 2.6 mM yielded the greatest dry tensile strength and water resistance, which indicated that a moderate protein denaturation might be favorable to the adhesion of SPI. Both modified and unmodified SPI showed greater adhesive strength at their optimal pH values. Modified SPI showed greatest adhesive strength at pH 7, whereas unmodified SPI showed greatest adhesive strength at pH 4.5; the tensile strength of modified SPI was greater than that of unmodified SPI. The protein-denaturation temperature and the enthalpy of modified SPI adhesives were also analyzed by using DSC. Denaturation of the native structure of SPI increased as detergent concentration increased.

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KEY WORDS: Adhesive, denaturation, detergent, pH, protein modification, soy protein, thermal property, tensile strength, water resistance.

Synthetic petroleum-polymer materials have achieved great success since the beginning of the last century, but the environmental impact of petroleum-based polymers and natural resource limitation on petroleum-based polymers have prompted the rising need for biodegradable, renewable, inexpensive substitutes (1). Soy protein, the major component of soybeans (30 to 45%), is readily available from renewable resources, for example, as a by-product from oil extraction processing. Soy proteins can be used as alternatives to partly replace petroleum polymers in the manufacture of adhesives, plastics, and various binders (2).

Soy proteins are complex macromolecules that contain a number of chemically linked amino acid monomers, which together form polypeptide chains, constituting the primary structure. The α-helix and β-sheet patterns of the polypeptide chains are called secondary structure. A number of side chains are connected to these amino acid monomers and interact with each other, mainly through hydrogen and disulfide bonds, to form tertiary or quaternary structures. Functional properties of proteins are strongly correlated to their structure; these structural features can be changed by physical, chemical, or enzymatic treatment (3). The native structure of soy protein can be modified to increase the bonding strength (2,4–6). The performance of soy protein adhesives is dependent on the dispersion and unfolding of protein in solution. Unfolded protein molecules have increased surface area, which will improve interaction with the substrate (7).

Soy proteins have a highly ordered structure, with most of the hydrophilic groups exposed on the outside and most of the hydrophobic groups buried inside (8). The structure and distribution of hydrophobic and hydrophilic groups on the protein surface greatly affect protein–protein and protein–substrate interactions and therefore must affect soy protein gluing strength (5). In recent years, attempts have been made to improve adhesion strength and water resistance of soy proteins by modifying soybean protein isolate (SPI). Modification reagents include sodium hydroxide, trypsin, guanidine hydrochloride, and anionic detergent such as SDS (1,9). With modification, the protein conformation changes, and hydrophobic groups buried inside the protein can be exposed. The unfolded structures, as well as exposed hydrophobic groups, increase the protein–substrate contact area and hydrophobicity and thus increase water resistance (2).

Cationic detergents such as hexadecyltrimethyl ammonium bromide (CTAB), dodecyltrimethyl ammonium bromide, and the like bind globular protein and induce conformational changes (BSA, gelatin, etc.). These changes are mainly due to electrostatic and hydrophobic interactions (10,11). No information is available about the effects of cationic detergent on the performance of soy protein adhesives. Detergents are water-soluble, surface-active agents composed of a hydrophobic portion, usually a long alkyl chain, that is attached to functional groups that are hydrophilic or that enhance water solubility. Cationic detergents feature a positive charge that is present in the hydrophilic portion of the molecule after dissociation in aqueous solution (12). The binding isotherm indicates that binding between the ionic detergent and protein begins with electrostatic binding, in which the head groups of the detergents bind to groups of opposite charge on the protein. Cooperative binding occurs after electrostatic binding and is hydrophobic in nature: The hydrophobic part of the detergent penetrates into the hydrophobic part of the protein, resulting in

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conformation-stabilizing protein–protein interactions being replaced by detergent–protein interactions (13,14). We assumed that the detergent–protein interaction may improve the adhesion of the protein.

