J Polym Environ (2011) 19:755–765
DOI 10.1007/s10924-011-0305-5

ORIGINAL PAPER

Adhesive Performance of Sorghum Protein Extracted from Sorghum DDGS and Flour

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Published online: 1 June 2011

Abstract Distillers dried grains with solubles (DDGS) is the main co-product from grain-based ethanol production. The objective of this research was to compare the adhesive performance of three types of sorghum proteins: acetic acid-extracted sorghum protein from DDGS (PI), aqueous ethanol-extracted sorghum protein from DDGS (PII) and acetic acid-extracted sorghum protein from sorghum flour (PF). Physicochemical properties including amino acid composition, and rheological, thermal and morphological properties also were characterized. Results showed that PI had the best adhesion performance in terms of dry, wet and soak adhesion strength, followed by PF and PII. The wet strength of PI at a concentration of 12% protein assembled at 150 °C was 3.15 MPa, compared to 2.17 MPa and 2.59 MPa for PII and PF, respectively. DSC thermograms indicated that the PF protein isolates contained higher levels of carbohydrates than PI and PII; such non-protein contaminants in the PF isolate could be the reason for its lower adhesion strength than PI. In addition, PI might have more hydrophobic amino acids aligned at the protein-wood interface than PII, which could explain the better water resistance of PI. The optimum sorghum protein concentration and pressing temperature for maximum adhesion strength was 12% and 150 °C. PI had a significantly higher wet strength (3.15 MPa) than unmodified soy protein (1.63 MPa for soy protein). The high percentage of hydrophobic amino acids in PI (57%) was likely a key factor in the increased water resistance of PI compared with soy protein (36% hydrophobic amino acids). These results indicated that sorghum protein has huge potential as an alternative to petroleum-based adhesives.

Keywords Sorghum protein · DDGS · Adhesion · Water resistance · Amino acids

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Introduction

About 20 billion pounds of adhesives and resins are used annually in the United States in plywood, particleboard, lamination, and various composites for construction, packaging, furniture, etc. [1]. These adhesives are derived mostly from petroleum-based chemicals such as phenol–formaldehyde and urea–formaldehyde resins. Formaldehyde-based adhesives took over the adhesive market in the mid-twentieth century due to their long shelf lives and water resistance [2]. However, due to finite petroleum resources, non-uniform distribution of these resources, volatile prices and environmental concerns, the adhesive industry is increasingly interested in bio-based adhesives. Various natural resources including animal glues, fish glues, casein and vegetable protein glues, starch glues and blood albumen glues have been used for the wood industry. Several new technologies have been investigated to explore the adhesive potential of various biomaterials, including tannins, lignins, carbohydrates, unsaturated oils, liquefied wood rice bran, soy protein and organisms [3–5]. Soy protein isolates (SPI) gained much attention over the last century as bio-based and

renewable materials. Much research has been conducted on the use of soy proteins as adhesives in the past 10 years [6, 7], with most of this work focused on improving water resistance through increasing the hydrophobicity of soy proteins via chemical modification [8].

Isolated sorghum proteins, another bio-based material, have been shown to be capable of producing biodegradable films and have been used as extenders in plywood adhesives and in other low-cost adhesives, wallboard and packaging materials [9–11]. Soy proteins containing a mixed content of hydrophilic and hydrophobic amino acids are less hydrophobic than sorghum kafirins [12]. We speculated that sorghum proteins may also have similar functions as soy proteins used as adhesives, and therefore might provide better water resistance than soy protein when used as adhesive.

Grain sorghum is the third most important cereal crop in United States and the fifth in the world, and the United States is the number-one producer and exporter of sorghum [13]. About 10 million acres of sorghum are harvested and used mainly as animal feed in United States each year. Approximately 25% of the country's sorghum crop is used for ethanol production [14]. Distillers dried grains with solubles (DDGS) are a co-product of the distillation and dehydration process during ethanol production [15]. DDGS contains about 30–40% of protein and serves as an inexpensive source of protein. More than 700 million lb of sorghum protein would have been available from DDGS in 2009, when sorghum production was 500 million bushels in the United States [16].

Kafirins (prolamins) are the main components in sorghum protein, accounting for approximately 70–90% of the total storage protein [17]. Several methods have been investigated for extracting kafirins from sorghum flour or bran for food and other uses. Based on their solubility, kafirins can be extracted from sorghum or DDGS with alcohol [18–21], acetic acid [22], or alkaline sodium borate/SDS buffers [23–25]. Wang et al. [26] extracted sorghum protein from DDGS with NaOH-ethanol, acetic acid and HCl-ethanol, and found that using acetic acid and NaOH-ethanol as extraction solvents was more efficient for protein extraction than others tested. Building on this work, the objective of this study was to compare the adhesive performance of sorghum protein extracted using different methods and to characterize the physicochemical properties of the proteins including amino acid composition and rheological, thermal and morphological properties.

Materials and Methods

Materials

Sorghum DDGS with 14.4% moisture content (wet base) was provided by White Energy (Russell, KS). Decorticated sorghum grains were provided by USDA-ARS Center for Grain and Animal Health Research (Manhattan, KS). Sodium metabisulfite, sodium sulfite, glacial acetic acid, ethyl alcohol and petroleum ether were purchased from Fisher Scientific (Pittsburgh, PA). Absolute ethanol was purchased from Aaper Alcohol and Chemical Co. (Shelbyville, KY). Soy protein isolates (SPI) were provided by Bio-Materials and Technology Lab (Kansas State University, Manhattan, KS). Cherry wood samples with dimensions of 127 mm (length) × 50 mm (width) × 5 mm (thickness) were provided by Veneer One (Oceanside, NY).

