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Water-resistant plant protein-based nanofiber membranes

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ABSTRACT: Developing green and sustainable alternative materials to replace petroleum based ones is the need of the day. Such green materials are becoming popular because they can be composted once their useful life is over. In the current research, protein-based nanofibers were fabricated without the use of any toxic cross-linking agent. Defatted soy flour was purified using an acid-wash process to obtain material with higher protein content, blended with gluten, and successfully electrospun into nanofibers with the help of polyvinyl alcohol. Oxidation of sucrose with hydrogen peroxide (H_2O_2) was carried out to synthesize oxidized sugar-containing aldehyde (—CHO) groups and used as green cross-linker. The cross-linking quality of protein-based nanofibers modified by oxidized sugar was found to be similar to nanofibers cross-linked using toxic glyoxal and show good resistance to water. These novel green protein-based nanofibers can be useful in fabricating inexpensive products with very high specific surface area and highly porous structure. © 2015 The Authors Journal of Applied Polymer Science Published by Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41852.

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INTRODUCTION

Nanofiber-based membranes have been used in myriad of applications.[1–3] Nearly, all currently used membranes are made using nonbiodegradable polymers derived from petroleum.[4–6] For such nonbiodegradable materials, there are no environmentally acceptable end-of-life solutions as of now. Most of them, unfortunately, end up in landfills. Availability of environment-friendly, biodegradable, and fully sustainable plant-derived polymers such as proteins, starches, and cellulose have slowly begun to change this scenario. Plant-derived proteins and starches also tend to be inexpensive compared to petroleum-based polymers. Other factors contributing to the current "Green Movement" are the abundant availability of the biomass and the possibilities of water-based "green" processing. These advantages have also resulted in developing "green" nanofiber-based membranes as replacement for petroleum-derived nondegradable ones that are being used at present.[7–10] In contrast to the materials derived from petroleum, most plant-based materials can be easily composted after their intended life without harming the nature.

Electrospinning is a simple, low-cost, efficient technique to produce nanofibers.[11,12] It utilizes a high electrostatic field to generate nanofibers from a polymer fluid. Electrospun nanofibers often show large surface-to-weight (volume) ratio, high porosity, and excellent pore interconnectivity. These unique features together with the functionality from the material have opened up enormous potential to use nanofibers in diverse fields such as filtration, tissue engineering, sound absorption, or medicine.[13–16] In most of these cases, the nanofibers can be made using green polymers as well. Several reviews on electrospun biobased materials have been published in the past few years.[17–19] Most of the reported studies, however, are based on polypeptide-based materials such as silk fibroin, collagen, and chitosan. Most of these materials are expensive, and hence, they are used only in niche biomedical applications rather than mass-scale commodity-type applications.[20] Substituting these materials with inexpensive plant-based proteins can provide the means for overcoming some of the cost challenges and can also expand their applications. For example, soybean is one of the most abundant crops grown in the world and the protein derived from it is available commercially in

three different forms, namely defatted soy flour (SF), soy protein concentrate (SPC), and soy protein isolate (SPI). The SF obtained after extracting oil from soybeans contains about 52% protein, the SPC is the next purified version which contains 65–70% protein, and the most purified version, SPI, contains about 90% protein. The rest consists of carbohydrates, minerals, ash, and moisture. Of all these forms, SF is the least expensive (about \$0.50 kg^{-1}) variety. Further, a simple acid-wash process can be used to increase the protein content of SF to the level of about 70% found in SPC.[21] This method is based on precipitating the protein at its isoelectric point (pH 4.5) in water and removing most of the soluble nonprotein constituents, mostly low-molecular-weight carbohydrates. These constituents commonly include water-soluble and some low-molecular-weight nitrogenous substances and minerals. Gluten is another plant-derived material that contains high percentage of protein and is relatively inexpensive (about \$0.90 kg^{-1}). Gluten is composed of various proteins and is mostly obtained from wheat, barley, or rye. When wheat dough is washed to remove starch granules and water-soluble constituents, the rubbery mass that remains is termed as gluten. Depending on the thoroughness of washing, the dry solid gluten contains 75–85% protein and 5–10% lipids. The remaining 5–20% is nonstarch carbohydrates and starch. Gluten is unique in terms of the amino acid composition and contains high amounts of glutamic acid, proline, and low amounts of amino acids with charged side groups, including lysine and histidine.[22]