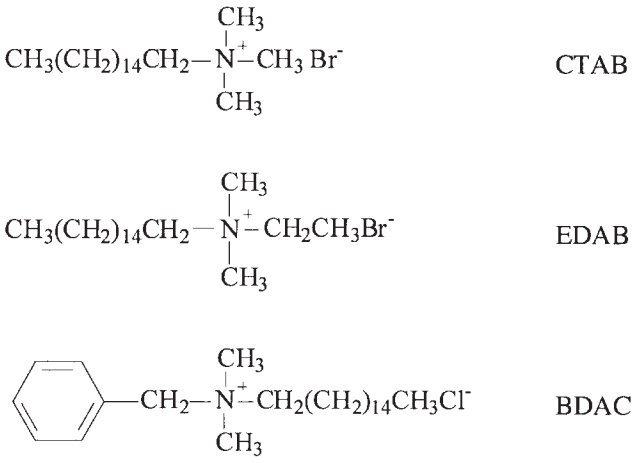
Different structures and concentrations of cationic detergents may affect soy protein adhesion. Additionally, the arrangement of protein subunits and tertiary and secondary structures can be altered by pH (15,16). The conformation and functional properties of protein, such as denaturation, solubility, gelation, and emulsifying and foaming activity, change with pH (16). Different pH values would also affect interaction between detergents and protein and thus affect adhesion. The objective of this research was to study the adhesiveness and thermal properties of soy protein adhesives as modified by selected cationic detergents and at different pH values.

MATERIALS AND METHODS

Materials. SPI, containing 85% (dry base) protein and 3% moisture, was extracted from defatted soybean flour (Cargill, Cedar Rapids, IA) by isoelectric point precipitation at pH 4.2. The precipitate was freeze-dried (Model 62111-0495 Freeze-Dryer; Virtis Co., Inc., Gardiner, NY) and then milled (Cyclone Sample Mill; UDY Corp., Fort Collins, CO) into a powder. Cherry woods with dimensions of 50 (width) × 127 (length) × 3 mm (thickness) were provided by Veneer One (Oceanside, NY).

CTAB, ethylhexadecyldimethyl ammonium bromide (EDAB), and benzyldimethylhexadecyl ammonium chloride (BDAC) were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Structures of these detergents are shown in Scheme 1. They have in common the same long alkyl chain containing 16 carbons and 2 methyl groups attaching to nitrogen, as well as another (different) group. The different groups are methyl in CTAB, ethyl in EDAB, and benzyl in BDAC.

Sample preparation. The cherry woods were preconditioned in a chamber (Model 518 controlled environment chamber; Electro-Tech Systems, Inc., Glenside, PA) at 23°C and 50% relative humidity (RH) for 7 d. The soy protein suspensions were made as follows: SPI was added to distilled water at room temperature to make a suspension and was stirred (magnetic stirrer) for 1 h; the pH of the suspension was adjusted by 2 N NaOH, if needed; and stock detergent solutions (20%, wt/vol) were added to make 1.3, 2.6, 5.2, and 7.8 mM detergent concentrations in SPI suspension and stirred for 3 h. The final 10% (wt/vol) protein suspensions were used as adhesive solutions. The SPI suspension was brushed onto one end of a piece of cherry wood until the entire area was completely wetted. The amount of adhesive applied on each piece was about 0.06 g and was controlled by using a pipette and a consistent brushing procedure. The area of application on each end was 127 × 20 mm. The brushing and setting procedure described by Mo *et al*. (5) was used. The two pieces of slurry-brushed cherry wood were allowed to rest at room temperature for 15 min and then assembled and pressed at a pressure of 3.57 MPa at 130°C for 5 min, using a Hot Press (Model 3890, Auto 'M,'; Carver Inc., Wabash, IN).



SCHEME 1

Thermal properties. Modification of soybean proteins can denature them to some extent and change their thermal transition properties, including denaturation temperature and enthalpy of denaturation. Thermal properties of modified protein adhesives were studied by using DSC (DSC 7; PerkinElmer, Norwalk, CT), which was calibrated with indium and zinc. All measurements were conducted under a nitrogen atmosphere. A large DSC pan was used with approximately 50 mg of modified protein suspension (10%, wt/vol). All samples were held at 25°C for 1 min and then scanned from 25 to 150°C at a heating rate of 10°C/min. All experiments were made in duplicate and average values were reported.

