Enhancing Strength of Wool Fiber Using a Soy Flour Sugar-Based “Green” Cross-linker

Namrata V. Patil and Anil N. Netravali*

Department of Fiber Science & Apparel Design, Cornell University, 37 Forest Home Dr., Ithaca, New York 14853, United States

Supporting Information

ABSTRACT: This study presents the preparation and use of a “green” cross-linker derived from a waste soy flour sugar (SFS) mixture to cross-link keratin in wool fibers to increase their tensile properties. Earlier studies of keratin cross-linking involved chemicals such as glyoxal and glutaraldehyde that are toxic to humans. In addition, their effectiveness in improving tensile properties has been significantly lower than obtained in this study using modified SFS. Characterization of SFS using 13C NMR revealed the presence of five sugars having different molecular lengths. Oxidation of SFS using sodium periodate resulted in multiple aldehyde groups, as confirmed by 1H NMR and attenuated total reflection Fourier-transform infrared (ATR-FTIR). The oxidized SFS (OSFS) when used to cross-link the amine groups from the wool keratin resulted in 36 and 56% increase in the tensile strength and Young’s modulus of the fibers, respectively. These significant increases in strength and Young’s modulus were a result of having multiple aldehyde groups on each sugar molecule as well as different molecular lengths of sugars, which favored cross-links of multiple lengths within the cortical cell matrix of wool fibers. The cross-linking between the aldehyde groups in OSFS and amine groups in wool fibers was confirmed using ATR-FTIR and from the color change resulting from the Maillard reaction as well as decrease in moisture absorption by the fibers. Stronger wool fibers can not only increase the efficiencies of wool fiber spinning and weaving and reduce yarn and fabric defects but can also allow spinning finer yarns from the same fibers. Oxidized sugars with optimum molecular lengths can be used to cross-link other biological proteins as well, replacing the currently used toxic cross-linkers.

INTRODUCTION

Wool is the most important animal fiber used in textiles and many other applications. It is a fully renewable but expensive fiber that is known for its comfort, warmth retention, moisture absorption, and elasticity.1,2 Although wool is most commonly obtained from sheep, hair from other animals, such as goats, llamas, and alpacas, are also used. The fleece (raw wool) obtained from the animals contains 30–70% impurities, such as sand, dirt, grease, dried sweat, etc., most of which are removed through the scouring process.3 The cleaned dry wool is commonly processed through a carding machine and comber to produce a continuous web or sliver (wool top) with individual fibers parallel to each other.4 The length of fibers in the sliver can vary from 2 to 6 in. depending on the wool variety and the processes used. Sliver is drawn to the desired linear density and twisted during spinning to form continuous yarn.4 Since wool fibers are inherently weak, fiber breakage during spinning and weaving processes, which are commonly carried out under tension, is a significant problem. Fiber breakages reduce the production efficiency, create fabric defects, and generate significant amounts of fiber and fabric wastes.5 Increasing the strength of the fibers can not only solve these issues but also allow spinning finer yarns from the same fibers, significantly increasing their value.

There have been many improvements in the genetic modifications of wool by selective breeding of sheep as well as by providing better nutrition to increase the length, fineness, yield, and strength of the fiber.5 Plasma treatment of wool fibers has also been shown to reduce fiber breakage during the spinning process.5 Genetic modifications and plasma treatments, however, can be expensive. Chemical cross-linking can be much less expensive and an easier way to enhance the tensile properties of the fiber. The chemical composition of wool has shown the presence of many polar and nonpolar amino acids. Amino acids with polar groups, e.g., in soy proteins, have shown excellent possibilities for chemical modifications through cross-linking.6 Although the exact content of polar amino acids varies on the basis of the source, high contents of amino acids, such as arginine (19.1%), serine (8.7%), glutamic acid (8.5%), and cystine (7.3%), have been found in merino wool.7 Amino acids with acidic side chains, such as glutamic acid, aspartic acid, asparagine, and glutamine, account for about 10% of the total amino acids. Amino acids with basic side chains, such as lysine, histidine, and tryptophan, account for 3.5%. Threonine and tyrosine are amino acids with hydroxyl groups in the side chain and account for 9% of the total amino acids.7 Glycine, leucine, proline, valine, alanine, isoleucine, and phenylalanine amino acids without reactive
groups on their side chains account for about 30% of amino acids.\(^7\) In most cross-linking cases involving proteins, bifunctional cross-linkers, such as glyoxal, glutaraldehyde, diisocyanates, and carbodiimides have been used.\(^8\) Some form-aldehyde-based cross-linkers have also been reported.\(^11\) These cross-linkers are skin irritant and toxic, not only to cells and biological systems but also to the environment.\(^9,12\) As a result, they pose a great danger to the health of the users. Formaldehyde has been classified as a carcinogen and is being banned in many places.