As mentioned earlier, there is great interest in developing green nanofiber membranes. Several studies have described production of nanofibers prepared by electrospinning of soy protein blends with polyvinyl alcohol (PVA), polylactic acid (PLA), zein or polyethylene oxide (PEO).[23–26] Despite the fact that protein-blend nanofibers have been reported recently, most of them are either water-soluble due to insufficient cross-linking or made insoluble in water by cross-linking using toxic agents. Cross-linking of polymers is one of the most common techniques to obtain enhanced resistance to water and to improve their physical and mechanical properties. Cross-linking is carried out using multifunctional cross-linking agents (cross-linkers) that are capable of chemically reacting with the functional groups present on proteins or other molecules. Protein structure is complex and contains several different amino acids with reactive groups. However, only a small number of functional groups can be targeted for cross-linking. In fact, only four protein functional groups account for most of the cross-linking modifications. These include the following: (a) primary amines (—NH_2) in lysine and arginine residues, (b) carboxyls (—COOH) in aspartic acid and glutamic acid, (c) hydroxyls (-OH) in serine, threonine tyrosine, and (d) sulfhydryls (—SH) in cysteine. For each of these functional or reactive groups present in proteins, there exist many reactive groups that can react with them and form a three-dimensional cross-linked structure.[27] Most commonly used cross-linkers for amine groups are bi-functional compounds, such as glutaraldehyde or glyoxal.[28,29] Both of these cross-linkers, however, are toxic and inappropriate from the environmentally-friendly point of view, and hence, green cross-linkers are preferred. The oxidized sugars (OS) have been found to be useful in such cases and are regarded as green cross-linkers for soy and other protein-based resins.[30–32]

Oxidized sugars are carbohydrates that are oxidized by weak oxidizing agents to generate compounds containing reactive aldehyde or carboxyl groups. The aldehyde groups in OS can cross-link the nucleophilic amino groups in protein-based resins utilizing the Maillard reaction and form bonds responsible for nondisintegration of soy protein-based resin in water.[33] Since OS can have multiple aldehyde groups, they can react with different protein molecules forming a cross-linked system. One of the major advantages of this reaction is that it can be carried out in an aqueous medium.

In this study, we report on the preparation and green cross-linking of very inexpensive protein-based nanofibers focusing on the use of SF. Being the least expensive source of the soy protein with the lowest content of protein, SF was used in this study. However, an acid-wash process was used to increase the protein content of SF to the level of about 70% found in commercial SPC. This "purified soy flour" (PSF) was used as the major constituent for fabrication of nanofiber membrane. The important key factors are as follows: (i) play significant role in electrospinning process of protein-based polymer solution and (ii) influence the morphology of resulted nanofiber membrane are summarized. Part of this study was to cross-link protein nanofibers by green cross-linkers to increase its moisture resistance and, thus, increase the durability. Sucrose was oxidized with H_2O_2 to synthesize OS which has been confirmed as a good cross-linker for protein-based nanofibers using Fourier transform infrared spectroscopy (FTIR) and solubility test. Finally, cross-linking quality of OS was compared with the properties of glyoxal cross-linked nanofibers. Such "green" nanofiber membranes may be used for filtration of dust, bacteria, or viruses and also in biotechnology applications.[34]

EXPERIMENTAL

Materials

Powdered PVA with molecular weight of 130,000 $g \cdot mol^{-1}$ was purchased from Sigma Aldrich, (St. Louis, MO), and gluten was purchased from MGP Ingredients, (Atchison, KS).

SF obtained from Archer Daniels Midland Company (Decatur, IL) was purified using an acid-wash process to obtain PSF with

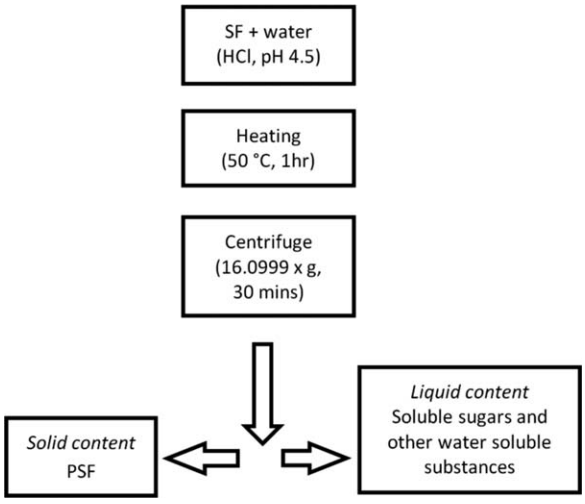

Figure 1. Scheme for purification of soy flour to obtain PSF.

