Green Resin from Forestry Waste Residue "Karanja (Pongamia pinnata) Seed Cake" for Biobased Composite Structures
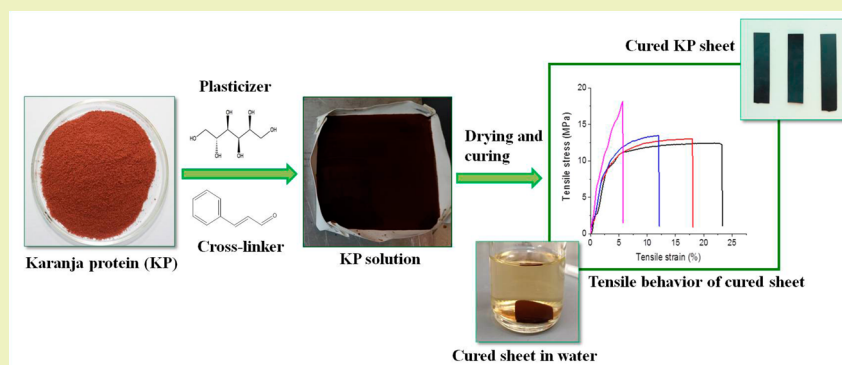
Muhammad M. Rahman and Anil N. Netravali*

Department of Fiber Science and Apparel Design, Cornell University, 37 Forest Home Drive, Ithaca, New York 14850, United States

ABSTRACT: A nonedible protein was extracted from defatted karanja (Pongamia pinnata) seedcake, so far considered as forestry waste residue after oil extraction. High average molecular weight and availability of reactive amino groups of karanja protein (KP), which can be cross-linked, provide the basis to form strong polymeric material. Green thermoset resin was developed from KP using a natural α,β-unsaturated aldehyde "cinnamaldehyde". In addition, a natural plasticizer (sorbitol) was used to reduce the brittleness of the resin. Mechanical and physical properties were characterized by solution casting resin films. The cross-linking and plasticizer content were optimized to get the best overall performance. A combination of 5% plasticizer content and 12% cross-linking resulted in best mechanical properties. The modified KP resin provides a green, sustainable, and biobased alternative to not only the petroleum-based resins but also to the edible protein-based resins such as soy protein. The KP resin would be inexpensive and useful to fabricate biobased green composite structures.

KEYWORDS: Bioresin, Plasticization, Cross-linking, Protein film, Pongamia pinnata (Karanja)

INTRODUCTION

Ecological awareness and economic concerns have created strong interest in developing environments friendly and biodegradable green materials from low cost or free natural resources as substitutes for nonrenewable petroleum-based materials. Much research has been done to utilize agricultural raw materials for the production of biodegradable systems.[1−7] Plant proteins, being natural polymers, constitute a viable source of the biodegradable system through the formation of numerous intermolecular bonds due to the presence of polar amino acids and, hence, can offer a wide range of potential functional properties as a biobased resin for composite structures.[8−14]

Until now, research has been focused on proteins derived from edible oilseeds such as soybean, peanut, wheat, etc. to produce resins commercially.[15−19] Because these proteins are edible for humans as well as animals, it would be preferable to utilize nonedible proteins instead. Proteins from oil-rich seeds such as karanja (Pongamia pinnata) and jatropha (Jatropha curcus) are nonedible due to the presence of toxic and antinutritional compounds. As a result, they do not compete with the edible protein sources and can be used for applications in nonfood packaging as well as adhesives and resins in composites. In the recent past years, karanja seed has been utilized as a source of biodiesel production.[20,21] After oil extraction from the seeds, protein-rich defatted karanja seed cakes remain as waste residue that have very low value applications such as organic nitrogenous fertilizers or fuel sources.[22] The defatted karanja cake might have additional value if it could be applied in the nonfood applications as a green resin for biobased composite structures. One of the worries of using edible proteins, such as soy as resin in green composites, is that it may get affected through microbial activity or even eaten by insects during use. This makes it necessary to add antimicrobial compounds to the resin. In the case of karanja, however, the presence of toxic compounds may altogether eliminate the need for antimicrobials and antifungi compounds.[23]

Karanja (Pongamia pinnata) is one of the widely grown forest trees in south Asia. About 200 million tons of seeds are collected every year in India.[21] The seed contains 33−36% oil and 20−30% protein.[24] Karanjin, pongamal, and an unusual

amino acid, glabrin, are the toxic and antinutritional chemicals present in the seed that restrict its use as animal feed.[22] As a result, after oil extractions, this nonedible defatted seedcake becomes essentially free in South Asia regions. While the functional properties of soy and other edible proteins have been explored extensively, sheet forming abilities and properties using nonedible karanja protein (KP) from defatted karanja seed cake (KSC) have not been investigated yet.

The present study is focused on the potential of KP for development of green resin with acceptable properties for future applications in composite structures. It is well established that a major additive for protein-based sheet formation is plasticizer as the sheet becomes very brittle without it.[25] However, plasticizers decrease the mechanical stiffness by reducing intermolecular forces and increasing the mobility of polymeric chains by increasing the free volume of the system.[25]

Modifications of protein by chemical cross-linking provide an effective means to improve the functional performances of the plasticized sheets.[26] So far, synthesized aldehydes such as glyoxal, gluteraldehyde, and formaldehyde have been most commonly used as cross-linkers for protein-based sheets.[27] However, the inherent toxicity of these compounds restricts their use in biobased green applications. Recently, a naturally occurring low cost aromatic aldehyde "cinnamaldehyde" derived from the bark of cinnamon has been used to cross-link wheat proteins.[26,28] In addition, it acts as an antimicrobial agent that may be needed for structural applications.[29] Cinnamaldehyde, together with karanjin, pongamal, and glabrin can provide excellent protection from microbes and insects and, thus, increase the durability and life of the protein-based composites.

This study investigates mechanical, thermal, and physico-chemical properties of KP sheets. The effects of an increasing ratio of a natural polyol-based plasticizer "sorbitol" and natural cross-linker "cinnamaldehyde" on the properties of KP resin have been extensively investigated. Overall, the aim of the study was to explore a fully natural and biodegradable protein-based resin from an agricultural waste residue of nonedible seed for sustainable green composites and other applications.

■ EXPERIMENTAL PROCEDURE

Materials. Defatted KSC was purchased from The Ahimsa Alternative, Inc., Bloomington, MN. Analytical grade sodium hydroxide (NaOH) pellets, hydrochloric acid (37%), reagent grade sorbitol (≥98% purity), and cinnamaldehyde (≥93% purity) were purchased from Sigma-Aldrich Chemical Co., Allentown, PA. Tris-HCl, sodium dodecyl sulfate (SDS), dithiothreitol (DTT), NuPAGE 4X LDS loading buffer, NuPage Novex 10% Bis-Tris precast gels, NuPAGE MOPS SDS running buffer, and Invitrogen colloidal coomassie blue, required for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), were purchased from Life Technologies, Grand Island, NY.

Extraction of KP. KP was obtained by alkaline extraction and acid precipitation of defatted KSC.[30] Defatted KSC was ground into powder using a blender and passed through a 300 μm mesh sieve to obtain homogeneous microparticles. Sieved powder was then dispersed in deionized (DI) water at a ratio of 1:10 (w/v) and adjusted to pH 10.5 ± 0.2 using 1 M NaOH. The mixture was stirred at 350 rpm and 75 °C for 60 min. Then, the mixture was centrifuged at 3000g for 20 min at room temperature, and the supernatant was collected by discarding insoluble residues. The pH of the supernatant was adjusted to

4.5 ± 0.2 with 1 M HCl and stirred for 20 min at 350 rpm at room temperature to allow isoelectric precipitation. The suspension was centrifuged again at 3000g for 20 min. The resulting brown suspension, referred to as KP, was separated, freeze-dried, ground, and stored at room temperature before characterizing their properties.

