Alkali-Modified Soy Protein with Improved Adhesive and Hydrophobic Properties

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ABSTRACT: Adhesive and hydrophobic properties of alkali-modified soy protein (AMSP) and trypsin-modified soy protein (TMSP) on wood were investigated. Modifying soy protein (i) under moderate alkaline conditions (pH 10.0 at 50°C) and (ii) with trypsin, enhanced adhesive strengths (730 and 743 N, respectively) compared with unmodified soy protein (340 N). Hydrophobicities of AMSP, TMSP, and unmodified soy protein isolate by sodium dodecyl sulfate binding and 1-anilino-8-naphthalene sulfonate methods were 7.6, 6.4, 5.0 and 39, 27, 13, respectively. Modified soy protein adhesives with higher hydrophobicities (AMSP and TMSP) had enhanced water-resistance properties.
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KEY WORDS: Adhesive strength, alkaline modification, enzymatic modification, hydrophobicity, soy protein, wood.

Functional properties of protein result from its amino acid composition, its structure, and its interactions with other protein and substances present such as water, lipids, and cellulose (1). Physical, chemical, and enzymatic methods have been used to modify protein structures (2,3). Modifications, which change secondary, tertiary, or quaternary structure of the protein molecule with no breakage of covalent bonds, are generally called denaturation. The effects of denaturation on the functional properties of protein have been well documented (4–7). Most of these investigations focused on functional properties in food applications such as solubility, foaming, gelation, and viscosity (4).

Methods used to denature protein include exposure to heat, acid/alkali, organic solvents, detergents, and urea (4). Soy protein is widely used as a food ingredient, especially as meat substitutes (8–10), due to its desirable functional properties. Soy protein has also been used as an ingredient in wood adhesive and paper coating (11,12). These soy protein adhesives are prepared by treating defatted soy flour (soy meal) with alkali. The most effective treatment is increasing the pH of the soy meal to 11 or higher. Alkali treatment is used to disperse soy protein in water and to denature it. Since alkali treatment alone may not yield optimal results, combination treatments of alkaline pH coupled with a moderate temperature were explored.

Water resistance is an important glue property that determines adhesive bond durability (11). To improve water resistance of soy protein glue, several cross-linking agents, such as calcium salts and carbon disulfide, have been used (11). Adhesives prepared from soy meal have poor water resistance due to the presence of carbohydrates (11). Improved adhesive strength and water resistance have been observed in glue prepared from trypsin-modified soy protein (TMSP) (13). Since limited information is available on the adhesive and water-resistance properties of soy protein isolates (SPI) modified by combinations of alkali and heat treatments, we investigated the effects of these treatments.

EXPERIMENTAL PROCEDURES

Materials. SPI (ARDEX D) was obtained from Archer Daniels Midland Co. (Decatur, IL). Trypsin (type II from porcine pancreas, activity 1,500 units/mg) was purchased from Sigma Chemical Co. (St. Louis, MO). Soft maple wood blocks (5 × 2 × 0.3 cm) were purchased from White River Hardwoods, Woodworks, Inc. (Fayetteville, AR).

Preparation of modified protein: alkali-modified soy proteins (AMSP). Ten grams of SPI was suspended in 140 mL deionized water and stirred (magnetic stirrer) for 10 min for uniform dispersion. Each suspension was then adjusted to a pH of 8.0, 9.0, 10.0, 11.0, or 12.0 using 1N NaOH; covered with aluminum foil, and incubated in a shaker bath at 180 rpm for 1 h at 30, 40, 50, 60, or 70°C. The resulting AMSP was frozen at –5°C, freeze-dried, and stored at ambient temperature (25°C).

Preparation of modified protein: TMSP. TMSP was prepared according to the method described by Kalapathy *et al.* (13). A 7% solution of SPI with trypsin (E/S = 1:50, pH 8.0) was incubated at 37°C for 1 h in a shaker (180 rpm). The enzyme was inactivated by heating at 90°C for 3 min, and the product was frozen and freeze-dried.