Soybean, a legume species, is an important agricultural and industrial crop. It is one of the major oil seeds produced in the U.S. and worldwide. Soybean makes up over half of all oil seeds in the world market. There has been an increase in the use of soybean oil to produce biodiesel in the last few years.\(^13\) Apart from oil, soybeans are also a major source of edible plant-based protein. Defatted soy flour (SF) is obtained as a by-product after extracting oil from soybeans. It consists of 50−54% protein, 30−32% carbohydrate, 2−3% dietary fibers, and other minor components, such as minerals, ash, and moisture.\(^14\) SF is purified to obtain soy protein concentrate (SPC) and further purified to get soy protein isolate (SPI). The purification process involves removing the 30−32% carbohydrates present in SF. The carbohydrate mixture, a by-product of SPC and SPI production, is generally discarded as waste.\(^15\) It consists of five different sugars: monosaccharides (fructose and glucose), disaccharide (sucrose), trisaccharide (raffinose), and tetrasaccharide (stachyose).\(^14\) Raffinose and stachyose are not digestible by humans or animals. These sugars, as a mixture, can be modified and utilized for nonedible purposes.\(^15\)

The main goal of this research was to cross-link protein (keratin) in wool fiber using a natural “green” cross-linker formulated using soy flour sugars (SFS) and enhance the tensile properties. SFS, extracted from SF, was characterized, chemically modified, and used as an inexpensive and nontoxic cross-linker for keratin, the protein in wool. The sugars in SFS were oxidized (OSFS) using sodium periodate (NaIO\(_4\)) to obtain aldehyde groups on them, as shown in Figure 1. Oxidation of the sugar mixture in SFS produces multiple lengths of oxidized sugars containing aldehyde groups. Although the high number of functional (aldehyde) groups obtained can provide chemical reaction with a majority of the amine groups in keratin, the presence of different sugars, i.e., different molecular lengths, improves the possibility of reaching all reactive sites in keratin. These reactions lead to formation of both intermolecular as well as intramolecular linkages in the proteins, forming a cross-linked system. The effects of chemical cross-linking on the performance properties of wool fibers such as tensile properties were studied.

### RESULTS AND DISCUSSION

**Characteristics of SFS.** \(^13\)C NMR has been an important tool for the structural elucidation of carbohydrates.\(^16,17\) Figure 2 shows the \(^13\)C NMR spectra of various sugars, such as fructose, glucose, sucrose, raffinose, stachyose, and the SFS obtained in this study. As seen in Figure 2, all pure sugars showed chemical shifts between 60 and 110 ppm.\(^17−19\) Fructose and glucose are reducing monosaccharides consisting of six carbons each. The aqueous solutions of these sugars are oxidized using sodium periodate (NaIO\(_4\)) to obtain aldehyde groups on them, as shown in Figure 1.
monosaccharides consist of equilibrium mixtures of their tautomers. In solution, fructose exists as an equilibrium of fructopyranose, fructofuranose, and other forms, including acyclic structures. Glucose exists in α and β pyranose together with its open-chain forms. The 13C NMR spectra of fructose (Figure 2a) and glucose (Figure 2b) show more than six carbons because they show tautomeric structures as they are present in aqueous solution. Sucrose is a nonreducing disaccharide made up of fructose and glucose. 13C NMR spectrum of sucrose is shown in Figure 2c. The 12 carbons from sucrose are seen between 60 and 110 ppm. Raffinose is a nonreducing trisaccharide composed of galactose, glucose, and fructose consisting of 18 carbons, as seen in its spectrum shown in Figure 2d. Stachyose is also a nonreducing tetrasaccharide but consists of two galactose units, one glucose and one fructose unit with a total of 24 carbons, as seen in its spectrum shown in Figure 2e. The spectrum of SFS (Figure 2f) shows chemical shifts between 60 and 110 ppm, as seen in all other sugars mentioned above, confirming the presence of different sugars in the SFS. Obendorf et al. have shown that the embryos of soybean seed accumulate sucrose, rafinose, and stachyose during seed development and maturation. Qi and Netravali showed that SFS extracted from the same SF as in the present case, consisted of 21.21 g/L sucrose, 11.92 g/L stachyose, 1.92 g/L rafinose, and 1.59 g/L fructose (combined), 1.59 g/L fructose and glucose (combined), 1.59 g/L fructose and glucose (combined). Table 1 shows the proposed oxidation reaction of sucrose and stachyose derivatives (Figure 1). These aldehyde groups were confirmed through the ATR-FTIR spectrum of OSFS, as shown in Figure 3a through the absorption peak at 1720 cm−1. A similar peak at 1718 cm−1 was seen by Jalaja and James after oxidizing sucrose using NaIO₄. The peak intensities of both 1250 and 1411 cm−1, which correspond to the C−OH bending

![Figure 3. (a) ATR-FTIR spectra and (b) 1H NMR spectra of SFS and OSFS.](image)

Table 1. Percent Content of Different Sugars in SFS

| Sugar          | Percentage |
|----------------|------------|
| fructose + glucose | 5.24%      |
| sucrose         | 57.90%     |
| rafinose        | 4.33%      |
| stachyose       | 32.53%     |