Preparation of KP Resin Sheets. KP sheets were prepared by using a modification of the method carried out for soy protein sheet formation in our laboratory.[8,11,13,14] At first, KP was mixed with DI water at a ratio of 1:10 (w/v) by a magnetic stirrer at 350 rpm for 15 min. After homogenization of mixtures, the pH of the solution was adjusted to 10.5 ± 0.2 using a 1 M NaOH solution and was stirred further for 30 min at 80 °C. The solution was dried on a Teflon-coated glass plate for 20 h in an air-circulating oven maintained at 35 °C after which the oven temperature was further raised to 45 °C for the next 4 h. Finally, the dried KP sheets were cured using a Carver Hydraulic hot press at 130 °C for 25 min under a pressure of 2.5 MPa. Sheets made of pure protein itself were too brittle to handle. A predetermined amount of sorbitol was added to the KP solution prior to casting, as plasticizer, to overcome the brittleness. The plasticizer content was varied to obtain the best properties of the KP resin sheets. A similar processing technique was used for plasticized KP sheets, as described above. Cross-linked KP sheets were obtained by adding cinnamaldehyde to plasticized KP solutions in different proportions to study the effect of cross-linking and to obtain the best properties of KP resin sheets. The predetermined amount of cross-linker was added to the plasticized solutions after 20 min, and stirring was continued at 350 rpm and 80 °C for 10 min. The same drying and curing cycle was followed in each category as described above. All KP sheets were conditioned at 21 °C and 65% RH for 24 h before doing the tests.

Characterization of KP. Crude protein, crude fat, starch, water, ethanol soluble carbohydrate, simple sugars, crude fibers, ash, and moisture contents of defatted KSC and KP were determined according to standard Association of Analytical Communities (AOAC) methods. The protein content was obtained by a Leco FP-528 nitrogen/protein analyzer using a conversion factor of 6.25.[31] All analyses were conducted by Dairy One, Ithaca, NY, in triplicate to confirm reproducibility.

Molecular weight distributions of KP were determined by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE).[32] KP was first solubilized in 100 mM tris−HCl (pH 8.0) and 1.0% SDS. Ten microliters of protein−buffer mixtures (0.5 μL solubilized protein, 6 μL H$_2$O, 1 μL DTT, 2.5 μL NuPAGE 4X LDS loading buffer) were injected onto each lane in NuPAGE Novex 10% Bis-Tris precast gels (1 mm thick with 10 lanes) with a NuPAGE MOPS SDS running buffer. Electrophoresis was carried out for about 50 min at a 200 V constant. After electrophoresis, protein-containing gel was stained with Invitrogen Colloidal Coomassie Blue overnight, destained with water for 3 h, and photographed. Densitometric quantification of molecular bands obtained from SDS-PAGE was determined using ImageJ software.

The amino acid composition analysis of KP was conducted according to AOAC Official Method 982.30 E by high performance liquid chromatography (HPLC).

Characterization of KP Resin Sheets. The thicknesses of the KP sheets were measured to the nearest 0.001 mm with a hand-held Digimatic micrometer. Five thickness measurements were taken for each specimen, one at the center and four

2319

dx.doi.org/10.1021/sc500095r | ACS Sustainable Chem. Eng. 2014, 2, 2318−2328

Table 1. Proximate Composition of Defatted Karanja Seed Cake (KSC) and Extracted Karanja Protein (KP)

constituent	defatted KSC	extracted KP	determination method
moisture (%)	7.9 ± 1.6	5.9 ± 0.3	AOAC 930.15
crude protein (%)	26.6 ± 2.6	60.0 ± 1.6	AOAC 992.23
crude fat (%)	13.4 ± 2.4	6.9 ± 1.7	AOAC 2003.05
starch (%)	12.4 ± 0.8	0.5 ± 0.2	YSI 2700 SELECT analyzer
water-soluble carbohydrate (%)	13.4 ± 1.2	3.8 ± 0.9	UV spectrophotometry
simple sugars (%)	9.8 ± 1.9	5.6 ± 2.2	UV spectrophotometry
crude fiber (%)	3.1 ± 0.5	−	AOAC 962.09
ash (%)	3.8 ± 0.7	4.6 ± 0.9	AOAC 942.05

around the perimeter, and the mean was used for assessing mechanical properties.

Color parameters of the sheets were measured using a Macbeth Color-eye spectrophotometer. Sheet specimens were placed on a white plate, and the CIELAB color scale was used to measure color in terms of whiteness index (WI). WI of the KP sheets was calculated using eq 1

$$\text{WI} = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2} \qquad (1)$$

where $L^* = 0$ (black) to $L^* = 100$ (white), $-a^*$ (greenness) to $+a^*$ (redness), and $-b^*$ (blueness) to $+b^*$ (yellowness). Standard values for the white calibration plate were $L^* = 95.99$, $a^* = -0.04$, and $b^* = 1.01$. Values were expressed as means of 10 measurements on different areas of each film.

The moisture contents (MC) of the KP sheets were measured according to a method described by Rhim et al.[33] The specimens were weighed (W_1), subsequently dried in an air-circulating oven at 105 °C for 24 h, and reweighed (W_2) to determine MC values. Four replicates were carried out for each specimen composition. MC values were calculated using eq 2

$$\text{MC } (\%) = \left(1 - \frac{W_2}{W_1}\right)100 \qquad (2)$$

Mechanical properties of the KP resin sheets were determined according to ASTM D 880-02 with an Instron universal tensile testing machine. Young's modulus, tensile strength, and fracture strain were measured from the test. Sheets with dimensions of 10 mm × 60 mm were tested at a strain rate of 1 min^{-1} and a gauge length of 30 mm after conditioning. A minimum of 10 specimens were tested for each composition.

Thermal degradation behaviors of the resin sheets were investigated using thermogravimetric analysis, TGA (TGA-2050, TA Instruments, Inc., DE). Specimens weighing approximately 6 mg were scanned from 30 to 600 °C at a heating rate of 10 °C/min under a flow of 60 mL/min nitrogen gas. The onset degradation temperatures of the KP sheets were determined from this analysis using Universal analysis software (TA Instruments). Three specimens from each composition were investigated to ensure reproducibility.

Differential scanning calorimetry (DSC) was performed using DSC 2920 calorimeter (TA Instruments, Inc., DE) to determine the glass transition temperatures of the resin sheets containing various amounts of plasticizer. Specimens (approximately 5 mg) were put individually in hermetically sealed T-zero aluminum pans to prevent any mass loss. DSC tests were performed by heating specimens from 30 to 110 °C at a rate of 10 °C/min, holding at that temperature for 1 min, and then cooling to 30 °C at a cooling rate of 20 °C/min (first scan) before carrying out the second heating scan to 180 °C at a

heating rate of 10 °C/min. The first heating scan was performed to erase the thermal history, if any, of the specimens. The glass transition temperatures (T_g) of the KP sheets were determined from the second heating scans using Universal analysis software. The T_g values of KP sheets were considered to be the midpoint temperatures of the shift in the baseline from the discontinuity of specific heat. Three specimens from each composition were investigated to ensure repeatability.

Attenuated total reflectance−Fourier transform infrared (ATR-FTIR) spectra of the resin sheets were collected using a Nicolet Magna 560 FTIR spectrometer with a split pea accessory. All spectra were obtained with an average of 64 scans recorded from 4000 to 800 cm^{-1} wavenumbers at a resolution of 4 cm^{-1}.

Water solubilities (WS) of the KP sheets were measured according to a method described by Cuq et al.[34] with some minor modifications. The specimens were dried in an air-circulating oven at 105 °C for 24 h and weighed (W_2). After weighing, specimens were immersed in 30 mL of DI water in a beaker for 24 h at 25 °C and occasionally stirred. Beakers were covered with Parafilm "M" wrap to avoid evaporation of volatile materials. Then, specimens were dried in an air-circulating oven at 105 °C for 24 h and weighed (W_3). WS values were calculated using eq 3

$$\text{WS } (\%) = \left(1 - \frac{W_3}{W_2}\right)100 \qquad (3)$$

Four replicates of the analysis were carried out for each specimen composition.

Also, the KP sheets were immersed in 50 mL DI water in a glass bottle and placed on a platform shaker (Innova 2300, New Brunswick Scientific Inc., New Brunswick, NJ) at 80 °C and 150 rpm for 5 and 10 h. Photographic images were taken after shaking for 5 and 10 h.