Protein Extraction Using Acetic Acid

Acetic acid-extracted sorghum protein was prepared according to the method described by Taylor et al. [22]. Sorghum DDGS was milled with a cyclone sample mill (Udy Corp., Fort Collins, CO) into a powder with a particle size of < 0.5 mm then presoaked in 4 volumes of 0.5% (w/w) sodium metabisulfite for 16 h at 70 rpm agitation in a Gyromax 939 XL incubator shaker (Amerex Instruments, Inc., Lafayette, CA). After soaking, the samples were centrifuged (Thermo IEC, Needham Heights, MA) at $3{,}500\times g$ for 10 min and the supernatant discarded. Pellets remaining after centrifugation were then mixed with 5 volumes of glacial acetic acid and stirred for 1 h. The mixture was centrifuged at $3{,}500\times g$ for 10 min and the supernatant was decanted through a six-layer cheesecloth to remove the oil layer on top. The pH of the supernatant was slowly adjusted to 5.0 with 50% (w/v) NaOH in a beaker placed in an ice water bath. The mixture was kept overnight at 4 °C and then centrifuged at $3{,}500\times g$ for 10 min. The precipitates were rinsed and washed with distilled water by centrifuging at $3{,}500\times g$ for 10 min 3 times and then oven-dried at 49 °C. The protein was defatted 3 times by mixing with a fivefold weight of petroleum ether followed by shaking in the incubator shaker at 70 rpm for 5 min and centrifuging at $3{,}500\times g$ for 10 min, then kept under a fume hood overnight to evaporate the solvent.

Protein Extraction Using Ethanol

The ethanol extract method described by Emmambux and Taylor [27] was used. Milled sorghum DDGS was mixed with tenfold 70% ethanol, 0.35% NaOH (w/v) and 0.5% sodium metadisulfite (w/v). The mixture was placed in a water bath at 70 °C, stirred for 1 h, and then centrifuged at $3{,}500\times g$ for 10 min. The ethanol content in the supernatant was diluted to 40%, and the suspension was put in a freezer at −20 °C overnight. The suspension was centrifuged at $3{,}500\times g$ for 10 min, and the precipitates were rinsed and

washed 3 times with distilled water and oven-dried overnight at 49 °C. The product was defatted following the procedure described previously and milled into powder.

Chemical Analysis

Protein content was measured using nitrogen combustion via a LECO FP-2000 nitrogen determinator (St. Joseph, MI) according to AOAC method 990.03 [28]. Nitrogen was converted to protein using a factor of 6.25. Fat content was determined by using the Soxhlet petroleum-ether extraction method according to AOAC method 920.39C for cereal fat and expressed as the weight percentage on dry basis [29]. Crude fiber was determined according to AOCS-approved procedure Ba 6a-05 [30].

Amino Acid Composition Analysis

Samples were weighed and then placed in about 0.5 mL of 6 N HCl solution along with the internal standard and hydrolyzed at 110 °C for 20 h. An aliquot, typically 10 or 20 µl, was then diluted to 250 µl with 0.4 M borate buffer to dilute the sample and raise the pH. After precolumn derivatization with o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC), 1 µl of this diluent was injected into an HPLC system with a C18 column (Hypersil AA-ODS, 2.1 × 200 mm, 5 µm). Mobile phase A was 20 mM sodium acetate buffer with 0.018% (v/v) triethylamine, 0.05 mM EDTA, and 0.3% tetrahydrofuran, pH adjusted to 7.2 using acetic acid. Mobile phase B was 100 mM sodium acetate:acetonitrile: methanol (20:40:40, v/v). The elution conditions went from 100% A to 60% B in 17 min at 0.45 mL/min. Amino acid derivatives were detected with a fluorescent detector at 340/450 nm (excitation/emission) for primary amino acids and 266/305 nm for secondary amino acids. Human serum albumin was used as a control, and norvaline and sarcosine were used as internal standards.

Rheological Properties

Rheological measurements of sorghum protein suspension with different concentrations were performed by using a Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, MA) with a CP 4/40 cone and plate fixture (4° cone angle, 40-mm cone diameter). The distance between cone and plate was set to 150 µm for all measurements. Experiments were conducted under steady shear flow at 23 °C. Shear rates ranged from 10 to 240 s^{-1} at 10 s^{-1} increment. All experiments were done in duplicate, and average values were reported.

Morphological Properties

A model CM 100 transmission electron microscopy (FEI Company, Hillsboro, OR) was operated at 100 kV. Protein samples (3% in acetic acid, w/w) were absorbed for approximately 30 s at room temperature onto Formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Sciences, Fort Washington, PA) and stained with 2% (w/v) uranyl acetate (Ladd Research Industries, Inc., Burlington, VT) for 60 s at room temperature before being viewed by transmission electron microscopy (TEM).

Differential Scanning Calorimetry

Thermal transition properties of protein samples were measured with a TA Instruments DSC Q200 V24.4 instrument (TA Instruments, New Castle, DE) that was calibrated with indium and zinc before making official measurements. Samples of sorghum proteins weighing approximately 10–15 mg were measured in a hermetic aluminum pan under a nitrogen atmosphere with a gas flow rate of 50 mL/min. All samples were heated from 25 to 280 °C at a heating rate of 10 °C/min in an inert environment. All experiments were performed in duplicate.