Tensile strength. After pressing, the glued wood assemblies were conditioned at 23°C and 50% RH for 2 d and were then cut into five, 20 mm-wide specimens. The cut specimens were conditioned for another 5 d before testing. Three adhesion tensile strengths were tested, including dry strength, soak strength, and wet strength. The wood specimens for dry strength testing were prepared and tested by using an Instron instrument (Model 4465; Canton, MA) according to ASTM Standard Method D 2339-98 (17).

Water resistance was measured according to ASTM Standard Methods D 1183-96 (18) and ASTM D 1151-00 (19). The preconditioned specimens were soaked in tap water at 23°C for 48 h, and then the specimens were tested immediately for wet strength. For the soak strength test, the specimens were conditioned at 23°C and 50% RH for another 7 d after 48 h of soaking before they were tested. Wood failure data were estimated by naked eye observation with the help of a magnifier and reported as percentages along with the tensile strength data.

Crosshead speed for tensile strength testing was 1.6 mm/min. Stresses at maximum load were recorded as tensile strength. Reported results were the average of five samples.

Experimental design and data analysis. A 3 × 5 full-factorial experimental design was used to study the effects of detergents and concentrations on the performance of modified protein adhesives. A 2 × 4 full-factorial experimental design was

used to study the effects of pH on the performance of modified-protein adhesives. ANOVA and LSD were performed according to SAS (SAS Institute, Cary, NC, 1995).

RESULTS AND DISCUSSION

Effect of detergent concentration on thermal properties. Loss of a certain degree of native structure could favor protein adhesive performance. Most proteins with an ordered native structure undergo a transition on addition of denaturation reagents because of the interaction with them (1). After complete denaturation, these proteins become random coils. If the denaturation reagent cannot completely denature the protein, the remaining native structures of protein can be measured by DSC as significant uptake of heat in the DSC thermogram. Results showed that both concentration and type of detergent had a significant effect on the denaturation temperature (T_d) and enthalpy (ΔH_d) of 7S and 11S globulins (Table 1). The thermal denaturation transition temperatures of 7S and 11S globulins for unmodified SPI adhesive (control) are 73.8 and 88.5°C, respectively. This is in accord with previous studies (20,21). The T_d of 7S and 11S changed slightly at detergent concentrations less than 5.2 mM but decreased significantly at detergent concentrations greater than 5.2 mM, which means the modified SPI treated with low concentrations of detergent was still thermally stable (Table 1). The enthalpy of 7S decreased as the detergent concentration increased to 5.2 mM, indicating the native 7S structure was partly unfolded during the detergent modification. No denaturation transition for 7S was detected at 7.8 mM concentrations of EDAB and BDAC, indicating that the modification with 7.8 mM detergent concentration could completely denature 7S globulins. At a lower concentration, the T_d of modified SPI was higher than that for the unmodified SPI, suggesting the modified SPI had a different structure from that of unmodified SPI. At a low degree of denaturation, detergent might unfold less stable structures in the protein, while more stable structures remain undenatured, which might result in a higher T_d (22). More extensive denaturation occurred at a higher concentration, and T_d was lower (4–6,23). T_d for the modified SPI treated with detergent was different from that for the unmodified SPI, suggesting the modified SPI had a different structure. The T_d for 7S at 1.3–5.2 mM detergent concentrations might suggest a mildly unfolded protein structure, whereas the lower T_d at 7.8 mM detergent concentration might suggest an extensively unfolded structure or complete denaturation. At 2.6 mM, BDAC induced more change of T_d for 7S, followed by EDAB, then CTAB. The 11S with lower T_d at different detergent concentrations might suggest a different unfolding mechanism from that of 7S. The higher detergent concentration could induce more unfolding of 7S, but not 11S, as indicated by enthalpy. The difference in T_d and ΔH_d between the 7S and 11S might reside in their different structures. The 7S globulin exists as trimeric complexes, whereas 11S has a hexameric form (24). Acidic and basic polypeptides in the 11S hexamer are connected by disulfide bonds (25). The content of tryptophan and sulfur-containing amino acids of 7S globulins is very small in comparison with that of 11S globulins, and 7S globulins are not able to form disulfide bonds (26). These disulfide bonds in the hexameric form of 11S make it more thermally stable than 7S (27).