Statistical evaluations were carried out by analysis of variance (ANOVA) followed by multiple comparison tests using the Tukey−Kramer's honest significant difference (HSD) at 95% confidence level. All of the analyses were performed using JMP statistical software (SAS Institute, NC).

■ RESULTS AND DISCUSSION

Proximate Composition of Defatted KSC and KP. Proximate composition of the defatted KSC and extracted KP is shown in Table 1 along with the determination methods used for obtaining the constituents. As shown in Table 1, the protein content in defatted KSC was about 27%. A very high fat content of over 13% was obtained for the seed cake. Fat content mainly depends on the oil extraction method or expeller efficiency. Usually, solvent extraction is a much more efficient method compared to expel or screw-pressing to extract

fat from seedcake.[35] KP, after lab processing, had about 60% protein, and the protein extraction yield was 0.17 ± 0.02 g KP/g defatted KSC. Protein recovery and content are not only dependent on the processing parameters during extraction but are also affected by the oil extraction method prior to protein extraction.[36] High temperature and organic solvents used during oil extraction may cause protein denaturation that reduces the protein solubility in solvents and, hence, total extractability of the protein.[35] Protein solubility is also affected by the composition of the amino acids of protein that is related to surface hydrophobic and hydrophilic interactions with water.[37]

Amino Acid Composition of KP. Table 2 shows amino acid composition of the KP. As shown in Table 2, about 41% of

Table 2. Amino Acid Composition of KP

classification	amino acids	KP (g/16 g nitrogen)
polar charged	arginine	5.6
	lysine	7.8
	histidine	2.6
	aspartic acid	11.8
	glutamic acid	15.1
polar neutral	cystine	2.0
	tyrosine	3.8
	serine	4.9
	threonine	3.6
nonpolar	alanine	4.2
	glycine	4.5
	leucine	9.1
	isoleucine	3.9
	methionine	1.0
	Proline	5.3
	Valine	5.4
	phenylalanine	6.2
	tryptophan	1.2

the total amino acids present in KP are hydrophobic or nonpolar, whereas about 43% of total amino acids have polar groups that can participate in various ionic interactions. The presence of arginine (∼6%), lysine (∼8%), cystine (∼2%), tyrosine (∼4%), and histidine (∼3%) with reactive groups allows certain chemical modifications of protein through cross-linking. The composition of amino acids in KP was comparable to that of conventional soy protein, reported in the literature.[38] The significant presence of lysine, arginine, cystine, tyrosine, and histidine may facilitate the formation of 3-dimensional covalent cross-linking interactions in KP over soy protein.

Molecular Weight Profile of KP. Figure 1 shows the SDS-PAGE patterns of extracted KP to determine the molecular weight distributions. Lane 1 in Figure 1 is the marker, while lane 2 is for the KP in which several bands can be observed. A very broad ranging band was observed between 120 and 250 kDa, which comprises about 40% of total protein according to densitometric quantification by ImageJ analysis. There are also two bands ranging in the 15−25 and 45−65 kDa regions, which have about 20% and 35% of the total proteins, respectively. An unclear band (5% of total protein) was observed between 25 and 35 kDa. These results suggest that the average molecular weight of KP subunits is significantly higher than that of soy protein, which could be favorable to real-life applications such as fiber-reinforced composite structures where higher resin properties would be desirable.[39]
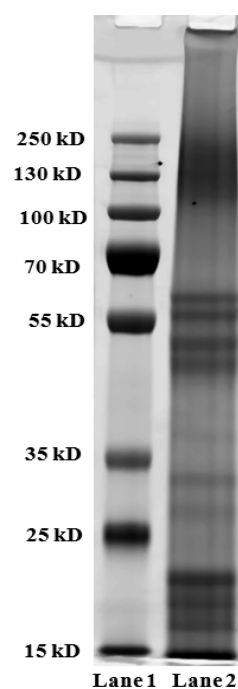

Figure 1. SDS-PAGE patterns of extracted karanja protein (KP). Lane 1: molecular weight marker. Lane 2: 5 μg of karanja protein.

Properties of Unmodified KP Sheets. Pure KP sheets with a smooth homogeneous surface and thickness of 0.3 ± 0.02 mm were produced without any additives/modifiers such as plasticizers or cross-linkers. The small variation in thickness might be a result of the differences in processing of a sheet-forming solution and shrinking of the sheet during the evaporation of the solvent, water in this case.[40] However, the difference in sheet thickness was not significant (p-value > 0.05). The moisture content of the KP sheets was 12.2 ± 0.4%, and the remaining material was dry matter contents. Young's modulus was found to be 622.7 ± 79.8 MPa. However, a tensile property measurement was not possible because of the high brittleness and poor handling. The degradation onset temperature of the unmodified KP sheets was 259.5 ± 1.2 °C. The color of the sheets was dark brown with a whiteness index of 54.3 ± 0.07. It should be noted that the dark color of the resin is not an important factor if it is used as resin in composites or as adhesive. The unmodified KP sheet will be used as a control for subsequent experiments using plasticization of KP sheets.

Effect of Plasticization on KP Sheets. A plasticizer, being a small molecule, increases the free volume in the protein system and, thus, increases the compliance and ductility.[41] Also, protein−protein interactions can be varied by varying the contents of the plasticizer, which determines the properties of the sheet.[42] The addition of a plasticizer in the KP sheet-forming solution was necessary as the sheet without the plasticizer was too brittle to be tested or handled. Sorbitol, one of the most common polyol type plasticizers for protein-based polymers, was used in this study. A range of 5−15% (on dry protein wt) sorbitol content was selected to study the effect of plasticizer content and seek optimal properties that would provide a compromise between strength and elasticity.

Increased levels of sorbitol did not exhibit any statistically significant differences in the final thickness values of the KP sheets (results not shown) (p-value > 0.05). The average thickness of sorbitol-plasticized KP sheets was 0.3 ± 0.03 mm.

Table 3. Moisture Content (MC) of KP Sheets as a Function of Sorbitol Contents

sorbitol contents	control	5% (w/w)	10% (w/w)	15% (w/w)
MC (%)	$12.2^a \pm 0.38^a$	14.0 ± 0.14^b	15.0 ± 0.40^c	15.8 ± 0.58^c

[a]Means not connected by same letters are significantly different at 95% confidence level through the Tukey−Kramer HSD test.
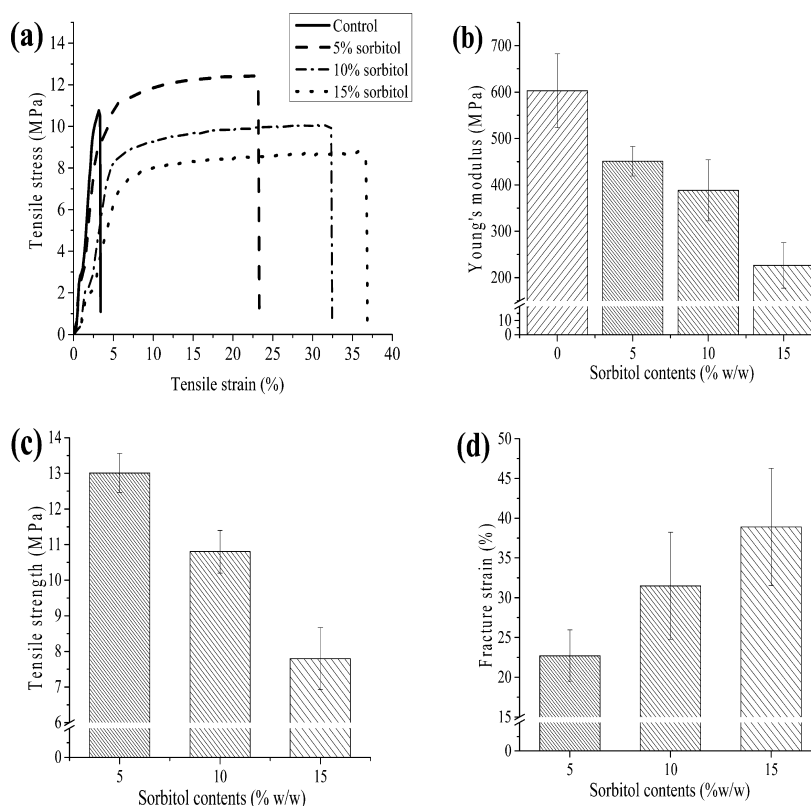

Figure 2. Effect of sorbitol on the tensile properties of KP sheets. (a) Representative stress−strain curve for control and sorbitol-plasticized KP sheets. (b) Young's modulus, (c) tensile strength, and (d) fracture strain of KP sheets as a function of sorbitol contents.