Wood Specimen Preparation

Cherry wood samples were preconditioned in a controlled-environment chamber (Model 518, Electro-tech systems, Inc., Glenside, PA) for 7 days at 25 °C and 50% relative humidity (RH). Sorghum proteins that were suspended in acetic acid at different concentrations and stirred with a magnetic stirrer for 3 h were brushed separately along the edges of two pieces of cherry wood, with an application area of 127 mm × 20 mm, until the entire area was completely covered. The amount of adhesive applied on each piece was about 0.06 g, controlled using a pipette and consistent brushing procedure. The brushing and setting procedure followed the method described by Mo et al. [31]. The two pieces of suspension-brushed cherry wood were allowed to rest open at room temperature for 15 min, then were assembled and pressed at a pressure of 3.57 MPa at 130 °C, 150 °C or 170 °C for 10 min, respectively, using a hot press (Model 3890 Auto 'M', Carver Inc., Wabash, IN).

Mechanical Properties

After pressing, the glued-wood assemblies were conditioned at 23 °C and 50% RH for 2 days and then were cut into 5 127 mm (length) × 20 mm (width) × 5 mm (thickness) specimens. The cut specimens were again conditioned for another 5 days at 23 °C and 50% RH before the dry test. Three adhesion strengths were tested:

dry strength, soak strength and wet strength. Wood specimens for dry strength testing were prepared and tested using an Instron (Model 4465, Canton, MA) according to ASTM Standard Method D2339-98 [32]. Crosshead speed of Instron for adhesion strength testing was 1.6 m/min. Tensile strength at the maximum load was recorded as adhesion strength. Reported results are an average of five samples.

Water resistance was determined by measuring wet and soak strengths according to ASTM Standard Methods D1183-96 [33] and D1151-00 [34], respectively. Preconditioned specimens were soaked in tap water at 23 °C for 48 h then tested immediately for wet strength. For the soak strength test, specimens were soaked in tap water at 23 °C for 48 h then conditioned at 23 °C and 50% RH for another 7 days before testing.

Statistical Analysis

Except for mechanical property evaluation which took an average of 5 samples, all experiments were carried out in duplicate. Data were analyzed by using the analysis of variance (ANOVA) and least-significant difference (LSD) at the 0.05 level according to procedures in the SAS statistical software package (SAS Institute 2005, Cary, NC).

Results and Discussions

Chemical Composition of Sorghum Protein from DDGS and Sorghum Flour

Chemical composition of sorghum protein extracted under different methods is summarized in Table 1. The purity of ethanol-extracted protein (PII) (93.05%) was higher than that of the acetic acid-extracted sorghum protein from DDGS (PI) (87.77%). When using acetic acid as buffer to extract protein from DDGS, NaOH solution was used to adjust the pH value to 5 to precipitate the protein. During this step, sodium acetate salt could be formed, which may explain why PI had lower purity than PII if the formed sodium acetate salt was not washed out completely [35]. In addition, more protein was extracted from DDGS than directly from sorghum flour (PF) (71.82%). This might be due to strong association between protein and non-protein components in sorghum flour, such as carbohydrate, as was the case for sorghum protein extraction conducted by Wang et al. [26]. However, the protein purities were lower than the values reported by Wang et al. [26].

Table 1 Chemical composition of sorghum DDGS, sorghum flour, and sorghum proteins

Type of raw materials	Composition of extracted protein (%, dry basis)		
	Protein	Lipids	Crude fiber
DDGS	30.84	8.95	5.27
Sorghum flour	8.70	1.18	1.25
PI[1]	87.77b[4]	1.40b	0.79b
PII[2]	93.05a	2.60a	0.97a
PF[3]	71.82c	2.30a	0.84b

[1] PI = acetic acid-extracted sorghum protein from DDGS

[2] PII = ethanol-extracted sorghum protein from DDGS

[3] PF = acetic acid-extracted sorghum protein from sorghum flour

[4] Means in the same column followed by different letters are significantly different at $p < 0.05$

Amino Acid Composition

Results of amino acid analysis are shown in Table 2. Compared to the results in Table 1, the protein purities for the three proteins calculated based on the accumulation of total amino acids were much lower than the values obtained by the LECO FP-2000 nitrogen determinator (St. Joseph, MI) method. This could be attributed to the existence of non-protein nitrogen in DDGS or sorghum flour [17, 36], or the destruction of tryptophan and cysteine by the liquid HCl hydrolysis assay during the amino acid composition test. Because the total amount of amino acids did not include tryptophan and cysteine, estimates of total protein and concentration would be slightly lower than the true number shown in Table 2. This difference in protein could also be due to the nitrogen conversion factor: 6.25 is in approved methods, but some good data says the conversion factor for sorghum should be 5.8 [37].

Table 2 also shows that the molar concentration of glutamic acid, leucine and asparagine in DDGS was decreased by 6.89, 10.65, and 29.79%, respectively, compared to sorghum flour. However, isoleucine, lysine, methionine, tyrosine, arginine, threonine, glycine, and histidine in DDGS increased by a larger extent, from 10.65 to 152.92%, among which histidine, lysine, methionine and threonine are essential amino acids. The increase of essential amino acids during the fermentation process improved sorghum protein's nutritional value and could be partly due to the usage of yeast during fermentation [36, 38].

Different amino acid compositions were detected in the extracted sorghum kafirins compared with DDGS and sorghum flour. Increases in glutamic acid, alanine, tyrosine and leucine were observed in extracted kafirins while histidine, glycine, threonine, arginine, methionine and lysine decreased extensively compared with DDGS and sorghum flour. Similar results were also reported by Cookman and Glatz [38]. Four major classes of proteins in sorghum are the glutelins, kafirins, albumins and globulins [39], and each class has different amino acid profiles [36, 40, 41].