The major difference among the three cationic detergents is the group attached to the nitrogen beside the alkyl chain. The benzyl group in BDAC provides more hydrophobicity than does the ethyl group in EDAB, and the methyl group in CTAB

TABLE 1
DSC Analysis of the Effect of Cationic Detergent Concentration on Modified SPI[a,b]

Concentration	$\Delta\Delta H_d$ (J/g SPI)			T_d (°C)		
(mM)	CTAB	EDAB	BDAC	CTAB	EDAB	BDAC
7S						
0	$0.55^{a,x}$	$0.55^{a,x}$	$0.55^{a,x}$	$73.8^{a,b,x}$	$73.8^{b,x}$	$73.8^{b,x}$
1.3	$0.34^{b,x}$	$0.25^{b,x}$	$0.24^{b,x}$	$74.6^{a,x}$	$75.4^{a,x}$	$76.2^{a,x}$
2.6	$0.26^{c,x}$	$0.20^{b,y}$	$0.15^{b,z}$	$74.8^{a,z}$	$75.8^{a,y}$	$76.7^{a,x}$
5.2	$0.16^{d,x}$	$0.09^{c,y}$	$0.06^{c,y}$	$74.2^{a,x}$	$74.5^{a,b,x}$	$75.8^{a,x}$
7.8	0.06^{e}	—[c]	—	72.3^{b}	—	—
11S						
0	$3.52^{a,x}$	$3.52^{a,x}$	$3.52^{a,x}$	$88.5^{a,x}$	$88.5^{a,x}$	$88.5^{a,x}$
1.3	$3.54^{a,x}$	$3.66^{a,x}$	$3.85^{a,x}$	$86.8^{b,y}$	$87.9^{a,x,y}$	$88.6^{a,x}$
2.6	$3.65^{a,x}$	$3.56^{a,x}$	$3.60^{a,x}$	$86.4^{b,z}$	$87.8^{a,y}$	$88.9^{a,x}$
5.2	$3.84^{a,x}$	$3.77^{a,x}$	$3.54^{a,x}$	$86.8^{b,y}$	$86.9^{b,y}$	$89.0^{a,x}$
7.8	$3.78^{a,x}$	$3.50^{a,x}$	$3.53^{a,x}$	$86.8^{b,x}$	$86.8^{b,x}$	$86.0^{b,y}$

[a]Means with the same letters (a–e) within the same subunits category in the same column are not significantly different at $\alpha = 0.05$.
[b]Means with the same letters (x, y) within the same subunits category in the same row are not significantly different at $\alpha = 0.05$.
[c]No peak detected because of the complete denaturation of the protein. SPI, soy protein isolate; CTAB, hexadecyltrimethyl ammonium bromide; EDAB, ethylhexadecyldimethyl ammonium bromide; BDAC, benzyldimethylhexadecyl ammonium chloride.

TABLE 2
Effect of Detergent Concentration on Adhesive Performance of Modified Soy Protein Adhesives on Wood[a,b]