Degree of hydrolysis. Degree of hydrolysis was determined by measuring insoluble nitrogen in 10% trichloro

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acetic acid (TCA), as discussed by Edwards and Shipe (14). TCA-insoluble nitrogen was assayed by using the Kjeldhal method. The degree of hydrolysis (% DH) was calculated using the following equation:

$$\% \text{ DH} = N_b - N_a/N_b \quad [1]$$

where N_a = TCA-insoluble nitrogen after hydrolysis and N_b = TCA-insoluble nitrogen before hydrolysis.

Adhesive strength. The procedures used for gluing wood pieces and determining adhesive strength were those of Kalapathy *et al.* (13). Freeze-dried samples were used for all adhesive tests. Protein content (as-is basis) of all samples was 87% (Kjeldhal method, $N \times 6.25$). Moisture content (oven method, 130°C/2 h) of wood blocks were 8–9%. One hundred milligrams of 8.0% (w/w) protein solution was placed on each side of a wood block ($5 \times 2 \times 0.3$ cm) and spread on an area of 2×2 cm to give a protein concentration of 2.0 mg/cm^2. Two additional wood pieces of similar size were superimposed on these glued areas and pressed with a load of 5 kg for 2 h. 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 Corp., Canton, MA) by pulling apart from two edges at a loading rate of 20 mm/min (tension mode); this force was expressed as the adhesive strength of the protein glue.

Water soaking test. The glued wood pieces were placed in a container (4 L capacity), submerged in 4 L tap water (23–25°C), and allowed to soak for 24 h (a weight was used to submerge the wood pieces). The wood pieces were air-dried at ambient temperature (25°C) for 10 h in a fume hood with fan-forced air circulation. The dried wood pieces were examined for delamination. The wood pieces were then subjected to an additional four cycles of soaking and air-drying treatments. After each cycle, wood pieces were examined for delamination; these pieces were removed. The total number of glue joints that delaminated was used as an indicator of the water resistance of the protein adhesive.

Hydrophobicity determination. Surface hydrophobicity of modified proteins were determined by using the sodium dodecyl sulfate (SDS) binding method (15) and the 1-anilino-8-naphthalene sulfonate (ANS) method (16). In the SDS binding method, a 0.1% protein solution in 0.07 mM SDS was prepared and allowed to stand for 30 min. SDS–protein solution was dialyzed against 0.02 M buffer (phosphate buffer for pH 7.0 and 8.0; bicarbonate buffer for pH 9.0 and 10.0) for 48 h. One milliliter dialyzed solution was transferred into a 25-mL screw-capped test tube containing 10.0 mL chloroform and was mixed by shaking. Methylene blue solution (2.5 mL of 0.0024%) was added to the contents, mixed, and centrifuged at $800 \times g$ to separate the water and insoluble protein from the chloroform. The absorbance of the SDS–methylene blue mixture in the chloroform layer was measured at 655 nm. A calibration curve, obtained using the above method with known amounts of SDS, was used to determine the amount of SDS bound to proteins. SDS binding capacity (μg of SDS bound to 1 mg protein) was expressed as a measure of hydrophobicity of proteins.

In the hydrophobic fluorescence probe method, protein samples having concentrations ranging from 0.0015 to 0.015% were prepared by serially diluting a stock solution having a concentration of 0.015% with 0.01 M buffer (phosphate buffer for pHs 7.0 and 8.0; bicarbonate buffer for pHs 9.0 and 10.0). Ten microliters ANS (8 mM in 0.01 M buffer) were added to 2.0 mL of protein solution. Fluorescence intensity of ANS–protein conjugates was measured with a Kontron Model SF23/B Spectrofluorometer (Kontron Ltd., Zurich, Switzerland), using excitation and emission wavelengths of 390 and 470 nm, respectively. The slope of the fluorescence intensity vs. the percentage of the protein concentration was calculated by linear regression and was used as an index of the protein hydrophobicity.

Statistical analysis. Analysis of variance (SAS Institute, Cary, NC) was used for data analysis and least significant differences were computed at the 5% level.