Also, the whiteness index was found to be 54.4 ± 0.06, comparable to the control. A significant effect on the thickness and color of the KP sheets was not observed for plasticization with sorbitol.

Table 3 shows the moisture content (MC) of the KP sheets as a function of sorbitol contents. As expected, the sorbitol-plasticized sheet showed a higher MC compared to the nonplasticized or control one. The hydroxyl groups present on sorbitol makes KP more hydrophilic. Thus, in addition to sorbitol plasticizing the resin, the additional moisture absorbed by KP plasticizes it further. The effect of sorbitol on the MC was significant (p-value < 0.05) according to statistical analysis.

Effect of sorbitol on the tensile properties of KP sheets is shown in Figure 2. Figure 2(a) shows the representative tensile stress−strain curves for the control and sorbitol-plasticized KP sheets. Control KP sheets were brittle and did not show any yielding as in the case of plasticized KP sheets. With the addition of sorbitol, yielding after the initial elastic region confirmed the plasticization. The plastic region was observed to increase, as expected, with the sorbitol content. Young's modulus, tensile strength, and fracture strain of KP sheets as a function of sorbitol contents are shown in Figure 2(b−d), respectively. Although some specimens were tested, the control sheets were too brittle to conduct tensile tests with reliability. Only Young's modulus data of the control sheets, which is not affected by the brittle fracture, can be considered reliable for

comparison. As expected, Young's modulus and tensile strength decreased with increasing sorbitol content. KP sheets with 15% sorbitol led to a Young's modulus of 226 MPa, about 2.8 times lower than that of the control KP sheets, 623 MPa, and for KP sheets with 5% sorbitol (451 MPa), the reduction in Young's modulus was about 30% compared to the control. At the same time, the tensile strength of 5% sorbitol-plasticized KP sheets showed an average value of 13.1 MPa, whereas 15% sorbitol-plasticized KP sheets had 7.8 MPa, about a 40% decrease in tensile strength for an increase in 10% plasticizer in KP. However, the fracture strain of the KP sheets, as expected, increased continuously with increasing sorbitol content. The fracture strain reported 23% for 5% sorbitol-plasticized specimens and was increased to 39% for 15% sorbitol-plasticized specimens. However, differences in the properties were significant (p-value < 0.05) according to Tukey−Kramer's HSD tests.

Figure 3(a) shows representative TGA curve for control and sorbitol-plasticized KP sheets, and Figure 3(b) shows the degradation onset temperature (T_d) of KP sheets as a function of sorbitol content. As shown in Figure 3(b), increasing the sorbitol contents in KP sheets resulted in a drop in T_d. Among the plasticized specimens, the highest T_d value was observed for the 5% sorbitol-plasticized KP sheet (253.3 ± 2.1 °C), a decrease of 6.2 °C compared to the control (not plasticized) sheets (259.5 ± 1.2 °C). The T_d values of the 15% sorbitol-

2322

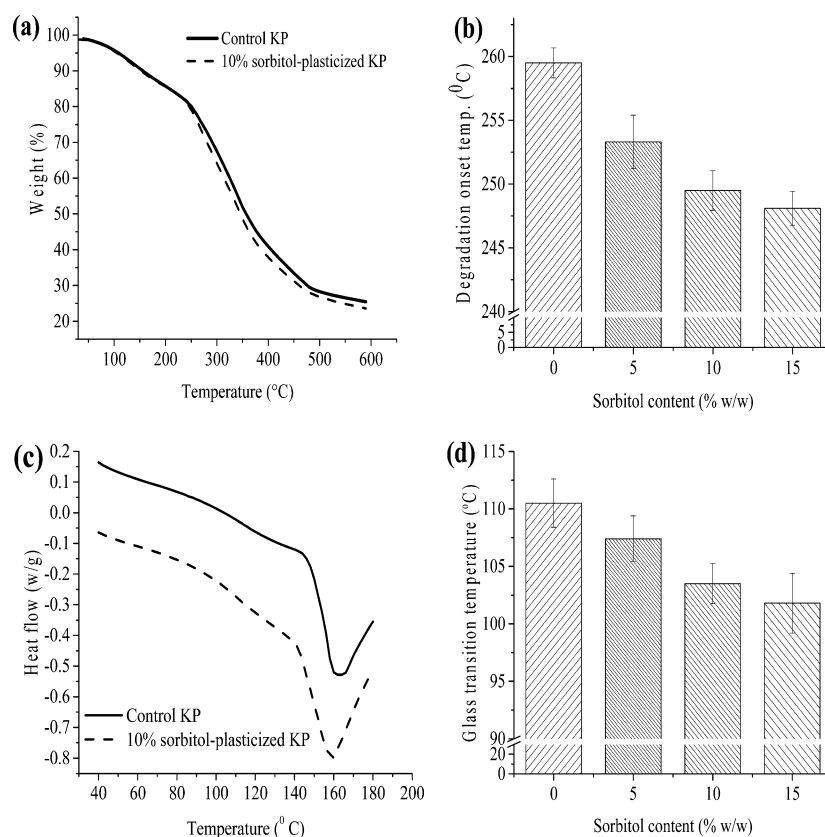dx.doi.org/10.1021/sc500095r | ACS Sustainable Chem. Eng. 2014, 2, 2318−2328

Figure 3. Effect of sorbitol on the thermal degradation behavior of KP sheets. (a) Representative TGA curve for control and sorbitol-plasticized KP sheets. (b) Degradation onset temperature of KP sheets as a function of sorbitol contents. (c) Representative DSC curve for control and sorbitol-plasticized KP sheets. (d) Apparent glass transition temperature of KP sheets as a function of sorbitol contents.

plasticized KP sheets were almost 11.0 °C lower than the control. In summary, the T_d values of sorbitol-plasticized KP sheets were significantly lower compared to that of the control sheet (p-value < 0.05). Glass transition temperatures of various KP sheets were measured from the DSC thermograms. Figure 3(c,d) shows the effect of plasticizers on the glass transition temperature (T_g) of KP sheets. As shown in Figure 3(d), the T_g values of the KP sheets decreased with an increase in plasticizer content. The T_g value of the nonplasticized sheet was found at about 110 °C and shifted lower to about 107, 104, and 102 °C after the addition of 5%, 10%, and 15% sorbitol, respectively, confirming its plasticizing effect.

Generally, the hydroxyl groups of sorbitol molecules interact with protein molecules at amino, carboxyl, and hydroxyl sites by forming hydrogen bonds with them. This results in reduced intermolecular and intramolecular interactions between the protein chains. Hydrogen bonding, within KP, might occur among −NH$_2$ groups in arginine and lysine, −NH− groups in proline and histidine, −OH groups in tyrosine, threonine, and serine, and −COOH groups in glutamic and aspartic acids. Sorbitol hinders the protein−protein molecular interaction by breaking the H bonds along the chains and reduces the functional properties.[41]

ATR-FTIR spectroscopic analysis was carried out to get an insight on the structural changes in KP and its effect on physicochemical properties by sorbitol-plasticization. Figure 4 shows the ATR-FTIR spectra of the control and 15% sorbitol-plasticized KP sheets. The spectra showed an extremely broad band ranging between 3500 and 3100 cm^{-1}, which corresponds to the stretching vibration of hydroxyl (O−H) groups from
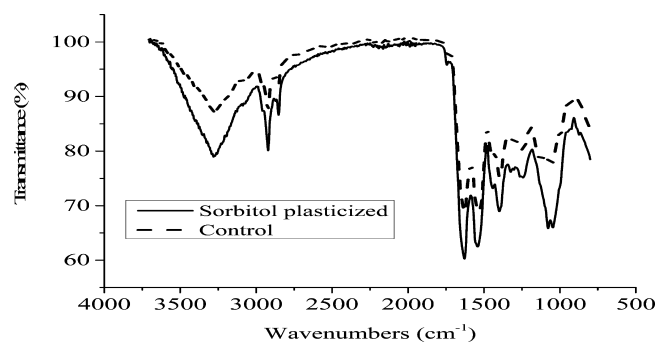