Concentrations	Tensile strength (MPa)			Difference[c] (%)		
(mM)	CTAB	EDAB	BDAC	CTAB	EDAB	BDAC
Dry strength						
0	$4.75^{b,c,x}$	$4.75^{c,x}$	$4.75^{c,x}$			
	WF 50[d]	WF 50	WF 50			
1.3	$5.10^{a,b,x}$	$4.98^{b,c,x}$	$5.06^{b,c,x}$	7.4	4.8	6.5
	WF 50	WF 50	WF 50			
2.6	$5.64^{a,x}$	$5.65^{a,x}$	$5.66^{a,x}$	18.7	19.0	19.2
	WF 90	WF 90	WF 90			
5.2	$5.20^{a,b,x}$	$5.41^{a,b,x}$	$5.44^{a,b,x}$	9.5	13.9	14.5
	WF 80	WF 80	WF 80			
7.8	$4.52^{c,x}$	$4.80^{c,x}$	$4.71^{c,x}$	–4.8	1.1	0
	WF 50	WF 50	WF 50			
Wet strength						
0	$1.34^{b,x}$	$1.34^{b,c,x}$	$1.34^{b,x}$			
	WF 5	WF 5	WF 5			
1.3	$1.66^{a,x}$	$1.51^{b,x}$	$1.54^{b,x}$	23.9	12.7	14.9
	WF 10	WF 10	WF 10			
2.6	$1.78^{a,y}$	$1.99^{a,x}$	$1.98^{a,x}$	32.8	48.5	47.7
	WF 10	WF 30	WF 30			
5.2	$1.55^{a,x}$	$1.62^{b,x}$	$1.59^{b,x}$	15.7	20.9	18.7
	WF 10	WF 10	WF 10			
7.8	$1.15^{b,x}$	$1.13^{c,x}$	$0.88^{c,y}$	–14.2	–15.7	–34.3
	WF 0	WF 0	WF 0			
Soak strength						
0	$3.83^{b,x}$	$3.83^{b,x}$	$3.83^{b,x}$			
	WF 40	WF 40	WF 40			
1.3	$4.06^{b,x}$	$4.06^{b,x}$	$4.00^{b,x}$	6.0	6.0	4.4
	WF 40	WF 40	WF 40			
2.6	$5.25^{a,x}$	$5.08^{a,x}$	$5.30^{a,x}$	37.1	32.6	38.4
	WF 80	WF 70	WF 70			
5.2	$4.98^{a,x}$	$4.92^{a,x}$	$5.21^{a,x}$	30.0	28.5	36.0
	WF 60	WF 70	WF 70			
7.8	$4.08^{b,x,y}$	$3.80^{b,y}$	$4.18^{b,x}$	25.0	0	9.1
	WF 30	WF 30	WF 40			

[a]Means with the same letters (a–e) within the same subunits category in the same column are not significantly different at $\alpha = 0.05$.
[b]Means with the same letters (x, y) within the same subunits category in the same row are not significantly different at $\alpha = 0.05$.
[c]The difference (%) is calculated by modified-control/control × 100.
[d]Percentage of wood failure (WF). For abbreviations see Table 1.

provides the least (28). This molecular difference could result in the difference in T_d. The detergent can interact with protein by electrostatic and hydrophobic reaction. The large extent of hydrophobic interactions provided by BDAC could contribute to the smaller ΔH_d and larger T_d value for 7S. Proteins modified with BDAC showed similar or lower ΔH_d than those with EDAB and CTAB at the concentrations studied here (Table 1). BDAC carries a chloride ion, whereas the other two detergents (CTAB and EDAB) carry bromide ions. According to the Hofmeister series (29), chloride ion is a protein structure-stabilizer, whereas the bromide ion is a protein structure-destabilizer, suggesting that the cation carried by BDAC could induce more denaturation than the cations carried by CTAB and EDAB. Because ΔH_d is an estimation of the thermal energy required to denature the protein, this means that BDAC induced more denaturation in 7S than the other two detergents, resulting in the most unfolded protein structure formed in SPI modified by BDAC, with less unfolded structure by EDAB, and the least unfolded structure by CTAB. The enthalpies of 11S globulins did not show a significant difference among different treatments and the control, according to the statistical analysis, indicating the detergents did not have much effect on the denaturation of 11S. EDAB and BDAC did not have a significant effect on the peak temperatures and enthalpies of 7S and 11S at 1.3 and 2.6 mM. In general, SPI modified by CTAB had a lower T_d and larger ΔH_d (except ΔH_d for 11S) than the SPI modified by EDAB or BDAC.