Table 2 Molar percentages of amino acids of different proteins

Amino acids (%)	PI[1]	PII[2]	PF[3]	DDGS	Sorghum	SPI[4]
Aspartic (ASX)	5.2b[5]	4.9b	4.4b	5.2b	5.6b	8.5a
Glutamine (GLX)	20.1a	18.4ab	17.7ab	11.7c	16.7b	16.2b
Serine (SER)	5.7b	6.2b	6.3ab	6.4ab	6.5ab	7.1a
Histidine (HIS)	1.2a	1.4ab	0.6ab	2.2a	1.4ab	2.0ab
Glycine (GLY)	2.3b	2.8b	2.1b	7.0a	6.1a	7.6a
Threonine (THR)	3.1c	3.0c	3.4bc	4.6a	3.7b	4.6a
Alanine (ALA)	16.6a	16.9a	16.9a	14.7b	14.6b	6.6c
Arginine (ARG)	1.5e	1.5e	2.3d	3.8b	2.9c	7.7a
Tyrosine (TYR)	3.4a	3.3a	3.5a	2.3b	0.9c	2.5b
Valine (VAL)	4.7c	4.8c	5.1b	5.7a	5.3b	5.0bc
Methionine (MET)	0.4d	0.5e	0.7d	1.4a	0.8c	1.2b
Phenylalanine (PHE)	4.9ab	4.9ab	5.1a	4.4c	4.4c	4.5bc
Isoleucine (ILE)	4.3bc	4.3bc	4.5b	4.5b	4.0c	4.9a
Leucine (LEU)	16.7a	16.8a	16.8a	13.4b	14.2b	8.4c
Lysine (LYS)	0.2de	0.0e	0.6d	3.0b	2.2c	6.9a
Proline (PRO)	9.7a	10.3a	10.0a	9.7a	10.8a	6.3b
T-AA (%, m/m)	100a	100a	100a	100a	100a	100a
T-protein (%, w/w)	67.2a	69.9a	52.1b	22.4c	6.20d	67.3a

[1] PI = acetic acid-extracted sorghum protein from DDGS

[2] PII = ethanol-extracted sorghum protein from DDGS

[3] PF = acetic acid-extracted sorghum protein from sorghum flour

[4] SPI = soy protein isolates

[5] Means in the same row followed by different letters are significantly different at $p < 0.05$

For instance, Yousif and Tinay reported that the albumin and globulin proteins of sorghum had higher levels of lysine compared to kafirins [36]. This is most likely why the isolated kafirins showed different amino acid content compared with sorghum flour and DDGS.

Amino acids can be classified into different groups according to their polarity, structure, nutritional requirements, metabolic fate, etc. Based on their hydrophobicity, amino acids can be grouped into hydrophobic (non-polar amino acids) and hydrophilic (polar amino acids) types [42]. Alanine, methinine, phenylananine, isoleucine, leucine and proline belong to hydrophobic amino acids, and they accounted for 57.32% to 59.04% in the three extracted sorghum protein kafirins (Table 3). These values were higher than the numbers reported by Mokrane et al. [43], who reported a range of hydrophobic amino acids from 45 to 50% in sorghum protein. This difference might be attributed to the different sorghum sources or the measurement methods. Amino acid compositions can affect the adhesive performance of protein-based adhesives [44–46], which will be discussed in detail in the next section.

Table 3 Hydrophilcity properties of amino acids in different proteins

Amino acids (% of total)	PI[1]	PII[2]	PF[3]	DDGS	Sorghum	SPI
Hydrophobic[4]	57.32a[5]	58.49a	59.04a	53.79a	54.1a	36.89b
Hydrophilic[6]	42.68b	41.51b	40.96b	46.21b	45.9b	63.11a

SPI soy protein isolates

[1] PI = acetic acid-extracted sorghum protein from DDGS

[2] PII = ethanol-extracted sorghum protein from DDGS

[3] PF = acetic acid-extracted sorghum protein from sorghum flour

[4] Hydrophobic amino acid = alanine, methinine, phenylalanine, isoleucine, leucine and proline

[5] Means in the same row followed by different letters are significantly different at $p < 0.05$

[6] Hydrophilic amino acid = lysine, tyrosine, arginine, threonine, glycine, histidine, serine, glutamine and asparagine

Rheological Properties

Rheological properties of extracted proteins are shown in Fig. 1. The maximum viscosity was 1 Pa·s, about 1,000× the viscosity of water, which means that the sorghum protein suspensions had good flowability properties. Apparent viscosity increased as protein concentration increased and decreased as shear rate increased, indicating that sorghum proteins in suspension showed shear thinning properties, which can be expressed by the Herscher-Bulkley model: $\tau = \tau_0 + K\dot{\gamma}^n$ where τ_0 is the yield stress (N/m^2), τ is the shear stress (N/m^2), $\dot{\gamma}$ is the shear rate (s^{-1}), and n and K are the flow behavior index and the consistency index, respectively. The method of least squares was used to find the best-fitting equation: Estimate a $\tau_0(=\tau_{01})$ by extrapolating the plot τ versus $\dot{\gamma}$, plotting ln τ versus ln $\dot{\gamma}$, and getting K_1 and n_1 from linear regression by using Microsoft Excel. Then, K_1 and n_1 are put back into the equation, and ln τ versus ln $\dot{\gamma}^n$ is plotted to get τ_{02} and K_2 from linear regression. Finally, τ_{01} and τ_{02} are compared until $\tau_{01} = \tau_{02}$, then $\tau_0(=\tau_{01})$, $K(=K_1 = K_2)$, and n $(=n_1)$ are obtained. The values of τ_0, n, and K are summarized in Table 4.