Figure 4. ATR-FTIR spectra for control and 15% sorbitol-plasticized KP sheets.

adsorbed water. An increase in the amplitude of this peak in plasticized KP indicates an increase in water content that is in agreement with the MC values presented in Table 3.[43] A peak located around 1000−1100 cm^{-1} when sorbitol is present might be related to the possible plasticizer hydrogen bonding with the protein molecules.[44,45] An amide-I band arises from a C=O stretching vibration in the range between 1600 and 1700 cm^{-1} that is generally used for analysis of protein secondary structures. The amide-I band shifted to a higher wavenumber, from 1623 cm^{-1} (control KP) to 1627 cm^{-1} (plasticized KP), which indicates a reduction of β-sheet-like structures and promotion of α-helical or disordered structures.[46] This shift also suggested the alternation of hydrogen bonding in protein molecular chains in the presence of sorbitol.[47]
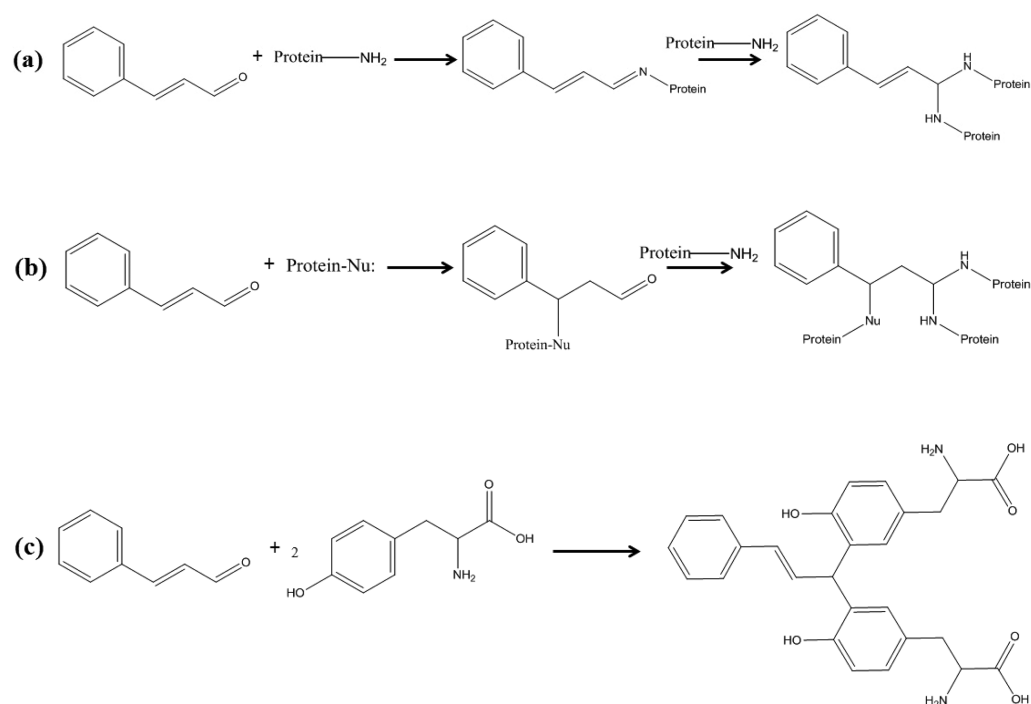
Figure 5. Possible reaction mechanisms involved in covalent cross-linking of karanja protein by cinnamaldehyde via (a) Schiff base formation between cinnamaldehyde and primary amine, (b) nucleophilic attack on the β-unsaturated carbon via a 1,4-Michael addition reaction, and (c) phenol groups of tyrosine residues with cinnamaldehyde[48−52]

Effect of Cross-Linking on KP Sheets. Sorbitol-plasticized KP sheets exhibited poor mechanical, thermal, and moisture resistance, especially in humid conditions. Therefore, cinnamaldehyde has been used as a natural cross-linker to enhance the resistance against humid conditions. Figure 5 shows possible reaction mechanisms for covalent cross-linking of cinnamaldehyde with protein. Basically, cinnamaldehyde possesses two electrophilic groups that could react directly with proteins via two different mechanisms, i.e., Schiff base formation to free amino groups of protein[48] or Michael addition to nucleophilic groups, i.e., thiol groups of cysteine residues and amino groups of lysine residues.[49−51] Also, aldehyde groups of cinnamaldehyde can react with phenol groups of tyrosine residues readily that resulted in cross-linking.[52] Considering one of the ideal applications of KP resin in fiber-reinforced composites, a sheet made with 5% sorbitol was selected as the control for noncross-linked specimens as the optimum amount of plasticizer due to its highest tensile strength and modulus with moderate fracture strain. The cross-linker has been varied between 0% and 16% (on dry protein wt) in 5% sorbitol-plasticized KP sheets.

KP Sheets with cross-linkers had an average thickness of 0.37 ± 0.02 mm. Formation of tighter and compact network structures due to cross-linking might result in higher thickness than the control specimens for the same consolidation pressure and temperature. However, the whiteness index of cross-linked KP sheets was about the same as compared to the control KP sheets, and the color change was not obvious. This is perhaps because the color of the control KP sheets was dark itself.

Figure 6 shows the SDS-PAGE patterns of the control and cross-linked (12% cinnamaldehyde) KP that was conducted to examine changes in the molecular weight distributions of KP, thus, confirming the cross-linking. The loadings of KP onto the gel were varied to different amounts, allowing a range of protein
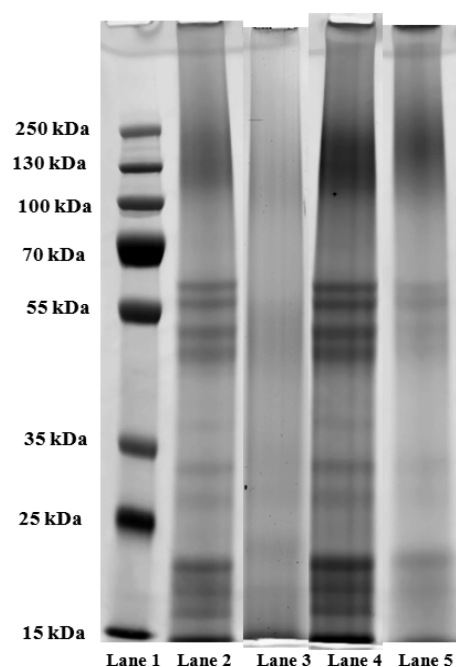

Figure 6. SDS-PAGE patterns of control (noncross-linked) and cross-linked (12% cinnamaldehyde) karanja protein (KP) for various concentrations. Lane 1: molecular weight marker. Lane 2: 2 μg of control KP. Lane 3: 2 μg of cross-linked KP. Lane 3: 5 μg of control KP. Lane 4: 5 μg of noncross-linked KP.

to be loaded to ensure visualization. At lower loadings of total protein, molecular weight distribution bands were not clearly visible in cross-linked KP, while a noticeable reduction in the intensity of the bands was observed at higher loadings as compared to control specimens. Aggregates of high molecular weight fractions were obtained on the top of the lane in both of

the cases during electrophoresis, reflecting low solubility of KP due to intermolecular cross-linking.[53] However, the very weak intensity observed at higher loadings is considered to be due to the presence of an insignificant amount of noncross-linked KP. Another reason might be intramolecular cross-linking at low molecular weight fractions that does not have significant influence on the increase in the molecular weight of protein subunits.[53]

Figure 7 shows the effect of cinnamaldehyde cross-linking on the water solubility (WS) and moisture contents (MC) of
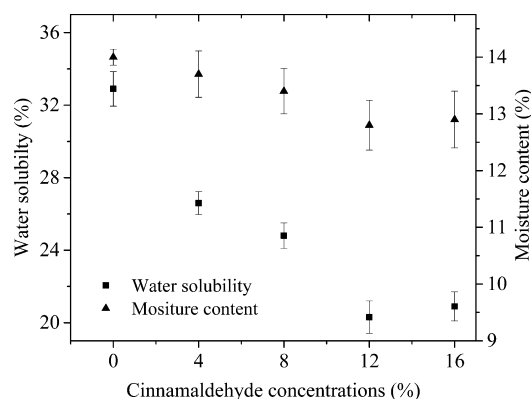