Effect of detergent concentration on adhesiveness. Dry, wet, and soak strengths of all detergent-modified SPI adhesives showed the same trend (Table 2). The high tensile strengths were accompanied by high wood failure percentage. Detergent concentration had a significant effect on the performance of all detergent-modified SPI adhesives. Dry and soak strengths of all three modified SPI showed an upward trend until 5.2 mM detergent concentration; there was no significant difference in tensile strength between SPI modified with 2.6 and 5.2 mM detergent

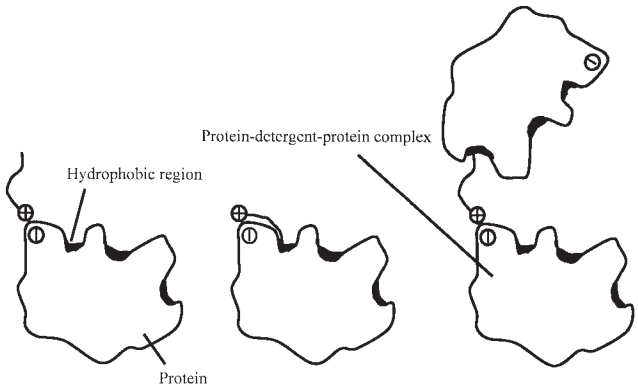


FIG. 1. Schematic illustration of the formation of the protein–detergent–protein complex (one detergent molecule is illustrated)

concentrations. Wet strength for SPI modified with CTAB showed no significant difference among 1.3, 2.6, and 5.2 mM detergent concentrations. Wet strengths of SPI modified with EDAB and BDAC showed significant differences among concentrations, with the greatest wet strength at 2.6 mM detergent concentration. Wet strengths of SPI modified with 2.6 mM EDAB and BDAC increased more than 47%, compared with that of the control. At detergent concentrations greater than 2.6 mM, wet strengths began to decrease. Molecular structures of the detergents are different, but not much difference was observed between the effects of EDAB- and BDAC-modified SPI adhesives. EDAB- and BDAC-modified SPI had greater wet strength than CTAB-modified SPI at 2.6 mM.

Cationic detergent is bound to protein by specific binding, followed by cooperative binding. Cooperative binding relies on surface hydrophobicity, especially in the area around potential specific binding sites of the protein. From the thermal study, the different concentrations induced different denaturation of the protein. These conformation changes resulted in different adhesive performance. The polar parts of different detergents might induce different conformation changes in protein. They might have different specific binding and cooperative binding because of the different properties of the polar groups, for example, steric hindrance. The steric hindrance of the benzyl group might be greater than that of the ethyl group followed by the methyl group, which could result in less specific and cooperative binding sites in 7S in BDAC than EDAB, followed by CTAB. The thermal study suggested that the conformation change induced by EDAB and BDAC might be at the same level. This could explain the similar adhesive test results at the same concentration levels for different detergents.

Both wet and soak strength are important properties that determine the durability of the adhesive bond for exterior applications. Water molecules penetrated into the glued areas and interacted with protein molecules and weakened the interface between proteins and wood during soaking. Concentration changes of the different detergents resulted in the same trend among the detergent-modified SPI adhesives. The interaction between detergent and protein resulted in the greatest wet strength for all detergents when 2.6 mM detergent concentration was used for all adhesives. From the thermal property results in Table 1, SPI modified with a detergent concentration of 2.6 mM had a different amount of denaturation than other concentrations. The extent of denaturation at 2.6 mM was less than that at detergent concentrations of 5.2 and 7.8 mM, but more than that at 1.3 mM. This suggests that a moderate amount of denaturation may favor the wet strength. At moderate denaturation, the hydrophobic part of the detergent might combine with the hydrophobic pockets of the protein, either the same protein on which specific binding happened or another protein nearby. This might increase the connection of protein molecules, forming a protein–detergent–protein complex in which a protein presents different conformations. This complex might have less polarity because of the decreased net charge from the specific binding (Fig. 1).

Detergents disrupted the hydrophobic and electrostatic bonds that maintained the native protein conformation and unfolded the protein structure. This would increase contact and interactions between protein and substrate and also increase entanglement and crosslinking during the curing process (4). After water penetrating and weakening the structure among protein molecules during soaking, the protein molecules were more attached to each other in treated proteins than in the control owing to the increased entanglement and crosslinking. This could decrease the number of protein groups that interact with the water molecule and thus could improve water resistance. An optimal detergent concentration level was essential for the best adhesion performance. The decrease in water resistance at higher detergent concentrations could be due to a superabundance of detergent molecules that interrupt the interaction among protein molecules. Excess detergents themselves are hydrophilic and thus absorb water molecules, which could have directly contributed to lower water resistance. Zhong and Sun (2) proposed this explanation for the decreased water resistance with high concentration of chemically modified soy protein plastics.