As seen from Table 1, sucrose and stachyose are present in considerable amounts in SFS, 58 and 32.5%, respectively, comprising over 90% of the total sugars. Fructose and glucose are reducing sugars and can exist in open-chain form in equilibrium, forming aldehyde or ketone groups. Unlike monosaccharides such as fructose and glucose, sucrose, rafinose, and stachyose are nonreducing sugars and do not exist in open-chain form and, importantly, none of them have aldehyde groups. However, they can be oxidized to convert the hydroxyl groups to aldehyde groups. As seen in Figure 1, NaIO₄ cleaves the vicinal diols and oxidizes the hydroxyl groups to aldehyde groups. Sucrose and stachyose have 5 and 11 secondary hydroxyl groups, respectively, which form the vicinal diols that can be broken and oxidized to 4 and 8 aldehyde groups, respectively (Figure 1). Thus, oxidation of sucrose and stachyose forms polyaldehyde (tetra-aldehyde sucrose and octa-aldehyde stachyose) derivatives (Figure 1). These aldehyde groups were confirmed through the ATR-FTIR spectrum of OSFS, as shown in Figure 3a through the absorption peak at 1720 cm−1. A similar peak at 1718 cm−1 was seen by Jalaja and James after oxidizing sucrose using NaIO₄. The peak intensities of both 1250 and 1411 cm−1, which correspond to the C−OH bending
in sugars, are seen to reduce as a result of oxidation of hydroxyls to aldehyde groups. Similarly, glucose, fructose, and raffinose in SFS get oxidized to form aldehyde groups as well. Since these three sugars account for less than 10% of the total sugars in the SFS solution, Figure 1 presents only the sucrose and stachyose reactions. The formation of polyaldehyde was also confirmed from the 1H NMR spectra. Figure 3b shows the 1H NMR spectra of SFS and OSFS. The spectrum of SFS shows characteristic sugar proton shifts at 5.4 ppm and between 4.2 and 3.2 ppm. The proton shift at 4.7 ppm is the solvent peak from D2O. The proton shifts between 3 and 4 ppm represent −CH and −CH2 in the sugars. The proton shifts at 4 and 4.2 ppm represent the protons from the vicinal diols of the sugars. The additional peak in OSFS at 8.3 ppm shows the formation of aldehyde groups upon oxidation of SFS. Liu et al. observed the free aldehyde peak upon oxidation of sucrose using NaIO4 between 8 and 8.5 ppm. The additional small proton shifts seen between 5 and 5.6 ppm show the formation of hemiacetals because of the intermolecular reaction between aldehyde and hydroxyl groups. Similar proton shifts were observed by Xu et al. and Liu et al. after oxidizing sucrose using NaIO4. The change in pH of SFS from 5.5 to 3 after oxidation also confirms the presence of aldehyde groups in OSFS.

Characteristics of Control and Cross-linked Wool Fibers. Figure 4 presents the ATR-FTIR spectra of wool fibers. Whereas Figure 4a shows the ATR-FTIR spectra of control and cross-linked wool fibers from 4000 to 500 cm−1, Figure 4b shows the spectra from 1800 to 1000 cm−1. The spectrum for untreated (control) wool fiber shows a broad peak at around 3268 cm−1. This peak is assigned to O−H stretching from adsorbed water and N−H bending vibrations from the amide A linkages. The peak at 2923 cm−1 is due to CH2 and CH3 stretching vibrations, whereas the peak at 1447 cm−1 is due to C−H bending in protein. The spectrum for control wool fiber also shows three main characteristic peaks between 1700 and 1200 cm−1. For example, the strong absorbance peak at 1628 cm−1 is associated with the C=O stretch from the amide I linkages. The medium strong absorbance peak at 1515 cm−1 is assigned to N−H in-plane bending in amide II linkages. The peak at 1233 cm−1 is assigned to the C−N stretch of the amide III linkages. The aldehyde groups of OSFS can react with the amine groups from keratin to form imine linkages, as shown in Figure 5. Oxidized sucrose present in OSFS has four aldehyde groups whereas stachyose, the longer molecule, has eight aldehyde groups, and, in theory, all aldehyde groups can react with the amine groups present in keratin to form cross-links. This cross-linking leads to the formation of imine linkages. It is, however, very difficult to see formation of new imine linkages in the cross-linked fibers due to spectral complexity of the proteins.
control and cross-linked wool fibers from 1800 to 1000 cm$^{-1}$. As can be seen in Figure 4b, the spectrum of cross-linked wool fibers shows an additional small peak at 1040 cm$^{-1}$, which corresponds to the C$-$O stretch in C$-$OH as well as C$-$C stretch in the sugars.23 This confirms the incorporation of OSFS within wool fibers. A similar additional peak at 1049 cm$^{-1}$ was observed after cross-linking soy proteins with oxidized sugars.15 Jalaja and James observed a peak at 1030 cm$^{-1}$ after cross-linking gelatin with oxidized sucrose.27 The shift in the amide I peak at 1628 cm$^{-1}$ shows that the secondary structure of wool has changed after cross-linking.23 The shift in the amide I peak at 1628 cm$^{-1}$ shows that the secondary structure of wool has changed after cross-linking.23 It was observed that the amide II peak changed from the sharp and narrow peak to a broad peak between 1510 and 1540 cm$^{-1}$ after cross-linking. A similar change in the amide II peak was observed when gelatin was cross-linked using glutaraldehyde.36 The cross-linking reaction between primary amine groups in collagen, and other proteins, typical to the Maillard reaction.15,36,38 Cross-linking of wool using OSFS was restricted to 140 and 150 °C because caramelization of sugars and subsequent pyrolysis is prominent at temperatures above 160 °C.53

Figure 6 shows typical stress–strain plots of control and cross-linked wool fibers. As seen in Figure 6, the stress–strain plots can be divided into three distinct regions: the initial Hookean region, yield region, and the postyield (strain hardening) region. Tensile properties of control and cross-linked fibers are summarized in Table 3. As seen in Figure 6, the initial Hookean region lies between 0 and 3.4% strain for both control and cross-linked fibers. This region exhibits a linear relationship between stress and strain. Wool protein, in the relaxed state, is called α-keratin, wherein the keratin molecules are unstrained and in their natural helical shape. At a low level of strain (~3.5%), the distortion involves extension of weaker bonds, such as hydrogen bonding within the amino acids (seen in Figure 5), Van der Waals forces, and Coulombic interactions.44 The folded α-helix structure of the fiber, hydrogen bonds between the helices, Coulombic interactions due to side chains, and some −COO$^{-}$ and $-$NH$_3^+$ groups oppose the distortion or strain. It was observed that the tensile stress of the fibers, at the end of the Hookean region, increased from 88 MPa to about 116 MPa, an increase of about 32%, after cross-linking with OSFS whereas the tensile strain reduced from 3.4 to 3.1%. Also, Young’s modulus of the fibers in the Hookean region increased from 2.5 to 3.9 GPa, an