RESULTS AND DISCUSSION

Adhesion. The adhesive strength of a protein glue depends on its ability to disperse in water and on the interaction of apolar and polar groups of the protein with wood material. In a native protein, the majority of polar and apolar groups is unavailable due to internal bonds resulting from Van der Waals forces, hydrogen bonds, and hydrophobic interactions. Dispersion and unfolding of a protein are enhanced by hydrolysis or by increasing the pH to a desirable value. As the protein molecule unfolds, polar and apolar groups are exposed and are able to interact with other materials. These interactions can lead to increased adhesive strength of modified soy protein with wood (11).

Adhesive strengths of AMSP resulting from treating at temperatures ranging 30 to 70°C and pH values ranging from 8.0 to 12.0 are shown in Figure 1. At pH 8.0 and 9.0, the adhesive strengths of soy protein progressively increased from 300 N to 689 and 712 N, respectively, as temperature increased from 30 to 70°C. At pH 10.0, 11.0, and 12.0, the adhesive strengths increased to 730, 743, and 788 N at 50, 50, and 40°C, respectively. Further increases in temperature (above 40–50°C) did not result in significantly greater ($P < 0.05$) adhesive strengths of AMSP. The optimum treatment conditions (pH/temperature) for producing AMSP with the highest adhesive strengths were 9.0/70, 10.0/50, 11.0/50, and 12.0/40°C. All of these alkali treatments under optimum pH/temperature conditions gave similar adhesive strengths. Furthermore, a higher pH (between 11.0 and 12.0) stains the wood. Hence a moderate pH/temperature combination of 10.0/50°C is desirable.

Water resistance. Water resistance is an important property that determines the durability of glue (11,17). A total of 30 blocks (three replicates with 10 blocks in each replicate) were tested for each sample (Table 1). The total number of blocks delaminated after four cycles using unmodified con-

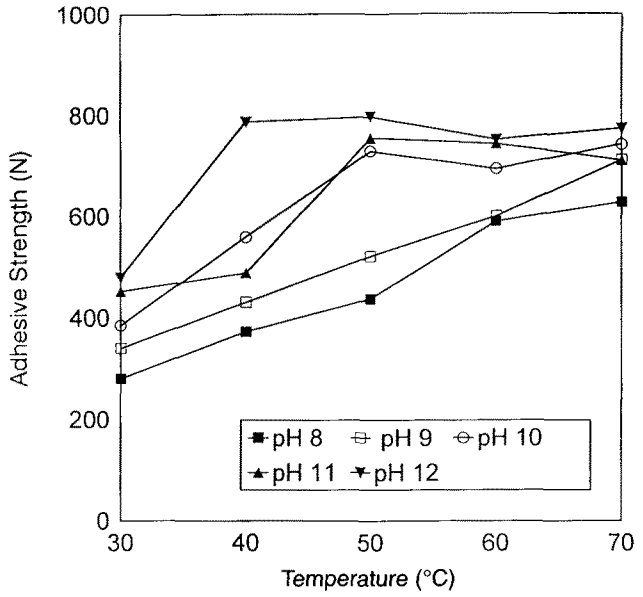

FIG. 1. Effects of treatment pH and temperature on adhesive strengths of modified soy protein preparations. Values are means of four measurements, and the standard error of the means was 21 to 44 N.

trol soy protein, AMSP, and TMSP were 26, 1, and 11, respectively. This showed that AMSP has improved water resistance. The hydrophobicities of AMSP, TMSP, and unmodified control measured by the SDS binding and ANS fluorescence probe methods confirmed these findings.

The hydrophobicities of unmodified control, AMSP, and TMSP were 5.0, 7.6, and 6.4 and 13, 39, and 27 by the SDS binding and ANS probe methods, respectively (Table 2). The hydrophobicity data by both SDS binding and ANS probe supported the results of the water-soaking tests: as glue hydrophobicity increased, water resistance of glue also increased. Both TMSP and AMSP gave similar adhesive strengths (743 and 730 N, respectively).

Trypsin modification involves limited hydrolysis (degree of hydrolysis 8%) of protein molecules, whereas alkali modification involves the unfolding of protein molecules. Both trypsin and alkali modifications lead to increased exposure of hydrophobic groups. At the conditions causing maximum adhesive strength, however, AMSP exposed more hydrophobic groups than did TMSP, as indicated by the significantly ($P < 0.05$) higher hydrophobicity (by SDS and ANS) of AMSP compared with that of TMSP.

