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ORIGINAL PAPER

Urea-Modified Soy Globulin Proteins (7S and 11S): Effect of Wettability and Secondary Structure on Adhesion

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Abstract This investigation characterized wettability and adhesive properties of the major soy protein components conglycinin (7S) and glycinin (11S) after urea modification. Modified 7S and 11S soy proteins were evaluated for gluing strength with pine, walnut, and cherry plywood and for wettability using a bubble shape analyzer. The results showed that different adhesives had varying degrees of wettability on the wood specimens. The 7S soy protein modified with urea had better wettability on cherry and walnut. The 11S soy protein modified with 1M urea had better wettability on pine. The 1M urea modification gave 11S soy protein the greatest bonding strength in all the wood specimens. The 3M urea modification gave 7S soy protein stronger adhesion on cherry and walnut than did 11S protein; but with pine, 11S soy protein had greater adhesion strength than 7S soy protein. Measurement of protein secondary structures indicated that the β-sheet played an important role in the adhesion strength of 3M urea-modified soy protein in cherry and walnut, while random coil was the major factor reducing adhesion strength of 7S soy protein modified with 1M urea.

Keywords Adhesion strength · 11S globulins · 7S globulins · *Protein modification* · Wettability

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Introduction

Soy-based adhesives were first developed in 1923. They are attractive because they are environmentally friendly, biodegradable and renewable. However, soy protein adhesives have relatively low gluing strength and water resistance. Most workers have investigated the adhesion mechanism and developed modified soy protein with greater adhesion strength and better water resistance [1–8]. Adhesion is a very complicated phenomenon that involves mechanical interlocking, adhesive penetration, physical attraction, and chemical bonding. However, the actual mechanism of adhesive attachment has never been clearly defined and universally accepted [8]. For good adhesion, the globular structure has to be broken because any good adhesive must consist of relatively large, flexible and interwoven polymer chains [9]. Chemical modifications were a means of breaking the internal bonds and unfolding the protein molecules. Alkali, urea and sodium dodecyl sulfate (SDS) improved the adhesion strength and water resistance of soy protein isolates [2–6]. The dominant storage proteins in soybean are globulins, which account for 50–90% of the total proteins [10]. Conglycinin (7S) and glycinin (11S) are the main storage globulins. Different structures and molecular properties of 7S and 11S cause variations in their functional properties, as well as, their adhesion performance. Studying 7S and 11S adhesion mechanism is beneficial to understand soybean protein adhesion mechanism. An adhesion problem involves the adhesive and the substrate. The adhesive property, substrate character and adhesive-substrate interfacial property affect the adhesion performance. Few reports have discussed adhesion mechanism of 7S and 11S after chemical modification. The objectives of this study were to characterize wettability and the adhesion strength of 7S and 11S

proteins, modified with urea, on walnut, cherry, and pine and to determine the secondary structures playing key roles on the adhesion strength of 7S and 11S proteins after modifying with urea.

Materials and Methods

Materials

Defatted soy flour was obtained from Shandong Gushen Group (Ling County, Shandong province, China) and used for the preparation of soybean globulins. Urea (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) was an analytical-grade reagent. Thickness and molecular weight cutoff of the dialyzing membrane were 0.023 mm and 14 kDa, respectively.

Isolation of 7S and 11S Globulins

The 7S and 11S fractions were separated from the soy flour by the method of Thanh and Shibasaki [11]. Defatted 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. The pH of the 7S-whey proteins was adjusted to 4.8, and the precipitate was redissolved in 30 mM Tris buffer (pH 8.0). 2 N NaOH solution was added dropwise while stirring until the protein dissolved (pH 6.2). The supernatant was 7S globulins collected by centrifugation (10,000*g* for 30 min at 4 °C). The 7S and 11S globulins were freeze-dried and then milled into a powder by mortar to a particle size of less than 1 mm. The freeze-dried 7S powder samples had an average protein content of 90.3% (dry basis, db), while that of 11S was 96.6% (db).

Protein Modification

Solutions of urea (1M, 3M) were prepared at room temperature. The concentrations of urea were chosen based on previous studies [5]. Ten grams of 7S or 11S fractions were suspended in 100 g of urea solution, stirred, and allowed to react for 6 h at room temperature.