Table 2. $L^*$, $a^*$, and $b^*$ Hunter Color Values of Control and Cross-Linked Fibers

| specimen       | $L^*$   | $a^*$   | $b^*$   |
|----------------|---------|---------|---------|
| control        | 78.02 ± 1.8 | −0.96 ± 0.02 | 3.80 ± 0.60 |
| wool-SFS       | 78.07 ± 2.1 | −0.98 ± 0.07 | 3.92 ± 0.98 |
| wool-OSFS 140  | 72.82 ± 2.4 | −1.01 ± 0.03 | 5.31 ± 1.49 |
| wool-OSFS 150  | 72.95 ± 2.3 | −1.02 ± 0.03 | 8.64 ± 3.01 |

As shown in Table 2, the control fibers showed $L^*$, $a^*$, and $b^*$ values of 78.02, −0.96, and 3.80, respectively. Wool fibers cross-linked using OSFS (wool-OSFS) showed significant increase in the $b^*$ (yellowness) values. The $b^*$ value increased from 3.80 for control fibers to 5.31 and 8.64 after cross-linking with OSFS at 140 and 150 °C for 20 min, respectively. The increase in $b^*$ after treating with OSFS is another evidence of cross-linking reaction between the oxidized sugars and the amino acids from wool keratin. The higher $b^*$ value for wool-OSFS at 150 °C (8.64) as compared to that for wool-OSFS at 140 °C (5.31) is due to the increased extent of cross-linking with the increase in the temperature. A similar change in color was observed when dialdehyde starch was used to cross-link amino groups in keratin. Since OSFS contains aldehyde groups due to caramelization of sugars, the yellow/brown coloration after cross-linking the proteins present in wool, zein, gelatin, soy protein isolate, soy flour, collagen, and other proteins, typical to the Maillard reaction.

Two types of browning have been observed after heating of sugars. The first one is caramelization, caused by heating of sugars, which breaks down the molecules giving the yellow/brown color. The second is the Maillard reaction, in which the browning is caused by heating reducing sugars in the presence of protein (amino groups). Reducing sugars in OSFS, such as fructose and glucose, contain aldehyde groups in the open-chain form, whereas nonreducing sugars, such as sucrose, stachyose, and raffinose, contain aldehyde groups due to oxidation. The Maillard reaction between aldehyde groups in OSFS and amino groups in keratin causes the increase in the $b^*$ value. To confirm that the change in color was due to the Maillard reaction (and not caramelization), wool sliver was treated with pure SFS solution at 150 °C for 20 min. The pictures of the treated wool slivers are shown in the Supporting Information (Figure S1). As can be seen from the Figure S1 and in Table 2, the $b^*$ value of SFS-treated wool (wool-SFS) sample is close to that of the pure wool sample, showing no evidence of caramelization. Thus, the increase in $b^*$ value for OSFS-treated samples proves that the browning is due to the Maillard reaction.15,38,42

Figure 6. Typical stress–strain plots for control and cross-linked wool fibers.
Table 3. Tensile Properties of the Control and Cross-linked Fibers

| specimen  | diameter (μm) | stress (MPa) | strain (%) | modulus (GPa) | stress (MPa) | strain (%) | modulus (GPa) | stress (MPa) | strain (%) | modulus (GPa) |
|-----------|---------------|--------------|------------|---------------|--------------|------------|---------------|--------------|------------|---------------|
| control   | 19.5 ± 1.8    | 88.2 ± 30.4  | 3.4 ± 1.4  | 119.8 ± 50.3  | 145.5 ± 30.3 | 3.9 ± 1.3  | 203.0 ± 40.8  | 276.0 ± 54.5 | 41.8 ± 5.9  | 203.0 ± 40.8  |
| cross-linked | 19.0 ± 1.3    | 116.8 ± 37.3 | 3.1 ± 1.3  | 158.5 ± 46.2  | 185.5 ± 50.3 | 3.9 ± 1.3  | 195.0 ± 50.2  | 276.0 ± 56.7 | 41.8 ± 5.9  | 203.0 ± 40.8  |

Beyond the initial 3% strain, the strain increases rapidly for a small increase in the stress. This region is called the “yield region”. The overall stress in the yield region increased from 88 MPa for control fibers to over 119 MPa, an over 35% increase, for cross-linked fibers. At the same time, the yield region, which extended from 3.4 to 25.3% for control fibers, changed from 3.1 to 21.7% for cross-linked fibers and the stress at the yield point increased from 117 to 146 MPa. The reduction in the tensile strain, from 25.3% for control to 21.7% for cross-linked fibers confirms the formation of cross-links between the peptide chains that restrict the molecular movement. The modulus in the yield region was also found to increase from 0.18 to 0.27 GPa after cross-linking (50% improvement in the modulus).