higher protein content. About 10 g of soy flour was dissolved in 100 mL deionized (DI) water, and the solution pH was lowered to 4.5 using HCl. The acidified SF solution was afterward heated to 50°C for 1 hr. Soy protein becomes insoluble in water at its isoelectric point (pH 4.5) and the molecules precipitate, while the sugars remain soluble. As a result, sugars can be filtered out easily from the SF solution increasing its protein content. Centrifuging was found to be useful to separate most of the soluble sugars leaving a PSF residue with a high protein concentration. For this reason, the SF solution was centrifuged for 30 min at 16.099 × g, when the precipitated protein was obtained in solid form. The scheme for the SF purification process to obtain PSF is shown in Figure 1.

Synthesis of cross-linker OS was performed by oxidation of sucrose. The oxidation reaction was carried out for 30 min at 45°C with occasional shaking. Finally, the solution was heated in an oven for two days at 45°C to complete oxidation.

Electrospinning of Nanofibers

Polymer solutions with different compositions of PSF, PVA, and gluten were blended to obtain different protein contents. To obtain the highest possible protein content in nanofibers which contained minimum number or no polymer beads or other defects was one of the main aims of this study. PVA was initially dissolved in DI water at room temperature overnight to obtain a polymer concentration of 14% (by wt.). Gluten and PSF were individually dissolved in water, and the solution pH was adjusted to 11 using NaOH while being heated at 60°C for 30 min. This step was performed to denature the protein and open up the molecules. Solution concentrations for gluten and PSF were kept at 10 and 12% (by wt.), respectively. Thereafter, the individually prepared solutions were mixed together at room temperature in different volume composition to obtain the desired blend proportions of proteins/PVA and stirred for 2 hrs. Various combinations of polymer solutions used in the study are presented in Table I. Triton X-100 (0.5 wt %) was added to all solutions as a nonionic surfactant to obtain uniform dispersion of the protein molecules. Nanofiber membranes were prepared by needle electrospinning for all polymer compositions. All electrospinning experiments were carried out at an applied voltage of 25 kV, polymer solution flow rate of 0.015 mL·min^{-1} and an electrode-collector distance 15 cm. Electrospun nanofibers were deposited on a polypropylene spun-bonded substrate.

Cross-Linking of Protein Nanofibers

Two different cross-linkers, glyoxal and OS, were used to obtain gluten/PSF/PVA resin with higher stability in water. Glyoxal is commercially available but more toxic option, in contrast, the laboratory synthesized OS was used as the green option. Glyoxal is known to cross-link proteins[35] and was used as a benchmark for comparing the quality of the cross-linking of gluten/PSF/PVA nanofibers by OS.

Table I. Polymer Blends Used for the Preparation of Nanofiber Membranes

Polymer blend	Dry basis composition
PSF/PVA	[36/64]
PSF/PVA	[46/54]
Gluten/PVA	[46/54]
Gluten/PSF/PVA	[36/26/38]
Gluten/PSF/PVA	[30/25/45]

Aqueous solution of glyoxal (40%) and 85% phosphoric acid (H_3PO_4), used as catalyst, were purchased from VWR International. As mentioned earlier, PVA was dissolved in DI water to obtain 14% concentration. Glyoxal with H_3PO_4 were added to PVA solutions in three different concentrations 2 hrs before blending with gluten/PSF polymer solution. PVA with gluten/PSF polymer solution was stirred for 2 hrs at room temperature thereafter. Dry basis composition of the final polymer blend was gluten/PSF/PVA [30/25/45], and the amount of glyoxal used was 5, 10, and 15% (by wt.). The surfactant, Triton X-100, was added in the amount of 0.5% (by wt.) to all solution mixtures, as the final step.

As stated earlier, OS was tested as the green cross-linker. Firstly, OS was added to gluten/PSF solutions in different amounts, separately, and stirred for 1 hr at 70°C. PVA solution in required proportion was added to gluten/PSF polymer solution afterward and stirred for 2 hrs at room temperature. The final polymer-blend composition on dry weight basis of gluten/PSF/PVA was [30/25/45] with OS in varying amounts of 5, 10, and 15% (by wt.). As in the case of glyoxal, Triton X-100 as surfactant, 0.5% (by wt. of total solids), was added to all solution mixtures.

The cross-linking reaction was completed to the maximum extent possible under the experimental conditions by heating the nanofiber membranes in an oven at 100°C for 30 min.