Wood Specimen Preparation

Three types of wood, ranging from soft to hard (pine, cherry, and walnut, respectively) were bought on the open market, put into polyethylene bags, and placed in silica gel desiccators under room conditions. The moisture content of the wood veneer was 10–11% (105 °C, 2 h). To ensure similar surface conditions, the wood veneer was prepared with the same planer (YB200C; Qingdao Dezhong Machinery Co., Ltd, Qingdao, Shandong province, China) using blade number 4, blade length of 800 mm and speed of 50 times/min. The method described by Sun and Bian [4] was used to prepare the wood specimens for testing. Each piece of wood was 3 (thickness) × 20 (width) × 50 (length) mm, and three pieces were glued together to form a specimen. One hundred milligrams of protein solutions [10.0% (w:w)] were placed on each side of the middle piece of wood and spread separately with one side (20 × 20 mm) of other two pieces until both sides of the marked area of 20 × 20 mm were wetted completely and uniformly. The glued area was 2 × 2 cm and the protein concentration was 2.0 ± 0.5 mg/cm^2. The wetted wood samples were allowed to rest at room temperature for about 10 min and were then assembled together by hand. Six specimens were then hot-pressed (Flatten Press Machine; Shanghai Light Industry Mechanical Ltd., Shanghai, China) at 120 °C and 1 MPa for 10 min. The pressed specimens were cooled and placed in polyethylene bags under room conditions for 4 days.

Contact Angle Measurement

Contact angles between adhesive and wood were measured with a bubble shape analyzer (model DSA100, Krűss Co., Germany). One droplet of liquid adhesive (about 2 μl) was dropped on the surface of the wood and contact angles were measured. Data reported are means of three replications. Non-modified 7S and 11S proteins were used as controls. Contact angle drop percentage was calculated by the following Eq. 1:

$$\text{Contact angle drop percentage} = \frac{\text{immediate contact angle} - \text{equilibrium contact angle}}{\text{immediate contact angle}} \times 100\% \quad (1)$$

Adhesion Strength Measurement

Shear strengths of wood specimens were determined by using a universal testing machine (model WDT-10; Shenzhen KaiQiangLi Mechanical Ltd., Shenzhen, China) operated at a speed of 10 mm/min. The force (N) required to break the glued wood specimen was recorded. Adhesion strength data reported are means of six replications. Non-modified 7S and 11S proteins were used as controls. Adhesion strength (MPa) was calculated by the following Eq. 2:

$$\text{Adhesion strength (MPa)} = \frac{\text{force (N)}}{\text{gluing area (m}^2\text{)}} \quad (2)$$

Secondary Structures of Proteins Measurement

Secondary structures of proteins after urea modification were measured with FTIR (model NEXUS, Thermo Electron Corporation, Madison, Wisconsin, USA). The corresponding unmodified globulins were used as controls. Sample solutions were dialyzed for 48 h to eliminate absorbance of urea at amide I (1,600–1,700 cm^{-1}). Spectra of dialyzed sample solutions were obtained using attenuated total reflectance (ATR) spectroscopy. Secondary structural features were calculated from the amide I (1,600–1,700 cm^{-1}) envelope by deconvolution, second derivative and curve fitting of Gaussian peaks to the original spectra.

Statistical Analysis

Analysis of variance (Statistical Program for Social Sciences, SPSS Institute, Chicago, USA) was used for data analysis, and least significant differences were computed at 5% probability level.

Results and Discussion

Wettability

Wettability of adhesive on the surface of the substrate is a precondition of better adherence. The contact angle is a parameter reflecting wettability. When the contact angle is less than 90°, wetting is adequate between the adhesive and the substrate [12]. The contact angle drop percentage is another indicator of wettability. A higher contact angle drop percentage or lower equilibrium contact angle mean better wettability of adhesive [13]. The wettability of 7S and 11S soy protein adhesives on different woods was evaluated by contact angles and contact angle drop percentages (Table 1). The contact angle data were all less than 90°, which indicated that these adhesives can wet all the wood samples sufficiently. Different adhesives had varying degrees of wettability on the wood specimens (Table 1).

The non-modified 7S soy proteins (7S/UnM) had better wettability on pine than did non-modified 11S soy proteins (11S/UnM) based on lower equilibrium contact angle and higher contact angle drop percentages. On walnut and cherry, 11S/UnM had better wettability than 7S/UnM. After modification, to 1M urea, the 7S soy proteins (7S/1M) had better wettability on walnut and cherry than did 11S/1M soy proteins as indicated by higher contact angle drop percentages. The 11S/1M adhesive had better wettability on pine than on other woods. At 3M urea modification, the wettability of 7S on all the wood samples was better than that of 11S as indicated by higher contact angle drop percentage data. Modification improved the wettability of the 7S fraction on walnut and cherry but made it worse on pine. After urea modification, the wettability of the 11S fraction decreased on walnut and cherry and the change of wettability on pine depended on the urea concentration.