Beyond the yield region, the wool fibers stiffen rapidly. This region is called the postyield region, and the stiffening phenomenon is called strain hardening. The postyield region terminates on the rupture of the fiber. As seen in Figure 6, the strain-hardening phenomenon in the postyield region is more prominent in the cross-linked fibers as compared to that in the control fibers. The tensile fracture stress of the fibers increased from 203 to 276 MPa after cross-linking, a 36% increase. The tensile strain was found to reduce from 47.4 to 41.8% after cross-linking. The secant modulus for the strain-hardening region increased from 0.35 to 0.53 GPa (51.4% increase) after cross-linking. Unpaired t-test showed that the increase in the modulus for all three regions after cross-linking of the fibers was statistically significant at the significance level of 0.05. As mentioned earlier, OSFS contains a mixture of different sugars having aldehyde groups. The major sugars present in OSFS sucrose and stachyose form tetra-aldehyde and octa-aldehyde, respectively, with different molecular lengths. This makes it easy to form various cross-links with the protein side chains and allows forming a better three-dimensional network within the fiber, leading to an increase in the tensile stress and modulus in all regions of the fiber stress–strain plots. Hassan et al. cross-linked wool fibers using four different cross-linkers and found that the tensile strength increased from 103 to 111.7, 115.2, 116.2, and 122 MPa for glyoxal, itaconic anhydride, naphthalene disulfonic acid, and succinic anhydride cross-linked wool fibers, respectively. They observed a maximum of 18.5% increase in the strength of the wool fibers after cross-linking with succinic anhydride. As seen earlier, cross-linking of fibers with OSFS showed an increase of about 36% in the tensile strength (from 203 to 276 MPa). This shows that the natural soy flour sugar-based green cross-linker is more effective in improving tensile properties of wool fibers than all other toxic bifunctional aldehyde-based cross-linkers currently used.
Keratin fibers have a tendency to absorb moisture, which plasticizes them and causes a decrease in Young’s modulus. The moisture content of the conditioned fibers reduced from 9.14% in control fibers to 6.6%, a decrease of about 28%, after cross-linking. As expected, the three-dimensional network obtained by cross-linking creates a more compact structure that acts as a moisture barrier. Reduced moisture absorption is beneficial since it can reduce the effect of moisture on fiber tensile properties. The cortex of the wool fiber is composed of ortho and para cortical cells. The para cortical cells contain disulfide (S–S) cross-links resulting from cystine amino acid, whereas the ortho cortical cells do not have S–S covalent cross-links, allowing them to absorb more moisture compared with para cortical cells. This results in ortho cortical cells swelling and elongating more than para cortical cells, which causes the crimp in the fiber. Absorbing less water could automatically reduce the undesired issues related to crimp.

**Surface Characteristics of Control and Cross-linked Wool Fibers.** Figure 7 shows scanning electron microscopy (SEM) images of the surfaces of the control and cross-linked wool fibers taken at different magnifications. Figure 7a,b shows control wool fibers with scales on the surface. These scales form the cuticle layer on the fiber surface. Figure 7c,d shows the surfaces of cross-linked fibers. When compared, the cross-linked fibers do not show any effect on the scalar structure of the fiber cuticle. No visible change or damage of scales can be
observed after cross-linking the fibers with OSFS. Oxidized sugar molecules from OSFS are small molecules that can penetrate inside the cortex of the fiber and cross-link them internally, enhancing the tensile properties, while leaving the surface unchanged.

Figure 8 shows SEM images of the fractured ends of control and cross-linked fibers taken at different magnifications. As seen in Figure 8, the fracture surfaces of both control and cross-linked fibers show similar fracture characteristics. It can also be seen from the tensile plots of the fibers (Figure 6) that the fibers do not fracture in a stepwise fashion but undergo a catastrophic failure after the strain hardening.

■ CONCLUSIONS

The present study has successfully demonstrated that the tensile performance of the wool fibers can be enhanced significantly using a green bio-based cross-linker. The utilization of SFS, a by-product with no potential application, showed promising results after oxidizing it to a polyaldehyde. This valorizes the by-product from soy processing industry and reduces the waste. The presence of different sugars in SFS was found to be beneficial by not only providing multiple aldehyde groups but also different molecular lengths, increasing the cross-linking efficiency. The higher cross-link density within the wool fiber improves its strength and modulus significantly. The room-temperature extraction and oxidation process used in this study is also an energy efficient way of making a natural, bio-based cross-linker for protein-based polymers. The availability of SFS at a very low cost and ease of oxidation reaction make it scalable at the commercial level. The method presented here can be easily extended for cross-linking other protein-based materials. The OSFS prepared in this study can easily replace currently used toxic cross-linkers, such as glyoxal and glutaraldehyde. Enhanced tensile properties of wool fibers can not only increase the efficiencies of wool fiber spinning and weaving by reducing breakages but can also reduce yarn and fabric defects. More importantly, stronger wool can allow spinning finer yarns from the same fibers, increasing their value significantly.

■ EXPERIMENTAL SECTION

Materials. Wool fibers in sliver form and defatted soy flour (SF) were provided by Raymond Woolen Mills, India and Archer Daniels Midland Co., Decatur, IL, respectively. Sodium periodate ≥ 99% and stachyose were purchased from Acros Organics, Bound Brook, NJ. Barium dichloride (BaCl2) was purchased from VWR, Rochester, NY. Glucose, fructose, sucrose, and raffinose were purchased from Sigma-Aldrich. Analytical grade sodium hydroxide (NaOH) pellets and hydrochloric acid 37% reagent grade (HCl) were also purchased from Sigma-Aldrich Chemical Co., Allentown, PA.