Solubility of Protein-Based Nanofibers

Nanofiber membranes prepared from gluten/PSF/PVA with 0, 5, 10, and 15% (by wt.) of glyoxal and OS were fully dried at 60°C for 24 hrs prior to any characterization. The unreacted protein extractions (solubles) were carried out in DI water using Erlenmeyer flasks that were placed on a shaker table (MAXQ 4450, Thermo Scientific) at 175 rpm for: (i) 3 hrs at 60°C, (ii) 6 hrs at 80°C, and (iii) 1 month at room temperature (21°C). The solid residual after extraction was collected using a Whatman filter paper (no. 4, QTY) and dried to constant weight (60°C for 24 hrs). The content of insoluble part (gel), g (%), was calculated according to the following eq. (1):

$$g(\%) = \left(\frac{w_e}{w_d}\right) \times 100 \quad (1)$$

where w_d and w_e are the weights of dry samples before and after extraction, respectively.[36–38]

Other Characterization of Protein-Based Nanofibers

The surface morphologies of nanofibers were characterized using a scanning electron microscope (SEM), LEO 1550 FE-SEM, Zeiss, at an accelerating voltage of 15 kV.

FTIR in attenuated total reflectance (ATR-FTIR) was recorded by Nicolet Magna-IR 560 (Thermo Scientific spectrophotometer). ATR-FTIR spectra were taken in the range of 4000–550 cm^{-1} wave numbers using a split peak accessory. Each scan was an average of 64 scans obtained at a resolution of 4 cm^{-1}

Table II. Protein Content in SPC, SF, PSF, and Gluten Analyzed by Elemental Analysis Technique

Type of protein source	Protein content [%]
SPC	64.4
SF	52.2
PSF	66.1
Gluten	77.7

wave number. Reproducibility was confirmed by repeating the ATR-FTIR analysis three times for each specimen prepared at different times. The spectra of sucrose and OS as well as nanofiber membranes made of gluten/PSF/PVA with 0, 5, and 10% OS before and after cross-linking were compared.

RESULTS AND DISCUSSION

Purification of SF

The protein contents in the SF, gluten, and laboratory prepared PSF were measured by elemental analysis. The average protein content values obtained from three separate tests are presented in Table II. From the results, it is clear that the purification of SF carried out in the laboratory was successful as the protein content in PSF reached 66.1%, up from 52% in SF and was comparable to the protein content of 65–70% found in commercial SPC. The repeatability of the purification process was confirmed by measuring the protein content of three independent purification tests. The standard deviation for the protein content for PSF was 1% of the average protein content value. Based on these results, it was concluded that it is possible to prepare PSF of consistent quality and comparable to the commercially available SPC, from SF using the acid-wash process. The lost material during the purification of SF amounted to about 44%. Commercial gluten was used without modification, since it contained high protein content of over 77%.

Morphology of Protein-Based Nanofibers

The electrospinning was performed to investigate the ability of nanofiber formation from polymer solutions that contained different amounts of proteins. As mentioned earlier, one of the main goals of this paper was to obtain the highest possible protein content in the nanofiber membranes with the least amount of beads and/or other defects. Pure PSF polymer solution heated to 60°C for 30 min and alkali-treated (pH 11) could not be spun into nanofibers. The continuous and uniform fiber formation of pure PSF by electrospinning process was seen as difficult, perhaps due to the complex helical conformations of soy protein in the aqueous solution.[40,41] However, when PVA, a linear polymer, was added, as a "helper polymer," to form PSF/PVA or gluten/PSF/PVA solutions, they were readily electrospun into nanofibers. Figures 2(a–e) show SEM images of nanofibers formed by PSF/PVA [46/54], [36/64], gluten/PVA [46/54], and gluten/PSF/PVA [36/26/38], or [30/25/45] compositions, respectively. As can be seen from Figure 2, electrospinning of PSF/PVA [46/54] or gluten/PVA [46/54] solutions led to nanofiber structure that contained a few polymer beads. The best nanofiber structure without polymer beads, however, was formed in the case of PSF/PVA [36/64] blend. To reach the maximum protein content in the nanofiber membrane, gluten/PSF/PVA compositions were electrospun. Nanofiber structure without any defect was obtained for gluten/PSF/PVA composition with dry basis close to [30/25/45]. Unfortunately, a decrease in PVA content in the polymer-blend gluten/PSF/PVA to [36/26/38] led to fiber structure with beads. The introduction of PVA in the mixed solution gluten/PSF/PVA increased the solution viscosity because of the ionic interactions between polymer molecules, which increased charge density of solution and led to uniform nanofibers without any bead formation.[42] These observations indicate that help from PVA is required to produce protein-based nanofibers, perhaps because of the helical nature of the protein. Nevertheless, protein content of up to 55% could be elctrospun into good nanofibers as shown in Figure 2(e).