For the hardwood, the 7S fraction modified by urea had better wettability than the 11S fraction. The drop percentages were in the order of 7S/1M/walnut > 7S/3M/cherry > 7S/1M/cherry > 7S/3M/walnut. On pine, the 11S/1M adhesive had the best wettability.

The 7S/3M and 11S/UnM adhesives had similar wettability on pine, as indicated by contact angle drop percentages that were not statistically different. Similarly, no difference in wettability was detected between 11S/1M and 11S/3M adhesives toward walnut. Wettability on cherry was significantly different among all adhesives.

There are many factors affecting the wettability of adhesive on woods, such as: surface component of wood [14], spring wood has better wettability than autumn wood [15, 16], alburnum (sapwood) has better wettability than duramen (heartwood) [17]. In our study, the nature of the surfaces of the different species wood we used may have affected their wettability. Because we bought them from the local market, we were unable to determine whether the samples were spring wood, autumn wood, sapwood, or heartwood, which could also affect wettability. We assumed that their effects were negligible in this paper.

Adhesion Strength

Adhesion strength of a protein glue depends on the protein's ability to disperse in water and the interaction of apolar and polar groups of the protein with the wood material [7]. The adhesion strengths of 7S and 11S modified with urea are shown in Fig. 1. Compared with unmodified proteins, the 1M urea-modified 7S fraction had improved adhesion strengths on pine and cherry while the 3M urea modification improved the adhesion strengths of 7S on walnut. For 11S fractions, the adhesion strengths improved on all woods after modification with 1M urea, but decreased on all wood samples after being modified with 3M urea. The 11S/UnM adhesive had greater shear strength than the 7S/UnM adhesive on all woods. The 7S/3M adhesive had greater shear strength than the 11S/3M adhesive with walnut and cherry. These results are in agreement with the observation of Mo et al. [18], who found that adhesive strength of the 7S/3M adhesive was higher than the 11S/3M adhesive applied to cherry wood. The 11S/1M adhesive gave the highest shear strength in all

Table 1 Wettability of different adhesives on pine, walnut and cherry

Adhesive	Wood	θ_i[a] (degree)	θ_e[b] (degree)	Contact angle drop percentage (%)
7S/UnM[c]	Pine	87.1a	8.45a	90.3a
	Walnut	89.5b	66.12b	26.1b
	Cherry	88.3c	60.3c	31.7c
7S/1M[d]	Pine	70.40d	19.80d	71.9d
	Walnut	39.30e	7.89e	79.9e
	Cherry	70.80f	23.38f	67.0f
7S/3M	Pine	81.50g	26.60g	67.4f
	Walnut	69.70h	24.47h	64.9g
	Cherry	71.20i	15.24i	78.6h
11S/UnM	Pine	83.7j	27.82j	66.8f
	Walnut	85.6k	24.25k	71.7d
	Cherry	88.2c	27.36l	69.0i
11S/1M	Pine	66.60l	16.99m	74.5j
	Walnut	59.00m	24.52h	58.4k
	Cherry	73.90n	39.48n	46.6l
11S/3M	Pine	71.20i	32.06i	55.0m
	Walnut	67.57o	27.70j	59.0k
	Cherry	46.80p	39.46n	15.7n

Mean values of three replications. Values in the same column followed by different online roman letters are significantly different ($P < 0.05$)