Extraction of Sugars from SF. SF (65 g) was added slowly to 400 mL of deionized (DI) water and stirred at 300 rpm at room temperature (RT) until a homogeneous SF mixture was obtained. The pH of the mixture was adjusted to 4.5 using HCl. At 4.5 pH, most of the amino acids present in the proteins from SF are at their isoelectric point and remain insoluble in water. The mixture was stirred overnight at 300 rpm at RT to dissolve all sugars present in SF into water. The sugars were then filtered using a microfiber polyester fabric to remove the insolubilized protein from SF. The pH of the filtered solution containing the sugars was then adjusted to 5.5 using NaOH, stirred for 6 h at RT, and filtered again to remove the small amount of the remaining protein having amino acids with isoelectric points close to 5.5. The filtered solution containing soy flour sugars is termed SFS. Two-hundred and fifty milliliters of SFS was obtained after filtering twice. Assuming that we extract 30% residual sugars from the SF, the final concentration of sugars after filtration in SFS was close to 5%.

Oxidation of SFS. Oxidation of SFS was carried out using NaIO4. Different molar ratios (MR), 0.5 to 2.5 MR, of NaIO4 to sugars were used to optimize the oxidizing reaction. (See Supporting Information Figure S2 for optimization of reaction.) The oxidation reaction was carried out in the dark for 22 h at RT with gentle stirring at 200 rpm. At the end of the reaction, the required amount of BaCl2 was added to the solution to stop sugars from further oxidation (see Supporting Information Figure S3). The solution was stirred for 5 min after addition of BaCl2 and then placed at 4 ºC for 1 h to allow complete precipitation of barium iodate Ba(IO3)2. It was then filtered to obtain the supernatant solution containing the mixture of oxidized SFS (OSFS). The pH of the prepared OSFS was found to be 3.

Wool Fiber Cross-linking. Wool sliver, 7.5 in. long, was cut and immersed in a flat form in the prepared OSFS solution for 10 min at RT in a rectangular Pyrex box. After 10 min of immersion in OSFS, the sliver was taken out and gently squeezed to remove excess solution. The wet sliver was immersed in the flat form again in the OSFS solution for 1 min at RT, then taken out and gently squeezed again to ensure uniform wet pickup by all fibers in the sliver with OSFS. The wet sliver was placed flat on a glass plate and cured in an air-circulated oven at 140 and 150 ºC for 20 min to allow the cross-linking between aldehyde groups from OSFS and amine groups in wool keratin, as shown later in Figure 4. The sliver was flipped upside down after the half curing time (10 min) in the oven to ensure a uniform treatment to all fibers. The sliver was taken out and placed flat in a Pyrex box containing DI water for washing. The cross-linked sliver was washed 2–3 times with water to remove all unreacted sugars from the fiber surface. The cross-linked fibers in the sliver were dried and conditioned at ASTM conditions of 65 ± 3% relative humidity (RH) and 21 ± 1 ºC for 24 h prior to any testing.

Characterization of SFS, OSFS, Control, and Cross-linked Wool. A complete 13C NMR analysis was performed to characterize SFS and various sugars present in SFS. The structural/chemical differences in SFS upon oxidizing were studied using 1H NMR. 13C and 1H NMR spectra were recorded on an INOVA 400 spectrometer (Varian Inc., Palo Alto, CA) using D2O as the solvent for both. Attenuated total reflectance Fourier-transform infrared (ATR-FTIR) analysis was done to characterize the effect of oxidation on SFS. ATR-FTIR was also used to study the cross-linking of wool. The ATR-FTIR spectra were collected using a Thermo Nicolet Magna-IR 560 spectrometer (Madison, WI) having a split pea accessory. Each scan was an average of 300 scans from 4000 to 500 cm−1 wavenumbers.

CIELAB color parameters of control and cross-linked wool fibers were measured using a Macbeth Color-eye spectrophotometer, Model M2020PT, (Newburgh, NY). The L*, a*, and b* values stand for L* = 0 (black) to L* = 100 (white), −a* (greenness) to +a* (redness), and −b* (blueness) to +b* (yellowness). A standard value for the white calibration tile
(L* = 95.91, a* = −0.43, b* = 1.01) was used to calibrate the spectrophotometer.

Tensile properties of single fibers were characterized using an Instron universal testing machine, model 5566 (Instron Corp., Canton, MA). Single wool fibers were individually mounted on rectangular paper tabs, and the two ends were secured using a self-adhesive tape. The diameters of every single fiber were measured using a calibrated optical microscope, Olympus, model BX51 (Melville, NY), at three different locations, and the average was used to calculate the tensile properties of each fiber. The fibers were conditioned and tested at 65 ± 3% RH and 21 ± 1 °C at a gauge length of 20 mm and a strain rate of 0.6 min⁻¹. Thirty single fibers were randomly chosen from different parts of the wool sliver of each type (control and cross-linked) for testing and statistical analysis. For the cross-linked fibers, 10 fibers were chosen from each of the three different slivers treated at different times using OSFS solutions prepared at different times to ensure reproducibility of the results. A Savitzky–Golay fitting was used to smooth the tensile stress–strain plots. Linear regression was performed on the smoothed plots to get accurate modulus values of the fibers. The unpaired t-test was used to test if the control and cross-linked fiber properties were statistically significant from each other.

To study the effect of cross-linking on the surface of the fibers and the fracture behavior of the control and cross-linked wool fibers, the surface and the fractured ends of the fibers fractured during the tensile tests were carefully mounted on standard aluminum stubs with double-sided electrically conductive carbon tapes and characterized using a Zeiss Gemini 500 scanning electron microscope, Germany, at 0.25 kV.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b00055.

Control and treated wool fibers; optimization of oxidation reaction parameters: effect of addition of OSFS with different molar ratios of NaIO₄/SFS (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**

*E-mail: ann2@cornell.edu.