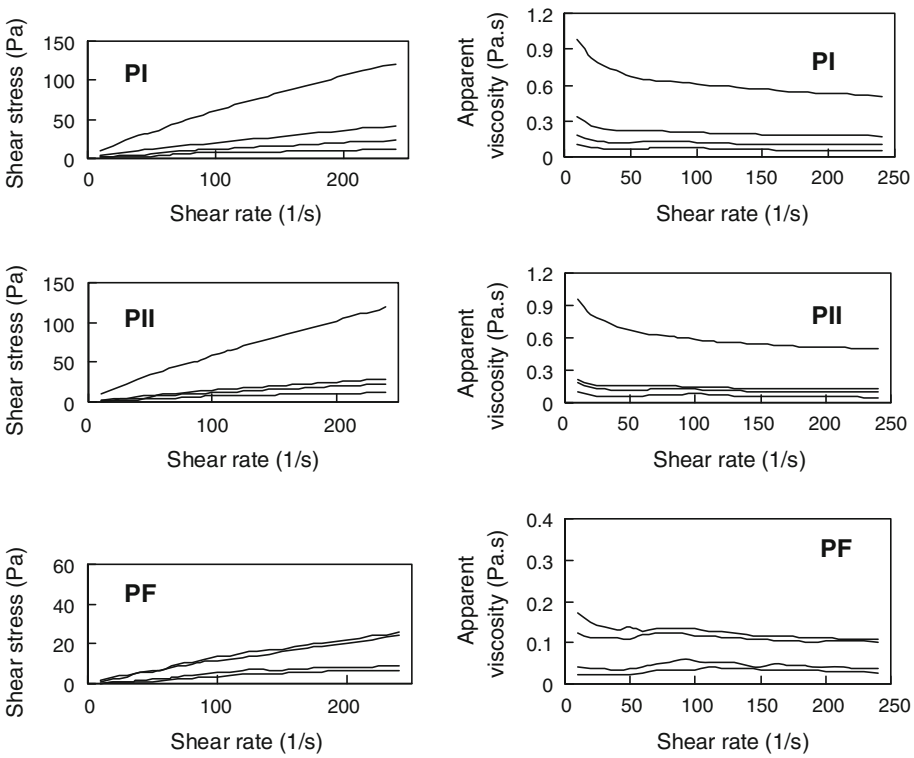

Fig. 1 Shear behavior of sorghum proteins. Curves from *bottom* to *top* are sorghum adhesives with 8, 10, 12, and 16% protein concentrations, respectively. *PI* acetic acid-extracted sorghum protein from DDGS, *PII* ethanol-extracted sorghum protein from DDGS, and *PF* acetic acid-extracted sorghum protein from sorghum flour

Table 4 Rheological parameters of sorghum proteins with different concentrations: yield stress (τ_0, N/m^2), flow behavior index (n), consistency index (K)

Rheological parameters	PI[1]				PII[2]				PF[3]			
	8%	10%	12%	16%	8%	10%	12%	16%	8%	10%	12%	16%
τ_0	0.20	0.10	0.10	0.20	0	0	0	0	0.01	0.10	0.40	4.00
n	0.55	0.49	0.60	0.61	0.63	0.53	0.69	0.64	0.49	0.57	0.61	0.83
K	0.38	0.65	0.96	0.87	0.08	0.09	0.27	0.25	0.83	0.96	0.96	1.21

[1] PI = acetic acid-extracted sorghum protein from DDGS

[2] PII = ethanol-extracted sorghum protein from DDGS

[3] PF = acetic acid-extracted sorghum protein from sorghum flour

Morphological Properties

Microstructures of protein dispersions are displayed in Fig. 2. The protein particles in suspension were evenly distributed and PI and PF had larger particles than PII. The configuration of PI might be different from PII. PI might have more hydrophobic amino acids aligned at the interface than PII, and more hydrophobic amino acids in PII may have been wrapped inside the particle. Some small particles surrounded the protein particle of the PF. Carbohydrate, including starch, was not fermented and remained in the sorghum flour. The strong association between protein and non-protein could have resulted in some non-protein residue left in the protein extract as indicated by the small particles present in the protein suspension made from sorghum flour protein.

DSC Thermal Transition Properties

As shown in Fig. 3, the DSC thermogram of PF exhibited large differences compared to the curves for PI and PII. A sharp and strong endothermic peak at about 57.9 °C was observed for PF, while only trivial endothermic peaks at around 63.8 and 62.5 °C were detected for PI and PII, respectively. The endothermic peak shown around this temperature range has been firmly related to starch gelatinization [47–49]. The strong signal at 57.9 °C observed in PF indicated more starch in the PF whereas PI and PII have less starch content as evidenced by the tiny peaks; these results also can be reflected by the lower protein purity of PF showed in Table 1. PF also exhibited another endothermic peak with the temperature of 110.5 °C, which probably represented protein denaturization. No peaks