Effects of adhesive pH on adhesive strength and hydrophobicity. The surface charge of a protein is a function of its pH. Hence the pH of the glue may affect its hydrophobic properties. AMSP obtained using moderate conditions of pH 10.0 and 50°C was selected to examine the effect of glue pH on adhesive and hydrophobic properties, since the product had comparatively high hydrophobicity. The pH of AMSP preparations (initial pH 10.0) was adjusted to 7.0, 8.0, or 9.0 after modification and was then freeze-dried.

The adjusted glue pH had no significant effect (data not shown) on the adhesive strength of glue. The hydrophobicity was affected, however, by adjusting the pH (Table 3). At pH 7.0, the SDS hydrophobicity dropped to a value of 6.4. At pH 8.0 and above, no significant change ($P < 0.05$) in hydrophobicity was observed. A similar trend in ANS hydrophobicity was observed with pH (see Table 3). The ANS method measured the hydrophobicity of the soluble protein, whereas the SDS binding method measured the hydrophobicities of both soluble and insoluble protein. Hydrophobic measurements by both methods gave similar results (14).

Since protein surface charge changes with pH, surface hydrophobicity will also be affected by pH. Between pH 8.0 and 10.0, however, no changes in hydrophobicity were observed. Apparently, in this alkaline media the hydrophobic groups may already be exposed and may remain so.

TABLE 1
Water Resistance of Modified and Unmodified Soy Protein Glues (percentage number of wood blocks delaminated after each cycle of water soaking test)

Sample	Cycle[a] 1	2	3	4	Total[b] delamination
Control	3.3	6.7	20.0	57.0	87.0
AMSP[c] (pH 10.0, 50°C)	0	0	0	3.3	3.3
TMSP[d]	0	10.0	10.0	16.7	36.7

[a]Means of three replicates.
[b]Values are significantly different from each other at $P < 0.05$.
[c]Alkali-modified soy protein.
[d]Trypsin-modified soy protein.

TABLE 2
Hydrophobicities of Modified and Unmodified Soy Protein as Measured by ANS and Sodium Dodecyl Sulfate (SDS) Binding Methods

Sample	ANS hydrophobicity[a,b]	SDS binding[a,c]
Control	13	5.0
AMSP[d] (pH 10, 50°C)	39	7.6
TMSP[d]	27	6.4

[a]Means of two measurements; values in the same column are significantly different from each other at $P < 0.05$. ANS, 1-anilino-8-naphthalene sulfonate.
[b]Fluorescence intensity/percentage protein concentration.
[c]μg of SDS/mg of protein.
[d]Abbreviations as in Table 1.

TABLE 3
Effects of pH on Hydrophobicities of AMSP (pH 10.0/50°C)[a]

pH of AMSP	ANS hydrophobicity[b,c]	SDS binding[b,d]
7.0	32[e]	6.4[e]
8.0	39[f]	7.7[f]
9.0	42[g]	8.2[g]
10.0	39[h]	7.6[h]

[a]Abbreviations as in Tables 1 and 2.
[b]Means of three measurements; values with different superscripts (e–h) in the same column are significantly different from each other at $P < 0.05$.
[c]Fluorescence intensity/percentage protein concentration.
[d]μg of SDS/mg of protein.

Conversely, at neutral pH, aggregation of protein causes changes in surface properties. It has been shown (14) that there is no common relationship between pH and surface hydrophobicity of protein. Furthermore, it is interesting that the surface charge had no effect on adhesive strength, which is determined by the interaction of protein with wood material. Water resistance of AMSP at pH 8.0 and 9.0 (data not shown) were similar to that of AMSP at pH 10.0 and was in agreement with their hydrophobicities. Hence, if the higher pH of 10.0 is detrimental to the quality of the glued wood, the pH of the AMSP can be brought down to 9.0 or 8.0 without adversely affecting adhesive strength or hydrophobic properties of AMSP-based adhesives. The findings presented here should be very useful in producing wood glue from soy protein with improved adhesion and water resistance.

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