Electrospinnable blend of gluten/PSF/PVA [30/25/45] was chosen for the cross-linking study because of its highest content of protein in the nanofibers and their good morphology. Two different cross-linkers (i) glyoxal and (ii) OS were used as mentioned earlier.

Electrospinning of gluten/PSF/PVA composition with 5, 10, and 15% of glyoxal led to very similar fiber structure and contained only a few polymer beads as can be seen in Figure 3. The beads formed during electrospinning process are possibly due to partially cross-linked polymer that is unable to straighten out. Electrospinning of gluten/PSF/PVA solution with 5, 10, and 15% of

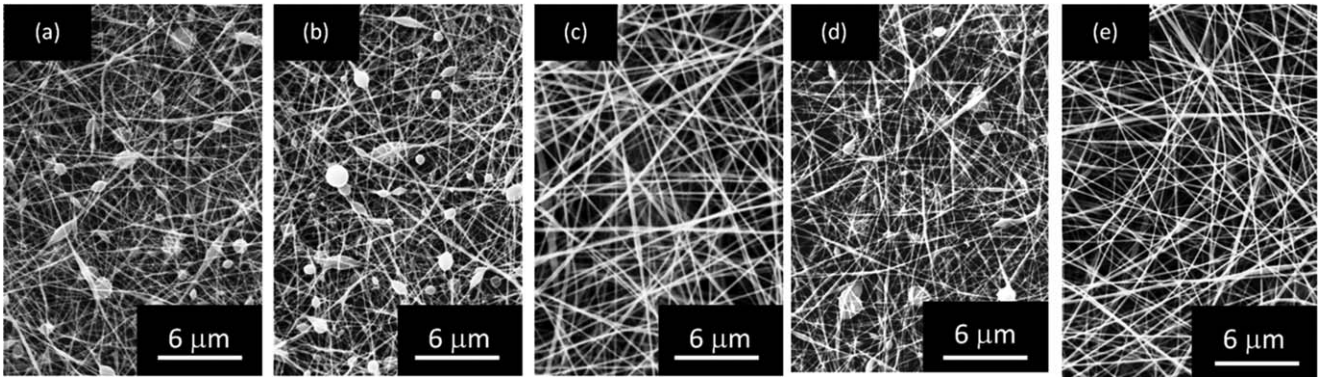

Figure 2. Nanofiber membranes consisting of (a) PSF/PVA [46/54], (b) gluten/PVA [46/54], (c) PSF/PVA [36/64], (d) gluten/PSF/PVA [36/26/38], and (e) gluten/PSF/PVA [30/25/45].