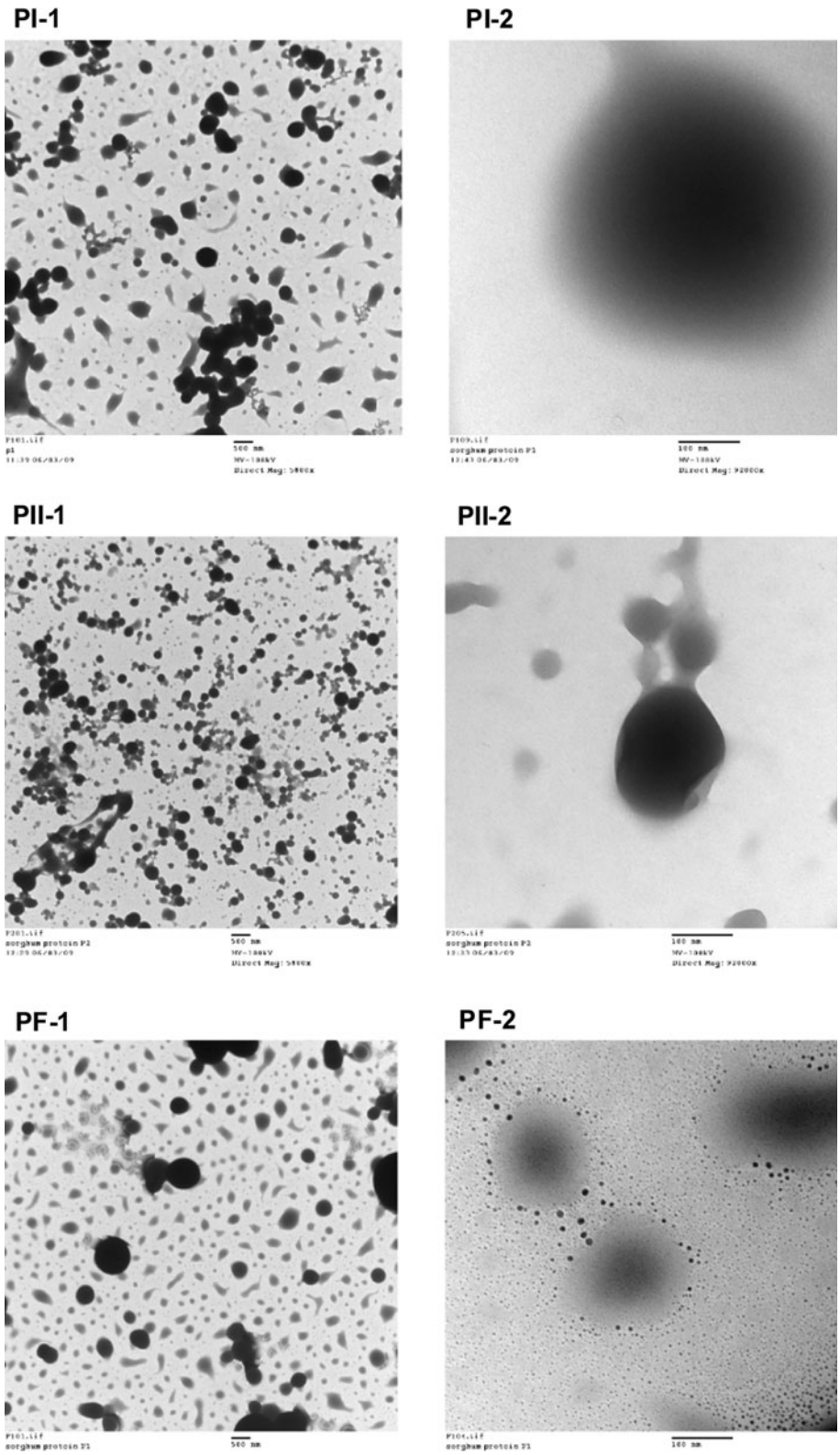

Fig. 2 TEM micrographs of sorghum proteins at 3% solid content. Magnification: PI-1-PF-1, ×5800; PI-2-PF-2, ×92 k. Scale bar represent 500 and 100 nm for ×5,800 and ×92 k magnifications, respectively. *PI* acetic acid-extracted sorghum protein from DDGS, *PII* ethanol-extracted sorghum protein from DDGS, and *PF* acetic acid-extracted sorghum protein from sorghum flour

around 110.5 °C were observed in PI and PII, indicating that protein in sorghum DDGS already had been denatured during the fermentation process. The results also confirmed the previous results reported by Zhao et al. [23] and Wang et al. [26], who suggested that protein cross-linkage and denaturation occurred during fermentation. The strong exothermic peaks that occurred with the onset temperature around 176.3, 184.8, and 164.1 °C for PI, PII, and PF,

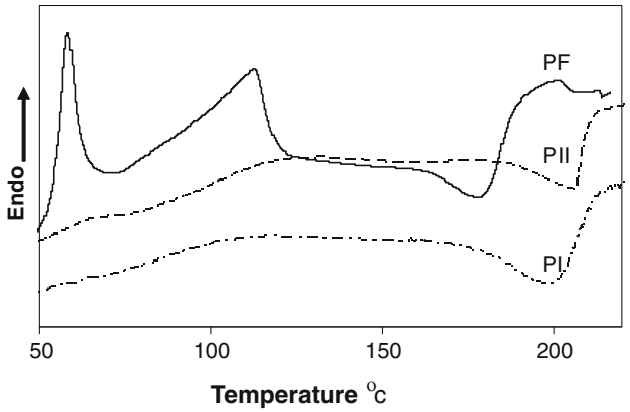

Fig. 3 Thermograms of the sorghum proteins; *PI* acetic acid-extracted sorghum protein from DDGS, *PII* ethanol-extracted sorghum protein from DDGS, and *PF* acetic acid-extracted sorghum protein from sorghum flour

respectively, might be attributed to protein aggregation, as suggested by Mo et al. [31]. Protein aggregation might have a negative effect on adhesion performance when considering that mechanical strength decreased significantly at the assembling temperature of 170 °C compared to 150 and 130 °C for PI and PF (Table 5). During protein aggregation, the exposed functional groups in sorghum protein-based adhesives that bonded with wood surface probably refolded again, resulting in the weak bonding capacity between adhesives and wood surface. However, the aggregation properties of sorghum protein need to be further studied to determine this conclusively.