Effect of pH on thermal properties and adhesiveness. From the thermal and tensile strength studies, EDAB- and BDAC-modified SPI showed higher wet strength at 2.6 mM. It was observed that EDAB dissolved faster than BDAC when making the detergent stock solutions. Therefore, 2.6 mM EDAB was chosen to study the effect of pH on the performance of modified SPI adhesive. A DSC thermogram of modified SPI adhesive is shown in Figure 2. Thermograms for the four selected pH values are quite different in both T_d and ΔH_d. There were two peaks for both pH 4.5 and 7, but only one peak for pH 10 or pH 2. The T_d of modified SPI at pH 2 is significantly lower than those of other pH values (Table 3), which corresponds to its structure change. It has been reported that at low pH values (<3), 11S globulins present as the 7S and/or 3S form (16). At a pH value of 2.75, AB, A, and B subunits of 11S globulins and the β subunit and small amounts of α and α′ subunits of 7S were presented as low-molecular-mass peptides (30). These low-molecular-mass peptides might result in the lower denaturation temperature observed at pH 2. The highest T_d happened at pH 4.5, which is in accord with the general opinion that globular proteins are most stable close

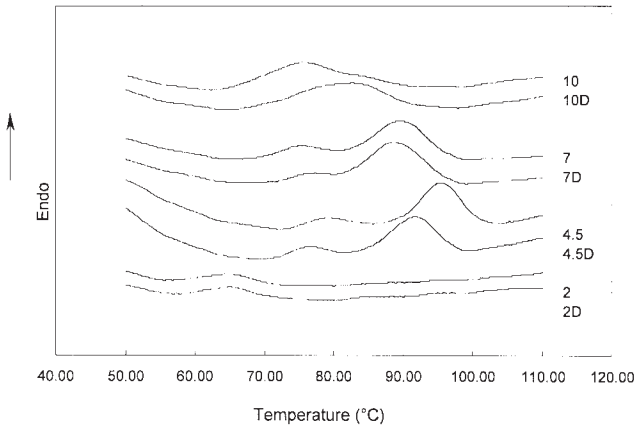


FIG. 2. DSC thermogram of unmodified soy protein isolate (SPI) and ethylhexadecyldimethyl ammonium bromide-modified SPI (designated by D) at pH values of 2, 4.5, 7, and 10.

to their apparent isoelectric point (15). This could result in the higher T_d at pH 4.5 than at pH 7. As shown in Figure 2, the thermogram showed a broader peak at pH 10. Petruccelli and Anon (31) found that as the pH increased from 7 to 10, the T_d of 7S globulins did not change, whereas the T_d of 11S globulins decreased approximately 10°C. The observed broader peak could result from the merger of the 7S globulin peak, which is at the same position, and the 11S globulin peak, which is about 10°C lower than that of pH 7 and falls into the same position as 7S. No significant differences in T_d and ΔH_d were detected between the control and modified SPI at pH 2 or pH 10. The detergent brought a positive charge to SPI at pH 4.5 and decreased the net charge at pH 7. This charge difference induced the different T_d values between the control and modified SPI, because proteins tend to be more stable when they have no net charge or when their charge is screened (20). There are no significant differences in ΔH_d for peak 1 and peak 2 between control and modified SPI at pH 4.5, indicating that no further denaturation has been induced by detergent for peak 1. Thermal behaviors for control and modified SPI were different when the pH was changed from 4.5 to 7. Decreased T_d values for peaks 1 and 2 for control SPI confirmed that higher stability happened at an isoelectric point. Decreased T_d for peak 2 for modified SPI could indicate unfolded structure induced by detergent.