[a] Immediate contact angle

[b] Equilibrium contact angle

[c] Unmodified soy protein

[d] Molar concentration of urea solution

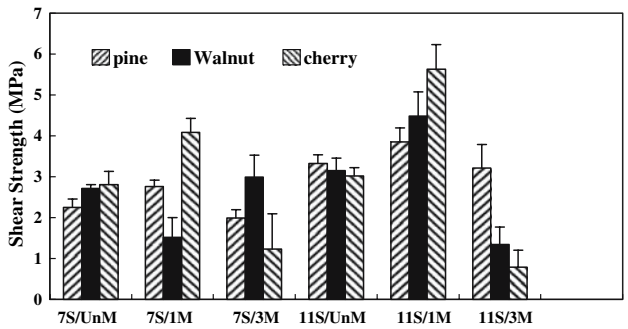

Fig. 1 Shear strengths of six different adhesives on pine, walnut, cherry plywood. Shear strengths values are means of six measurements

the wood samples. 7S/3M adhesive had better wettability than 11S/3M adhesive, which likely resulted in better gluing strength in the wood samples, except for pine. The 11S/1M adhesive had the highest shear strength on pine, which was probably influenced by its superior wettability on this wood. The 7S/3M adhesive had lower shear strength on pine despite good wettability indicators. The 11S/1M adhesive had a higher shear strength on hardwood even with lower wettability. These results indicated that, while wettability of adhesive is a strong influence on adherence, wettability was not the only factor affecting adhesion strength. This finding was suggested by the lack of difference noted in the wettability between 7S/3M and 11S/UnM with pine, although the adhesion strength of 11S/UnM on pine was higher than that of 7S/3M. Similarly, the adhesion strength of 11S/1M on walnut was higher than that of 11S/3M, but their wettabilities were not statistically different (Table 1).

Secondary Structures of Proteins

The secondary structure might be desirable for protein adhesion [5]. Proteins modified at relatively lower urea concentrations (e.g. 1M or 3M) may have been partly unfolded and have a certain amount of secondary structure, resulting in better shear strengths [5]. The assignment of the individual bands to secondary structure elements is based on FTIR data of proteins published recently [19–21]. The original FTIR spectrums are shown in Fig. 2. We assigned the α-helix structure to 1,649–1,660 cm^{-1}, the random coil structure to 1,638–1,648 cm^{-1}, the β-sheet structure to 1,606–1,637 cm^{-1}, and β-turn structure to 1,660–1,700 cm^{-1}. The α-helix content of 7S decreased and β-sheet increased notably after being modified with 3M urea. With 11S, the α-helix content increased and β-sheet decreased after being modified with 3M urea (Table 2). The β-sheet content of 7S/3M adhesive was higher than that of 11S/3M adhesive (45.5 and 33.4%, respectively). The 7S/3M had higher adhesion strengths on walnut and cherry than the 11S/3M adhesive (Fig. 1). These results indicated that the β-sheet played an important role on the adhesion strength of 3M urea-modified soy protein on walnut and cherry. The adhesion strengths of 11S/1M for the three types of plywood were the highest among all adhesives tested (Fig. 1). After urea modification, random

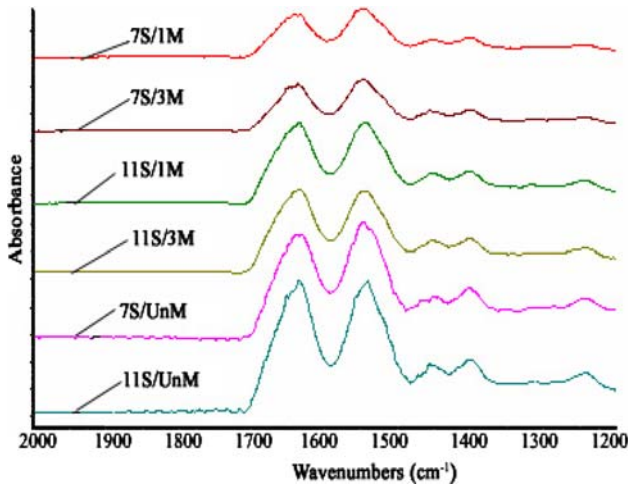

Fig. 2 Original FTIR spectrums of different adhesives after dialysis

Table 2 Secondary structures of proteins modified with urea (U) (1M, 3M) and unmodified (UnM) soy proteins

Different adhesives	α-Helix (%)	β-Sheet (%)	β-Turn (%)	Random coil (%)
7S/UnM[a]	28.48d	34.30a	20.90a	16.31a
7S/1M[b]	18.84c	33.27a	25.93b	21.96b,c
7S/3M	0.00a	45.49c	30.57c	23.95c
11S/UnM	14.29b	43.35c	26.66b	15.71a
11S/1M	17.00c	37.60b	27.74b	17.67a
11S/3M	17.97c	33.37a	27.58b	21.08b

Mean values of three replications. Values in the same column followed by different online roman letters are significantly different ($P < 0.05$)