Mechanical Properties of Sorghum Protein

Sorghum protein concentration, pressing temperature, extraction methods and protein sources all had significant effects on the adhesion properties of sorghum protein (Table 5). In general, wood failure happened to almost all of the dry and soaked samples, which indicated that the bonding strengths between cheery wood and sorghum adhesives were stronger than the mechanical strength of cheer wood when to resist the shear force. However, not wood failure, but adhesives failure was observed for wet samples. The adhesion strength of sorghum protein increased as the sorghum protein concentration increased and virtually leveled off when protein concentration increased to 16%. Increasing pressing temperature from 130 to 150 °C had a positive effect on protein adhesion strength, while increasing temperature to 170 °C had different effects on the adhesion properties of the three different types of sorghum protein isolates. PI and PF with 12% concentration exhibited a decrease in wet strength to 1.92 and 1.19 MPa from 3.15 and 2.59 MPa, respectively, as pressing temperature increased from 150 to 170 °C. However, for PII with a concentration of 12%, the wet strength increased to 2.51 from 2.17 MPa as pressing temperature increased to 170 °C from 150 °C. PI had better

Table 5 Adhesion performance of sorghum protein adhesives

Extraction methods	Wet strength (MPa)			Dry strength (MPa)			Soaked strength (MPa)		
	130 °C	150 °C	170 °C	130 °C	150 °C	170 °C	130 °C	150 °C	170 °C
8% protein content									
PI[1]	1.21 ± 0.15	1.71 ± 0.08	1.23 ± 0.22	4.82 ± 0.43	4.85 ± 0.42	5.52 ± 0.23	4.07 ± 0.16	4.70 ± 0.32	4.51 ± 0.08
PII[2]	1.22 ± 0.10	1.27 ± 0.07	1.81 ± 0.10	4.07 ± 0.32	4.01 ± 0.39	4.07 ± 0.36	3.63 ± 0.28	3.73 ± 0.17	3.91 ± 0.17
PF[3]	1.71 ± 0.26	1.67 ± 0.09	1.44 ± 0.08	4.69 ± 0.28	4.52 ± 0.13	4.51 ± 0.25	4.08 ± 0.29	4.22 ± 0.11	4.18 ± 0.16
10% protein content									
PI[1]	1.89 ± 0.09	2.32 ± 0.16	1.44 ± 0.08	5.05 ± 0.27	5.49 ± 0.30	5.25 ± 0.19	4.93 ± 0.18	4.90 ± 0.21	4.32 ± 0.08
PII[2]	1.32 ± 0.14	1.70 ± 0.15	1.85 ± 0.18	5.18 ± 0.07	4.62 ± 0.08	4.85 ± 0.19	4.79 ± 0.08	4.54 ± 0.32	4.61 ± 0.26
PF[3]	1.63 ± 0.14	1.88 ± 0.13	1.19 ± 0.07	4.68 ± 0.21	4.42 ± 0.29	4.17 ± 0.10	4.14 ± 0.05	4.32 ± 0.21	4.06 ± 0.10
12% protein content									
PI[1]	2.89 ± 0.18	3.15 ± 0.11	1.92 ± 0.10	5.08 ± 0.26	5.05 ± 0.17	4.78 ± 0.39	4.26 ± 0.13	4.42 ± 0.26	4.92 ± 0.48
PII[2]	1.61 ± 0.10	2.17 ± 0.04	2.51 ± 0.13	4.95 ± 0.29	5.02 ± 0.14	4.98 ± 0.13	4.23 ± 0.15	4.33 ± 0.35	4.61 ± 0.28
PF[3]	2.23 ± 0.13	2.59 ± 0.07	1.19 ± 0.14	4.70 ± 0.25	4.52 ± 0.16	4.49 ± 0.17	4.43 ± 0.12	4.35 ± 0.12	4.35 ± 0.06
16% protein content									
PI[1]	3.01 ± 0.10	3.29 ± 0.19	1.32 ± 0.10	5.16 ± 0.20	5.08 ± 0.30	4.85 ± 0.30	4.95 ± 0.17	4.71 ± 0.29	4.50 ± 0.14
PII[2]	1.49 ± 0.10	1.92 ± 0.09	2.42 ± 0.14	4.95 ± 0.19	5.13 ± 0.13	4.85 ± 0.10	4.90 ± 0.08	5.10 ± 0.15	5.44 ± 0.17
PF[3]	2.36 ± 0.22	2.45 ± 0.10	1.13 ± 0.04	5.00 ± 0.14	5.23 ± 0.08	4.36 ± 0.12	4.90 ± 0.20	4.89 ± 0.27	4.22 ± 0.24

[1] PI = acetic acid-extracted sorghum protein from DDGS

[2] PII = ethanol-extracted sorghum protein from DDGS

[3] PF = acetic acid-extracted sorghum protein from sorghum flour

adhesion performance in terms of wet, dry and soak strength than that of the PII and PF, especially for the wet strength. For example, the wet strength for PI was 3.15 MPa at a pressing temperature of 150 °C and a concentration of 12%, compared with 2.17 MPa for PII and 2.59 MPa for PF.

Adhesion between proteins and wood surfaces occurs as the protein spreads, wets and penetrates the porous wood structure to achieve mechanical interlocking, physical attraction and chemical bonding between wood and protein during the setting period, followed by entanglements and cross-link through physical attraction and chemical bonding between protein and wood and between protein and protein during thermal setting procedure [50]. With increasing protein concentration, more protein is available to bond with wood and deliver greater strength. However, as protein concentration increases beyond the optimum levels (>16%), protein–protein interaction could dominate the protein-wood interaction, resulting in uneven distribution of the proteins or insufficient exposure of hydrophobic groups at the wood surface. As a result, limited contribution to adhesion performance improvement was observed when protein concentration reached 16%. Increasing press temperature had marked effects on solvent evaporation, immobilization of protein molecules and possibility of chemical and physical interaction between proteins and the wood surface. On the other hand, when higher temperatures were applied to adhesives with high protein concentrations, more hydrophobic amino acids were available for hydrophobic interaction, which could lead to greater protein cohesion but could be detrimental to the bonding ability between protein and wood [5].