**ORCID**

Anil N. Netravali: 0000-0002-2391-7116

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This research was partially supported by New York Corn & Soybean Growers Association (NYCSGA). The use of Cornell Center for Materials Research (CCMR) shared facilities supported through the NSF MRSEC program (DMR 1719875) and of the Department of Fiber Science & Apparel Design facilities are also acknowledged.

**REFERENCES**

(1) Chen, D.; Tan, L.; Liu, H.; Tang, F.; Hu, J.; Li, Y. Fabrication of Fast-Absorbing and Quick-Drying Wool Fabrics with Good Washing Durability. *ChemSusChem* 2010, 3, 1031–1035.

(2) Chen, D.; Tan, L.; Liu, H.; Hu, J.; Li, Y.; Tang, F. Fabricating superhydrophilic wool fabrics. *Langmuir* 2009, 26, 4673–4679.

(3) Johnson, N. A.; Russell, I. *Advances in wool technology*; Elsevier: Woodhead Publishing: England, 2008.

(4) Prins, M. Advances in Wool Spinning Technology. In *Advances in Wool Technology*; Elsevier: Woodhead Publishing, 2009; pp 86–108.

(5) Barani, H.; Calvimontes, A. Effects of oxygen plasma treatment on the physical and chemical properties of wool fiber surface. *Plasma Chem. Plasma Process.* 2014, 34, 1291–1302.

(6) Rahman, M. M.; Netravali, A. N. Green resin from forestry waste residue “Karanja (Pongamia pinnata) seed cake” for biobased composite structures. *ACS Sustainable Chem. Eng.* 2014, 2, 2318–2328.

(7) Corfield, M. I.; Robson, A. The amino acid composition of wool. *Biochem. J.* 1955, 59, 62.

(8) Di Modica, G.; Marzona, M. Cross-linking of wool keratin by bifunctional aldehydes. *Text. Res. J.* 1971, 41, 701–705.

(9) Song, K.; Xu, H.; Mu, B.; Xie, K.; Yang, Y. Non-toxic and clean crosslinking system for protein materials: Effect of extenders on crosslinking performance. *J. Cleaner Prod.* 2017, 150, 214–223.

(10) Xu, H.; Song, K.; Mu, B.; Yang, Y. Green and Sustainable Technology for High-Efficiency and Low-Damage Manipulation of Densely Crosslinked Proteins. *ACS Omega* 2017, 2, 1760–1768.

(11) Reddie, R.; Nicholls, C. Some reactions between wool and formaldehyde. *Text. Res. J.* 1971, 41, 841–852.

(12) Mohsin, M.; Farooq, U.; Raza, Z. A.; Ahsan, M.; Afzal, A.; Nazir, A. Performance enhancement of wool fabric with environment-friendly bio-cross-linker. *J. Cleaner Prod.* 2014, 68, 130–134.

(13) Milazzo, M.; Spina, F.; Cavallaro, S.; Bart, J. Sustainable soy biodiesel. *Renewable Sustainable Energy Rev.* 2013, 27, 806–852.

(14) Qu, K.; Netravali, A. N. “Green” Composites Based on Bacterial Cellulose Produced Using Novel Low Cost Carbon Source and Soy Protein Resin. In *Recent Advances in Adhesion Science and Technology in Honor of Dr. Kush Mittal*; CRC Press, 2014.

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

(16) Duus, J. Ø.; Gottfredsen, C. H.; Bock, K. Carbohydrate structural determination by NMR spectroscopy: modern methods and limitations. *Chem. Rev.* 2000, 100, 4589–4614.

(17) Fort, D. A.; Swatloski, R. P.; Moyna, P.; Rogers, R. D.; Moyna, G. Use of ionic liquids in the study of fruit ripening by high-resolution 13C NMR spectroscopy: green solvents meet green bananas. *Chem. Commun.* 2006, 714–716.

(18) Kazalaki, A.; Misiai, M.; Sypros, A.; Dais, P. Identification and quantitative determination of carbohydrate molecules in Greek honey by employing 13 C NMR spectroscopy. *Anal. Methods* 2015, 7, 5962–5972.

(19) Bubb, W. A. NMR spectroscopy in the study of carbohydrates: Characterizing the structural complexity. *Concepts Magn. Reson., Part A* 2003, 19, 1–19.

(20) Barclay, T.; Ginic-Markovic, M.; Johnston, M. R.; Cooper, P.; Petrovsky, N. Observation of the keto tautomer of D-fructose in D2O using 1H NMR spectroscopy. *Carbohydr. Res.* 2012, 347, 136–141.

(21) Jarrell, H. C.; Conway, T. F.; Moyna, P.; Smith, I. C. Manifestation of anomeric form, ring structure, and linkage in the 13C-NMR spectra of oligomers and polymers containing D-fructose: maltulose, isomaltulose, sucrose, levulose, 1-ketose, nystose, inulin, and grass levan. *Carbohydr. Res.* 1979, 76, 45–57.

(22) Obendorf, R. L.; Sensenig, E. M.; Wu, J.; Ohashi, M.; O’Sullivan, T. E.; Kosina, S. M.; Schenbly, S. R. Soluble carbohydrates in mature soybean seed after feeding D-chiro-inositol, myo-inositol, or D-pinitol to stem-leaf-pod explants of low-raffinose, low-stachyose lines. *Plant Sci.* 2008, 175, 650–655.