Figure 7. Effect of cinnamaldehyde cross-linking on the water solubility and moisture contents of sorbitol-plasticized KP sheets.

sorbitol-plasticized KP sheets. As shown in Figure 7, WS has been decreased noticeably due to cross-linking. The WS value of the control KP sheets was around 33%, which was reduced to 20% after cross-linking with 12% cinnamaldehyde. An almost 40% reduction in WS was observed in cross-linked KP sheets with 12% cinnamaldehyde. A similar reduction in WS has been reported on other protein-based resins by cross-linking with glyoxal and gluteraldehyde.[54] Because the hydrophilic amine groups are consumed in cross-linking, reduction in WS by cinnamaldehyde treatment can be expected. MC values also decreased with cinnamaldehyde contents as shown in Figure 7. A significant decrease (p-value < 0.05) in MC was also observed in 12% cross-linked sheets (12.8%) as compared to the control sheets (14.0%). However, the variation was not statistically significant among the cross-linked specimens. Formation of tighter and compact network structures due to cross-linking resulted in higher moisture resistance for cross-linked specimens than the control specimens.

Figure 8 shows the photographs of the control (noncross-linked) and cross-linked KP sheets in water after high temperature agitation in water for 5 and 10 h. Because of the hydrophilicity of the control sheets, they initially showed softening and subsequently warped after 5 h. After 10 h of water immersion, the control sheet broke into 2 to 3 pieces. In the case of soy protein, the control resin sheet was completely disintegrated in water.[4] Soy proteins are completely soluble in water, whereas KP in the present study might be partially soluble because of the fat content of about 7%. Higher cystine contents in KP resulted in higher amounts of intramolecular and intermolecular disulfide linkages that might also make KP protein less soluble.[55] A similar phenomenon was also observed in the case of jatropha protein.[56] Hence, this phenomenon can be attributed to the variations in molecular weights as well as amino acid composition and sequences of KP, which is considered as an added advantage in composite applications.
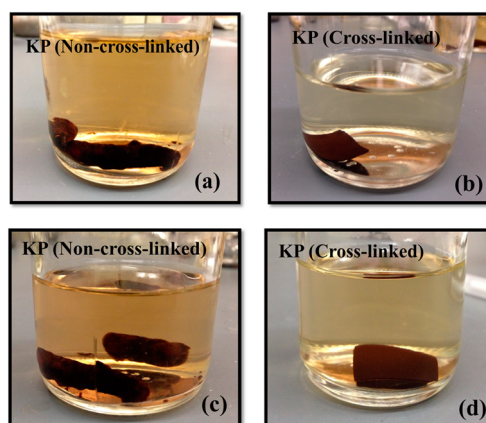

Figure 8. Photographs of KP sheets (noncross-linked and cross-linked) after shaking at 150 rpm in 80 °C temperature of water. (a,b) 5 h continuous shaking and (c,d) 10 h continuous shaking.

Cross-linked KP sheets remained mostly intact even after being continuously shaken in water at 80 °C for 10 h. Higher cross-linking made the structure rigid and less expandable with smaller capacity for softening and apparently did not allow it to warp due to the formation of an isotropic 3-dimensional structure. A similar behavior was also observed in biodegradable plastics made from cross-linked soy protein.[4]

Figure 9(a) shows typical tensile stress−strain plots of the control and cross-linked KP sheets, while Figure 9(b−d) shows Young's modulus, tensile strength, and fracture strain obtained from the stress−strain plots of the control and cross-linked KP sheets, respectively. With the addition of cinnamaldehyde up to 12%, the yield region after the initial elastic region decreased but increased at higher cinnamaldehyde addition. Also, the Young's modulus and tensile strength increased with an increase in cinnamaldehyde up to 12%. However, with any additional cinnamaldehyde, the Young's modulus and strength decreased, while the fracture strain increased. Cross-linked KP sheets with 12% cinnamaldehyde showed an increase in tensile strength by about 35% (17.6 MPa from 13.1 MPa for control), whereas the Young's modulus for cross-linked KP sheets increased almost 1.6 times (711 MPa from 451 MPa for control) in comparison to noncross-linked specimens. The addition of 12% cinnamaldehyde made the network rigid and decreased the fracture strain to 5% from 23% for the control sheets, a decrease of about 78%. However, increasing the cinnamaldehyde to 16% resulted in an increase in fracture strain. Also, tensile strength decreased to about 23% at 16% loading of cinnamaldehyde. These results suggest that 16% cinnamaldehyde in KP resin may leave some of it unreacted as a result of a nonstoichiometric ratio with lysine and arginine causing an excess of cinnamaldehyde, which acts as a plasticizer.

Representative TGA curves for the control and cross-linked (12% cinnamaldehyde) KP sheets are shown in Figure 10(a). Figure 10(b) shows the degradation onset temperature of KP sheets as a function of the increasing ratio of cinnamaldehyde. Cross-linking generally induces an increase in thermal stability by an appreciable increase in T_d.[57] As shown in Figure 10(b), the noncross-linked KP sheets had a T_d value of 253.3 °C, whereas maximum T_d values were observed at 267 °C for cross-linked KP sheets with 12% cinnamaldehyde. However, 8−16% cross-linking in plasticized KP did not show significant variations in T_d (p-value < 0.05).
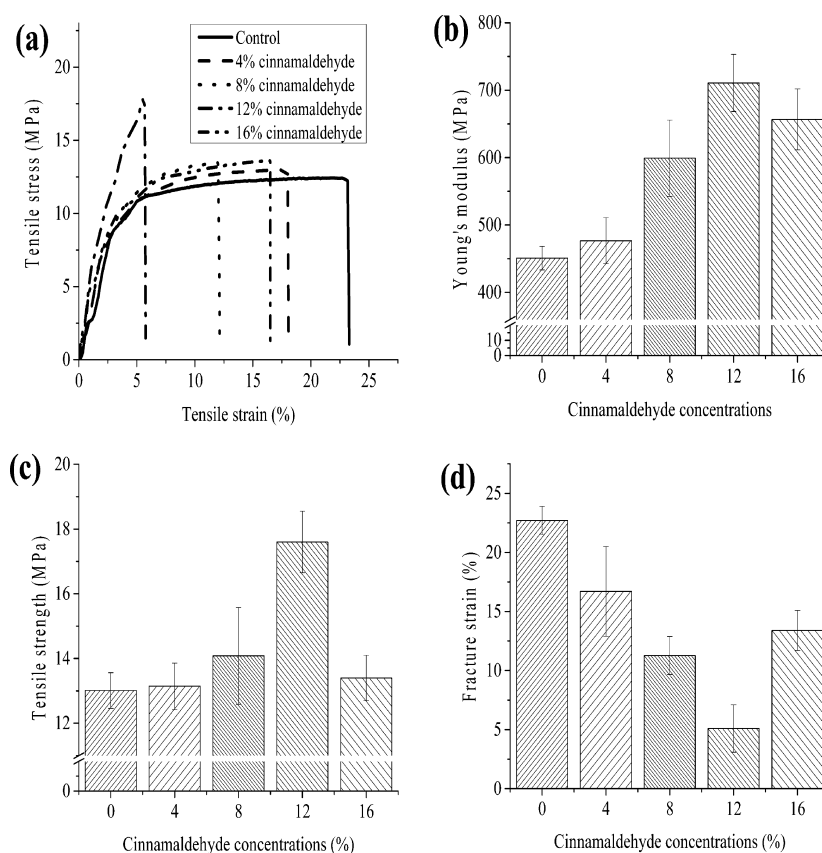
Figure 9. Effect of cinnamaldehyde on the tensile properties of KP sheets. (a) Representative stress−strain curve for control and cross-linked KP sheets. (b) Young's modulus, (c) tensile strength, and (d) fracture strain of control and cross-linked KP sheets.
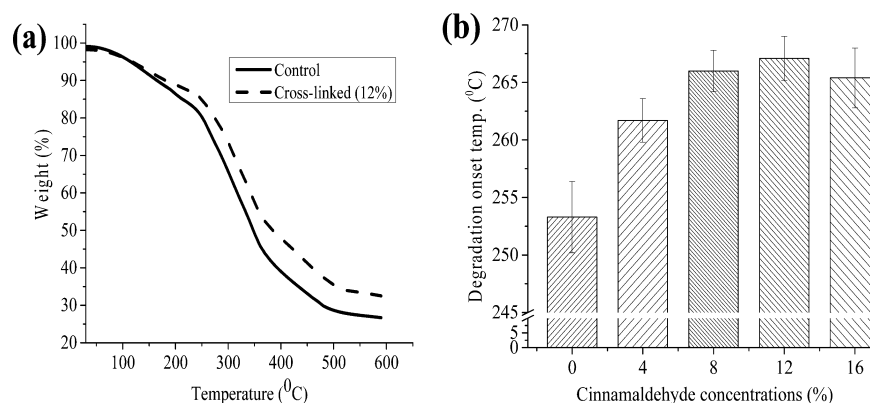