Lower protein purity (Fig. 3, PF) and the small non-protein particles surrounding the PF (Fig. 2, PF-2) extracted from sorghum flour could be why its adhesive strengths were lower than PI extracted from DDGS. The smaller particles of PII would make contact surface area between protein and protein not as great as the other two. Furthermore, as explained in the earlier section on morphological properties, PI might have more hydrophobic amino acids aligned at the interface than PII whereas more hydrophobic amino acids in PII may have been wrapped inside the particle, which could explain the better water resistance of PI.

Comparison of Sorghum Protein and Soy Protein Isolates (SPI) Adhesives

To date, soy protein-based adhesives have been considered the most promising bio-based adhesives, as they are thought to be partially capable of replacing petroleum-based adhesives because of their excellent adhesion performance on wood and other materials as reported by Wool and Sun [51]. Table 6 displays the comparison of adhesion performance between soy protein isolates (SPI) and different sorghum kafirin proteins (PI, PII, PF) at a concentration of 12%. Sorghum protein showed excellent water resistance, which has been the biggest challenge for soy protein adhesives. For instance, the wet strength of PI adhesive (2.89, 3.15 and 1.92 MPa) was much higher than those of unmodified soy protein adhesives (1.61, 1.63 and 1.98 MPa) under pressing temperatures of 130, 150 and 170 °C, respectively.

Table 6 Comparison of adhesion performance of sorghum protein adhesives and soy protein isolates at 12% protein concentration

Hot press condition	Sorghum protein (MPa)			SPI (MPa)
	PI[1]	PII[2]	PF[3]	
Wet strength				
130 °C	2.89 ± 0.18	1.61 ± 0.10	2.23 ± 0.13	1.61
150 °C	3.15 ± 0.11	2.17 ± 0.04	2.59 ± 0.07	1.63
170 °C	1.92 ± 0.10	2.51 ± 0.13	1.19 ± 0.14	1.98
Dry strength				
130 °C	5.08 ± 0.26	4.95 ± 0.29	4.70 ± 0.25	4.55
150 °C	5.05 ± 0.17	5.02 ± 0.14	4.52 ± 0.16	5.29
170 °C	4.78 ± 0.39	4.98 ± 0.13	4.49 ± 0.17	4.88
Soaked strength				
130 °C	4.26 ± 0.13	4.23 ± 0.15	4.43 ± 0.12	4.17
150 °C	4.42 ± 0.26	4.33 ± 0.35	4.35 ± 0.12	4.35
170 °C	4.92 ± 0.48	4.61 ± 0.28	4.35 ± 0.06	4.42

SPI soy protein isolates

[1] PI = acetic acid-extracted sorghum protein from DDGS

[2] PII = ethanol-extracted sorghum protein from DDGS

[3] PF = acetic acid-extracted sorghum protein from sorghum flour

Amino acid composition and the overall hydrophobicity of proteins are known to be essential factors affecting protein adhesive performance. As shown in Tables 2 and 3, large differences in amino acid composition between sorghum proteins and soy protein were observed. In terms of total hydrophobic amino acids, sorghum protein was much higher (about 58 mol %) than soy protein (~37 mol %). Hydrophobic protein adhesives repelled water when the assembled cherry wood samples were soaked, ensuring the interaction between the wood boards and the boundaries formed by the protein adhesives and wood surfaces remained intact. On the other hand, hydrophilic protein adhesives may absorb water when applied on cherry wood board, and this behavior could destroy the cohesion between adhesives and wood surface. Furthermore, cross-linking among kafirins may offer another reason why sorghum protein adhesives had better water resistance than soy protein. Kafirins are known to cross-link significantly when heated [52, 53] and may have done so when the adhesives were heated.

Another advantage of sorghum protein used as adhesive compared to soy protein is that sorghum protein adhesives need less energy than soy protein adhesive when assembling the wood board using a hot press method. Results have shown that 150 °C for sorghum protein was the optimum temperature whereas soy proteins typically need 170 °C or higher to achieve maximum strength (Table 5).

However, some challenges for the use of sorghum protein isolates as adhesives are notable. First, finding a low-cost solvent to dissolve the isolated sorghum kafirins for use as an adhesive was difficult; in contrast, uniform suspension of soy proteins can be obtained by mixing soy protein with water. In addition, the lower efficiency of sorghum protein recovery and complicated extraction procedures compared with soy protein are a concern. Further research is needed to improve the extraction of sorghum proteins.

Conclusions

Sorghum protein extracted from sorghum DDGS and sorghum flour with different methods had different adhesion performances. Results showed that PI had the best adhesion performance, followed by PF and PII, especially for wet strength. The wet strength of PI at a 12% concentration assembled at 150 °C was 3.15 MPa, compared to 2.17 MPa for PII and 2.59 MPa for PF. Low protein purity caused by non-protein materials of PF might be the main reason for its lower adhesion strength than PI. In addition, PI might have more hydrophobic amino acids aligned at the interface than PII, which could explain the better water resistance of PI. The optimum sorghum protein concentration and pressing temperature for maximum adhesion strength is 12% and 150 °C. Compared with soy protein-based adhesives, PI had advantages such as significantly higher water resistance and lower energy input. These results indicate that sorghum protein displays huge potential as an alternative to petroleum-based adhesives.

Acknowledgments The authors extend their gratitude to Dr. Dan Bolye for TEM tests. The authors also thank Dave Trumble for carrying out the chemical analysis. This article has a contribution No. 11-203-J from the Kansas Agricultural Experimental Station, Manhattan, KS 66506, USA.

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