[a] Unmodified soy protein

[b] Molar concentration of urea solution

coil contents of 7S were higher than that of 11S. Random coil contents also increased with increasing urea concentration (Table 2). Random coil contents of 11S/1M was lower than that of 7S/1M, which indicated that random coil was the major factor reducing adhesion strength of 7S modified with 1M urea. This is supported by our observation that the random coil content of 11S/UnM was lower than that of 7S/3M, and its adhesion strength on pine was higher than that of 7S/3M. A smooth surface structure, like pine wood, might have less micro random "finger joint" effects [4] and these random coil structure could penetrate though cell walls of fibers of the wood surface and become part of fiber component, which could be another reason for the low gluing strength.

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References

1. Kalapathy U, Hettiarachchy NS, Myers D, Hanna MA (1995) Modification of soy proteins and their adhesive properties on woods. J Am Oil Chem Soc 72:507–510
2. Hettiarachchy NS, Kalapathy U, Myers D (1995) Alkali-modified soy protein with improved adhesive and hydrophobic properties. J Am Oil Chem Soc 72:1461–1464
3. Kalapathy U, Hettiarachchy NS, Myers D, Rhee KC (1996) Alkali-modified soy proteins: effect of salts and disulfide bond cleavage on adhesion and viscosity. J Am Oil Chem Soc 73:1063–1066
4. Sun SX, Bian K (1999) Shear strength and water resistance of modified soy protein adhesives. J Am Oil Chem Soc 76:977–980
5. Huang WN, Sun X (2000) Adhesive properties of soy proteins modified by urea and guanidine hydrochloride. J Am Oil Chem Soc 77:101–104
6. Huang WN, Sun X (2000) Adhesive properties of soy proteins modified by sodium dodecyl sulfate and sodium dodecylbenzene sulfonate. J Am Oil Chem Soc 77:705–708
7. Zhong ZK, Sun X, Fang XH, Ratto JA (2001) Adhesion properties of soy protein with fiber cardboard. J Am Oil Chem Soc 78:37–41
8. Cheng EZ (Michael) (2004) Adhesion mechanism of soybean protein adhesives with cellulosic materials. Ph.D. Dissertation, Kansas State University, Manhattan, KS
9. Van der Leeden MC, Rutten AACM, Frens G (2000) How to develop globular proteins into adhesives. J Biotechnol 79:211–221
10. Wright DJ (1987) The seed globulins. In: Hudson BJF (ed) Developments in food proteins. Elsevier, London, pp 81–157
11. Thanh VH, Shibasaki K (1976) Major proteins of soybean seeds. A straightforward fractionation and their characterization. J Agric Food Chem 24:1117–1121
12. Wang MZ (1987) Adhesion of wood and metal. In: Wang MZ, Huang YC (eds) Adhesive application handbook, Chemical Industry Press, China, pp 374–407
13. Liu ZM (2002) Surface feature of wheat straw and adhesive joint mechanism of wheat straw particleboards. Ph.D. Dissertation, Northeast Forestry University, Harbin, Heilongjiang province
14. Hemingway RW (1969) Thermal instability of fats relative to surface wettabilty of yellow birch. Tappi J 52:2149–2155
15. Hse CY (1972) Wettability of southern pine veneer by phenol formaldehyde wood adhesives. For Prod J 12:452–461
16. Hse CY (1971) Properties of phenolic adhesives as related to bond quality in southern pine plywood. For Prod J 21:44–52
17. Kajita H (1992) Wettability of the surface of some American softwoods species. Mokuzai Gakk 38:516–521
18. Mo XQ, Sun X, Wang DH (2004) Thermal properties and adhesion strength of modified soybean storage proteins. J Am Oil Chem Soc 81:395–400
19. Zhang X, Huang LX, Nie SQ (2003) FTIR characteristic of the secondary structure of insulin encapsulated within liposome. J Chin Pharm Sci 12(1):11–14
20. Van de Weert M, Haris PI, Hennink WE, Crommelin DJA (2001) Fourier transform infrared spectrometric analysis of protein conformation: effect of sampling method and stress factors. Anal Biochem 297:160–169
21. Zhao XY, Chen FS, Xue WT, Lee LT (2007) FTIR spectra studies on the secondary structures of 7S and 11S globulins from soybean proteins using AOT reverse micellar extraction. Food Hydrocoll. doi:10.1016/j.foodhyd.2007.01.019