Figure 10. Effect of cinnamaldehyde on the thermal degradation behavior of plasticized KP sheet. (a) Representative TGA curve for control and cross-linked (12% cinnamaldehyde) KP sheets. (b) Degradation onset temperature of KP sheets as a function of an increasing ratio of cinnamaldehyde.

Comparison of modified KP sheets with various other edible protein sheets is difficult due to the differences in protein sources, modifiers used, and processing parameters, as well as protein contents. Still, if compared, KP (with about 60% protein content) sheets modified with 5% sorbitol and 12% cinnamaldehyde showed better mechanical, thermal, and physicochemical properties than some other edible proteins sheets.[58−61] The fracture strain can be manipulated, which explores a new nonedible source for food packaging industries, after toxic chemical treatment. Therefore, KP can be comparable and easily replace edible proteins considering the

functional performances and, hence, could be considered more sustainable.

This study introduces a green route of developing novel biodegradable nonedible protein-based resin from forestry waste residue "karanja seed cake". The study also demonstrated that KP sheet properties can be modified by manipulating plasticizers and cross-linkers. Investigation of plasticization by sorbitol in KP sheets revealed that an optimum content (5% sorbitol) in KP resulted in the best mechanical properties of KP resin under the current experimental conditions. An addition of 12% cinnamaldehyde as cross-linker in 5% sorbitol-plasticized KP sheets showed improved mechanical, thermal, and water

resistance. Overall, this paper explores the utilization of waste residues from forestry seed as a sustainable and "green" biobased resin to replace conventional petroleum-derived as well as edible biobased resins in fiber-reinforced composites.

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: (1) 607 255 1875. E-mail: ann2@cornell.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) Mekonnen, T.; Mussone, P.; Khalil, H.; Bressler, D. Progress in bio-based plastics and plasticizing modifications. *J. Mater. Chem. A* **2013**, *1*, 13379−13398.

(2) Zhang, H.; Mittal, G. Biodegradable protein-based films from plant resources: A review. *Environ. Prog. Sustainable Energy* **2010**, *29*, 203−220.

(3) Ghosh Dastidar, T.; Netravali, A. Cross-linked waxy maize starch-based "green" composites. *ACS Sustainable Chem. Eng.* **2013**, *1*, 1537−1544.

(4) Dastidar, T. G.; Netravali, A. N. A soy flour based thermoset resin without the use of any external crosslinker. *Green Chem.* **2013**, *15*, 3243−3251.

(5) Siqueira, G.; Bras, J.; Dufresne, A. Cellulosic bionanocomposites: A review of preparation, properties and applications. *Polymers* **2010**, *2*, 728−765.

(6) Siró, I.; Plackett, D. Microfibrillated cellulose and new nanocomposite materials: a review. *Cellulose* **2010**, *17*, 459−494.

(7) Liu, H.; Xie, F.; Yu, L.; Chen, L.; Li, L. Thermal processing of starch-based polymers. *Prog. Polym. Sci.* **2009**, *34*, 1348−1368.

(8) Chabba, S.; Matthews, G.; Netravali, A. Green" composites using cross-linked soy flour and flax yarns. *Green Chem.* **2005**, *7*, 576−581.

(9) Lodha, P.; Netravali, A. N. Characterization of stearic acid modified soy protein isolate resin and ramie fiber reinforced "green"composites. *Compos. Sci. Technol.* **2005**, *65*, 1211−1225.

(10) Chabba, S.; Netravali, A. N. 'Green' composites Part 1: Characterization of flax fabric and glutaraldehyde modified soy protein concentrate composites. *J. Mater. Sci.* **2005**, *40*, 6263−6273.

(11) Kim, J. T.; Netravali, A. N. Development of aligned-hemp yarn-reinforced green composites with soy protein resin: Effect of pH on mechanical and interfacial properties. *Compos. Sci. Technol.* **2011**, *71*, 541−547.

(12) Kim, J. T.; Netravali, A. N. Mercerization of sisal fibers: Effect of tension on mechanical properties of sisal fiber and fiber-reinforced composites. *Composites, Part A* **2010**, *41*, 1245−1252.

(13) Huang, X.; Netravali, A. Characterization of flax fiber reinforced soy protein resin based green composites modified with nano-clay particles. *Compos. Sci. Technol.* **2007**, *67*, 2005−2014.

(14) Huang, X.; Netravali, A. Biodegradable green composites made using bamboo micro/nano-fibrils and chemically modified soy protein resin. *Compos. Sci. Technol.* **2009**, *69*, 1009−1015.

(15) Wihodo, M.; Moraru, C. I. Physical and chemical methods used to enhance the structure and mechanical properties of protein films. A review. *J. Food Eng.* **2013**, *114*, 292−302.

(16) Debeaufort, F.; Quezada-Gallo, J. A.; Voilley, A. Edible films and coatings: Tomorrow's packagings: A review. *Crit. Rev. Food Sci.* **1998**, *38*, 299−313.

(17) Song, F.; Tang, D. L.; Wang, X. L.; Wang, Y. Z. Biodegradable soy protein isolate-based materials: A review. *Biomacromolecules* **2011**, *12*, 3369−3380.

(18) Zhang, X.; Hoobin, P.; Burgar, I.; Do, M. D. Chemical modification of wheat protein-based natural polymers: Cross-linking effect on mechanical properties and phase structures. *J. Agric. Food Chem.* **2006**, *54*, 9858−9865.

(19) Liu, C. C.; Tellez-Garay, A. M.; Castell-Perez, M. E. Physical and mechanical properties of peanut protein films. *LWT-Food Sci. Technol.* **2004**, *37*, 731−738.

(20) Sahoo, P.; Das, L.; Babu, M.; Naik, S. Biodiesel development from high acid value polanga seed oil and performance evaluation in a CI engine. *Fuel* **2007**, *86*, 448−454.

(21) Naik, M.; Meher, L.; Naik, S.; Das, L. Production of biodiesel from high free fatty acid Karanja (*Pongamia pinnata*) oil. *Biomass Bioenergy* **2008**, *32*, 354−357.

(22) Vinay, B.; Sindhu Kanya, T. Effect of detoxification on the functional and nutritional quality of proteins of karanja seed meal. *Food Chem.* **2008**, *106*, 77−84.

(23) Mishra, M.; Neelakantan, S.; Khandelwal, K.; Bhardwaj, S.; Vyas, S. Margosa (neem) seed cake as an inhibitor of nitrification. *Soil Biol. Biochem.* **1975**, *7*, 183−184.

(24) Vismaya; Sapna Eipeson, W.; Manjunatha, J.; Srinivas, P.; Sindhu Kanya, T. Extraction and recovery of karanjin: A value addition to karanja (*Pongamia pinnata)* seed oil. *Ind. Crops Prod.* **2010**, *32*, 118−122.

(25) Aguirre, A.; Borneo, R.; León, A. E. Properties of triticale protein films and their relation to plasticizing−antiplasticizing effects of glycerol and sorbitol. *Ind. Crops Prod.* **2013**, *50*, 297−303.