TABLE 3
DSC Analysis of pH Effect on SPI Modified with EDAB[a,b]

	$\Delta\Delta H_d$ (J/g SPI)		T_d (°C)	
pH	Control	EDAB	Control	EDAB
Peak 1				
2	$0.52^{b,x}$	$0.47^{b,x}$	$62.47^{c,x}$	$62.96^{b,x}$
4.5	$0.72^{b,x}$	$0.71^{b,x}$	$77.89^{a,x}$	$75.09^{a,y}$
7	$0.55^{b,x}$	$0.20^{c,y}$	$73.75^{b,y}$	$75.82^{a,x}$
10	$4.69^{a,x}$	$5.16^{a,x}$	$74.15^{b,x}$	$76.81^{a,x}$
Peak 2				
2	—[c]	—	—	—
4.5	$4.12^{a,x}$	$3.56^{a,x}$	$94.14^{a,x}$	$90.61^{a,y}$
7	$3.52^{a,x}$	$3.56^{a,x}$	$88.51^{b,x}$	$87.85^{b,x}$
10	—	—	—	—

[a]Means with the same letters (a–e) within the same subunits category in the same column are not significantly different at $\alpha = 0.05$.
[b]Means with the same letters (x, y) within the same subunits category in the same row are not significantly different at $\alpha = 0.05$.
[c]No peak detected. For abbreviations see Table 1.

TABLE 4
Effect of pH on Adhesive Performance of SPI Modified with EDAB[a,b]

pH	Control (MPa)	EDABm (MPa)	Difference[c] (%)
Dry			
2	$5.01^{a,b,x}$ WF 70[d]	$4.51^{c,y}$ WF 40	−9.98
4.5	$5.43^{a,x}$ WF 80	$5.19^{a,b,x}$ WF 80	−4.42
7	$4.75^{b,y}$ WF 50	$5.65^{a,x}$ WF 90	18.95
10	$4.89^{a,b,x}$ WF 50	$4.88^{b,c,x}$ WF 50	0
Wet			
2	$0.82^{b,x}$ WF 0	$0.73^{c,x}$ WF 0	−10.98
4.5	$1.45^{a,x}$ WF 10	$1.21^{b,y}$ WF 10	−16.55
7	$1.34^{a,y}$ WF 10	$1.95^{a,x}$ WF 30	45.52
10	$0.50^{c,x}$ WF 0	$0.51^{c,x}$ WF 0	2.0
Soak			
2	$4.48^{a,x}$ WF 50	$3.73^{c,y}$ WF 40	−16.74
4.5	$4.74^{a,x}$ WF 50	$4.07^{b,c,y}$ WF 40	−14.14
7	$3.83^{b,y}$ WF 40	$5.35^{a,x}$ WF 70	39.69
10	$3.72^{b,y}$ WF 40	$4.17^{b,x}$ WF 40	12.10

[a]Means with the same letters (a–e) within the same subunits category in the same column are not significantly different at $\alpha = 0.05$.
[b]Means with the same letters (x, y) within the same subunits category in the same row are not significantly different at $\alpha = 0.05$.
[c]The difference (%) is calculated by control-EDABm/control × 100.
[d]Percentage of wood failure (%). EDABm, modified EDAB; for other abbreviations see Table 1.

The pH had a significant effect on adhesive performance for both unmodified SPI and SPI modified by EDAB. The dry test and water-resistance tests of unmodified SPI showed the same trend (Table 4). The unmodified SPI reached the highest tensile strength at pH 4.5, and tensile strength declined as pH increased to 7 and 10. Dry tests and water-resistance tests of modified SPI also showed the same trend. With pH increasing, tensile strength kept increasing until pH 7, and then declined at pH 10. At pH 4.5, there was no significant difference in dry strength between the control and modified SPI, and the wet and soak strengths of the control were higher than for modified SPI. This might suggest a weak interaction between detergent and SPI because of the compact protein structure at pH 4.5 and weak accessibility of the hydrophobic pockets. At pH 7, tensile strengths of

modified SPI were significantly greater than that of unmodified SPI (about 19% for dry strength, 45% for wet strength, and 40% for soaked strength) (Table 4). From Table 4, the highest strength of modified SPI was higher than that of unmodified SPI. This difference between unmodified and modified SPI could be due to the detergent-induced change in net charge and hydrophobicity. This indicated that both electronic and hydrophobic interactions between SPI and detergent contributed to the improved adhesive performance.

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