(23) Anjos, O.; Campos, M. G.; Ruiz, P. C.; Antunes, P. Application of FTIR-ATR spectroscopy to the quantification of sugar in honey. *Food Chem.* 2015, 169, 218–223.
(24) Lin, C.-A.; Ayvaz, H.; Rodriguez-Saona, L. E. Application of portable and handheld infrared spectrometers for determination of sucrose levels in infant cereals. Food Anal. Methods 2014, 7, 1407−1414.
(25) Bureau, S.; Ruiz, D.; Reich, M.; Gouble, B.; Bertrand, D.; Audergon, J.-M.; Renard, C. M. Application of ATR-FTIR for a rapid and simultaneous determination of sugars and organic acids in apricot fruit. Food Chem. 2009, 115, 1133−1140.
(26) Xu, H.; Canisag, H.; Mu, B.; Yang, Y. Robust and flexible films from 100% starch cross-linked by bio-based disaccharide derivative. ACS Sustainable Chem. Eng. 2015, 3, 2631−2639.
(27) Jalaja, K.; James, N. R. Electrospun gelatin nanofibers: a facile cross-linking approach using oxidized sucrose. Int. J. Biol. Macromol. 2015, 73, 270−278.
(28) Ghosh, B.; Jones, A. D. Profiling, characterization, and analysis of natural and synthetic acylsugars (sugar esters). Anal. Methods 2017, 9, 892−905.
(29) Liu, P.; Xu, H.; Mi, X.; Xu, L.; Yang, Y. Oxidized Sucrose: A Potent and Biocompatible Crosslinker for Three-Dimensional Fibrous Protein Scaffolds. Macromol. Mater. Eng. 2015, 300, 414−422.
(30) Xu, W.; Ke, G.; Wu, J.; Wang, X. Modification of wool fiber using steam explosion. Eur. Polym. J. 2006, 42, 2168−2173.
(31) Yao, J.; Liu, Y.; Yang, S.; Liu, J. Characterization of Secondary Structure Transformation of Stretched and Slenderized Wool Fibers with FTIR Spectra. J. Eng. Fibers Fabr. 2008, 3, No. 155892500800300.
(32) Rahman, M. M.; Netravali, A. N. Micro-fibrillated cellulose reinforced eco-friendly polymeric resin from non-edible ‘Jatropha curcas’ seed waste after biodiesel production. RSC Adv. 2016, 6, 47101−47111.
(33) Lodha, P.; Netravali, A. N. Thermal and mechanical properties of environment-friendly ‘green’ plastics from stearic acid modified-soy protein isolate. Ind. Crops Prod. 2005, 21, 49−64.
(34) Chabba, S.; Matthews, G.; Netravali, A. N. ‘Green’ composites using cross-linked soy flour and flax yarns. Green Chem. 2005, 7, 576−581.
(35) Li, R.; Wang, D. Preparation of regenerated wool keratin films from wool keratin−ionic liquid solutions. J. Appl. Polym. Sci. 2013, 127, 2648−2653.
(36) Nguyen, T.-H.; Lee, B.-T. Fabrication and characterization of cross-linked gelatin electro-spun nano-fibers. J. Biomed. Sci. Eng. 2010, 3, 1117.
(37) Park, S.; Bae, D.; Rhee, K. Soy protein biopolymers cross-linked with glutaraldehyde. J. Am. Oil Chem. Soc. 2000, 77, 879−884.
(38) 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.
(39) Happich, W.; Winds, W.; Naghsji, J. Stabilization of wool by glutaraldehyde. Text. Res. J. 1965, 35, 850−852.
(40) Song, F.; Tang, D.-L.; Wang, X.-L.; Wang, Y.-Z. Biodegradable soy protein isolate-based materials: a review. Biomacromolecules 2011, 12, 3369−3380.
(41) Wang, L.-F.; Rhim, J.-W. Preparation and application of agar/alginate/collagen ternary blend functional food packaging films. Int. J. Biol. Macromol. 2015, 80, 460−468.
(42) Bhat, R.; Karim, A. Towards producing novel fish gelatin films by combination treatments of ultraviolet radiation and sugars (ribose and lactose) as cross-linking agents. J. Food. Sci. Technol. 2014, 51, 1326−1333.
(43) Jiang, B.; Liu, Y.; Bhandari, B.; Zhou, W. Impact of caramelization on the glass transition temperature of several caramelized sugars. Part I: chemical analyses. J. Agric. Food Chem. 2008, 56, 5138−5147.
(44) Feugelmam, M. Mechanical Properties and Structure of Alpha-Keratin Fibres: Wool, Human Hair and Related Fibres; UNSW Press, 1997.
(45) Popescu, C.; Wortmann, F.-J. Wool—Structure, Mechanical Properties and Technical Products Based on Animal Fibres. In Industrial Applications of Natural Fibres: Structure, Properties and Technical Applications; John Wiley & Sons, 2010; pp 255−266.
(46) Hassan, M. M.; Schiermeister, L.; Staiger, M. P. Sustainable Production of Carbon Fiber: Effect of Cross-Linking in Wool Fiber on Carbon Yields and Morphologies of Derived Carbon Fiber. ACS Sustainable Chem. Eng. 2015, 3, 2660−2668.
(47) Hossain, K. M. G.; González, M. D.; Juan, A. R.; Tzanov, T. Enzyme-mediated coupling of a bi-functional phenolic compound onto wool to enhance its physical, mechanical and functional properties. Enzyme Microb. Technol. 2010, 46, 326−330.
(48) Mayo, J., Jr.; Wetzel, E. Cut resistance and failure of high-performance single fibers. Text. Res. J. 2014, 84, 1233−1246.