(26) Balaguer, M. P.; Gómez-Estaca, J.; Gavara, R.; Hernandez-Munoz, P. Biochemical properties of bioplastics made from wheat gliadins cross-linked with cinnamaldehyde. *J. Agric. Food Chem.* **2011**, *59*, 13212−13220.

(27) Marquié, C.; Tessier, A. M.; Aymard, C.; Guilbert, S. HPLC determination of the reactive lysine content of cottonseed protein films to monitor the extent of cross-linking by formaldehyde, glutaraldehyde, and glyoxal. *J. Agric. Food Chem.* **1997**, *45*, 922−926.

(28) Balaguer, M. P.; Gómez-Estaca, J.; Gavara, R.; Hernandez-Munoz, P. Functional properties of bioplastics made from wheat gliadins modified with cinnamaldehyde. *J. Agric. Food Chem.* **2011**, *59*, 6689−6695.

(29) Ben Arfa, A.; Preziosi-Belloy, L.; Chalier, P.; Gontard, N. Antimicrobial paper based on a soy protein isolate or modified starch coating including carvacrol and cinnamaldehyde. *J. Agric. Food Chem.* **2007**, *55*, 2155−2162.

(30) Tzeng, Y. M.; Diosady, L. L.; Rubin, L. J. Production of canola protein materials by alkaline extraction, precipitation, and membrane processing. *J. Food Sci.* **1990**, *55*, 1147−1151.

(31) Rao, P. U. Chemical composition of defatted and debiterrer cake of Azadirachta indica. *J. Am. Oil Soc.* **1987**, *64*, 1348−1351.

(32) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *nature* **1970**, *227*, 680−685.

(33) Rhim, J. W.; Gennadios, A.; Weller, C. L.; Cezeirat, C.; Hanna, M. A. Soy protein isolate−dialdehyde starch films. *Ind. Crops Prod.* **1998**, *8*, 195−203.

(34) Cuq, B.; Gontard, N.; Cuq, J. L.; Guilbert, S. Functional properties of myofibrillar protein-based biopackaging as affected by film thickness. *J. Food Sci.* **1996**, *61*, 580−584.

(35) Saetae, D.; Kleekayai, T.; Jayasena, V.; Suntornsuk, W. Functional properties of protein isolate obtained from physic nut (*Jatropha curcas* L.) seed cake. *Food Sci. Biotechnol.* **2011**, *20*, 29−37.

(36) Lestari, D.; Mulder, W. J.; Sanders, J. P. Jatropha seed protein functional properties for technical applications. *Biochem. Eng. J.* **2011**, *53*, 297−304.

(37) Vodjani, F. Solubility. In *Methods of Testing Protein Functionality*; Hall, G. M., Ed.; Springer: New York, 1996; pp 11−60.

(38) Berk, Z. Technology of production of edible flours and protein products from soybeans. *Agric. Serv. Bull. (F. A. O.)* **1992**, *97*, 1−10.

2327

dx.doi.org/10.1021/sc500095r| *ACS Sustainable Chem. Eng.* 2014, 2, 2318−2328

(39) Liu, S.; Zhou, R.; Tian, S.; Gai, J. A study on subunit groups of soybean protein extracts under SDS-PAGE. *J. Am. Oil Chem. Soc.* **2007**, *84*, 793−801.

(40) Kokoszka, S.; Debeaufort, F.; Hambleton, A.; Lenart, A.; Voilley, A. Protein and glycerol contents affect physico-chemical properties of soy protein isolate-based edible films. *Innovative Food Sci. Emerging Technol.* **2010**, *11*, 503−510.

(41) Sears, J. K.; Darby, J. R. Mechanism of Plasticizer Action. In *The Technology of Plasticizers*; Sears, J. K.; Darby, J. R., Eds.; Wiley Interscience: New York, 1982; pp 35−77.

(42) Ayhllon-Meixueiro, F.; Vaca-Garcia, C.; Silvestre, F. Biodegradable films from isolate of sunflower (*Helianthus annuus*) proteins. *J. Agric. Food Chem.* **2000**, *48*, 3032−3036.

(43) Piermaria, J.; Bosch, A.; Pinotti, A.; Yantorno, O.; Garcia, M. A.; Abraham, A. G. Kefiran films plasticized with sugars and polyols: water vapor barrier and mechanical properties in relation to their microstructure analyzed by ATR/FT-IR spectroscopy. *Food Hydrocoll.* **2011**, *25*, 1261−1269.

(44) Blanco-Pascual, N.; Fernández-Martín, F.; Montero, P. Jumbo squid (*Dosidicus gigas)* myofibrillar protein concentrate for edible packaging films and storage stability. *LWT–Food Sci. Technol.* **2014**, *55*, 543−550.

(45) Bergo, P.; Sobral, P. Effects of plasticizer on physical properties of pigskin gelatin films. *Food Hydrocoll.* **2007**, *21*, 1285−1289.

(46) Ullah, A.; Vasanthan, T.; Bressler, D.; Elias, A. L.; Wu, J. Bioplastics from feather quill. *Biomacromolecules* **2011**, *12*, 3826−3832.

(47) Shi, W.; Dumont, M.-J. Processing and physical properties of canola protein isolate-based films. *Ind. Crops Prod.* **2014**, *52*, 269−277.

(48) Elahi, E. N.; Wright, Z.; Hinselwood, D.; Hotchkiss, S. A.; Basketter, D. A.; Smith Pease, C. K. Protein binding and metabolism influence the relative skin sensitization potential of cinnamic compounds. *Chem. Res. Toxicol.* **2004**, *17*, 301−310.

(49) Weibel, H.; Hansen, J. Interaction of cinnamaldehyde (a sensitizer in fragrance) with protein. *Contact Dermatitis* **1989**, *20*, 161−166.

(50) Macpherson, L. J.; Dubin, A. E.; Evans, M. J.; Marr, F.; Schultz, P. G.; Cravatt, B. F.; Patapoutian, A. Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature* **2007**, *445*, 541−545.

(51) Dupuis, G.; Benezra, C. *Allergic Contact Dermatitis to Simple Chemicals: A Molecular Approach*; Dekker: New York, 1982.

(52) Akabori, S.; Ohno, K. On splitting off the side chain from tyrosine by the action of aldehydes. *Proc. Jpn. Acad.* **1950**, *6*, 39−41.

(53) Park, S.; Bae, D.; Rhee, K. Soy protein biopolymers cross-linked with glutaraldehyde. *J. Am. Oil Chem. Soc.* **2000**, *77*, 879−884.

(54) Orliac, O.; Rouilly, A.; Silvestre, F.; Rigal, L. Effects of additives on the mechanical properties, hydrophobicity and water uptake of thermo-moulded films produced from sunflower protein isolate. *Polymer* **2002**, *43*, 5417−5425.

(55) Okamato, S. Factors affecting protein film formation. *Cereal Foods World* **1978**, *23*, 257−262.

(56) Hamarneh, A.; Heeres, H.; Broekhuis, A.; Picchioni, F. Extraction of *Jatropha curcas* proteins and application in polyketone-based wood adhesives. *Int. J. Adhes. Adhes.* **2010**, *30*, 615−625.

(57) Bigi, A.; Cojazzi, G.; Panzavolta, S.; Rubini, K.; Roveri, N. Mechanical and thermal properties of gelatin films at different degrees of glutaraldehyde crosslinking. *Biomaterials* **2001**, *22*, 763−768.

(58) Chen, L.; Reddy, N.; Wu, X.; Yang, Y. Thermoplastic films from wheat proteins. *Ind. Crops Prod.* **2012**, *35*, 70−76.

(59) Reddy, N.; Jiang, Q.; Yang, Y. Preparation and properties of peanut protein films crosslinked with citric acid. *Ind. Crops Prod.* **2012**, *39*, 26−30.

(60) Shi, K.; Yu, H.; Lakshmana Rao, S.; Lee, T.-C. Improved mechanical property and water resistance of zein films by plasticization with tributyl citrate. *J. Agric. Food Chem.* **2012**, *60*, 5988−5993.

(61) Rouilly, A.; Mériaux, A.; Geneau, C.; Silvestre, F.; Rigal, L. Film extrusion of sunflower protein isolate. *Polym. Eng. Sci.* **2006**, *46*, 1635−1640.