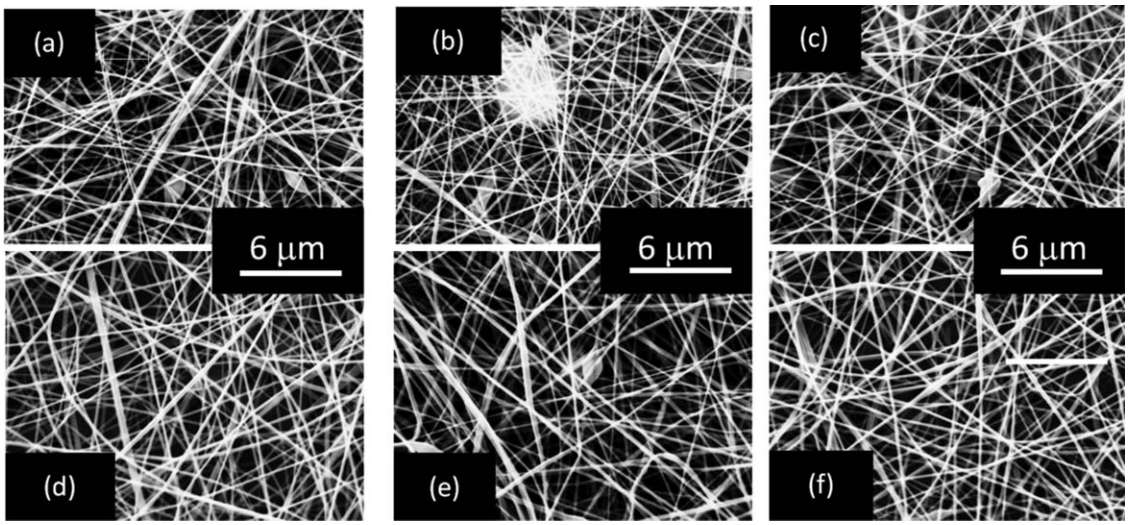

Figure 3. Nanofiber membranes gluten/PSF/PVA [30/25/45] cross-linked by glyoxal: (a) 5 wt %, (b) 10 wt %, and (c) 15 wt % and by OS: (d) 5 wt %, (e) 10 wt %, and (f) 15 wt %.

OS, however, led to the most uniform nanofiber structure with no polymer beads. It is clear from Figures 3(d–e) that the fibers are uniform, cylindrical shaped, and exhibit a narrow range of fiber diameters. This is perhaps because of the lower cross-linking density obtained with OS compared to that obtained by glyoxal.

ATR-FTIR Characterization of OS

Aldehydes (—CHO) are reactive varieties of more general functional group, carbonyl (C═O). The polarity of this bond (especially in the context of aldehydes) makes the carbon atom electrophilic and reactive to nucleophiles such as primary amines. Aldehydes are often used to cross-link proteins that contain amine groups as is the case in the present study.

Aldehyde groups can be created from oxidizable sugar groups.[39] In this study, oxidation of sucrose was carried out by H_2O_2 to generate dialdehyde molecules, which was used as the green cross-linker for gluten/PSF/PVA nanofibers. ATR-FTIR spectra of sucrose before and after the H_2O_2 oxidation are shown in Figure 4. The spectrum clearly shows the absorption peak at 1720 cm^{-1} which corresponds to carbonyl peaks from the oxidation of the primary alcohols to aldehydes. This peak is absent in the unreacted sugar which do not have carbonyl groups. It is also possible that OS contains some carboxyl (—COOH) groups which also results in absorption at 1720–1725 cm^{-1}. The aqueous process along with the use of H_2O_2 as a green and harmless oxidizing reagent for converting sucrose into a cross-linker can be considered as a "green" process as noted by earlier researchers.[30]

Chemical Characterization of Cross-Linked Protein-Based Nanofibers

Figure 5 shows ATR-FTIR spectra of gluten/PSF/PVA nanofiber membranes without cross-linker and after cross-linking reaction with 5 and 10 wt % of OS. The ATR-FTIR analysis of the nanofiber membranes was based on the identification of absorption bands related to the functional groups present in gluten, PSF, PVA, and OS.

A broad band at 3050–3550 cm^{-1} corresponds to hydroxyl (—OH) stretching vibration resulting from the presence of amino acids containing —OH and —COOH groups in proteins as well as the —OH groups in PVA and OS. These groups are

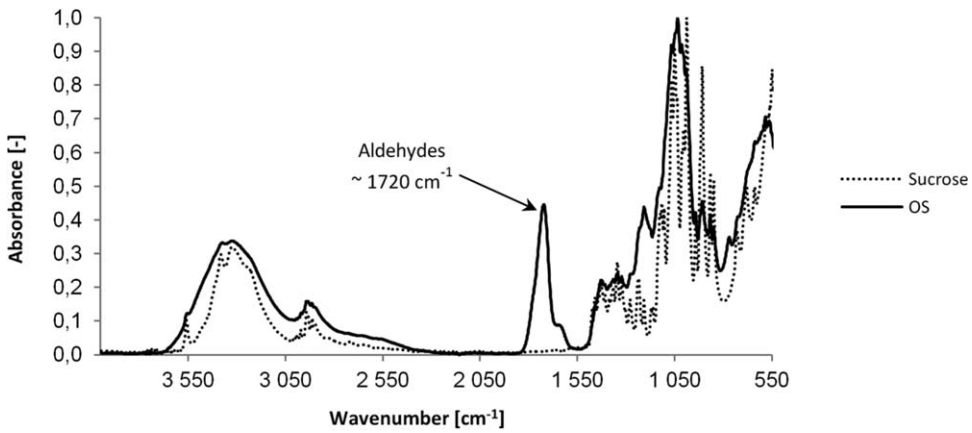

Figure 4. ATR-FTIR spectra of sucrose and OS.

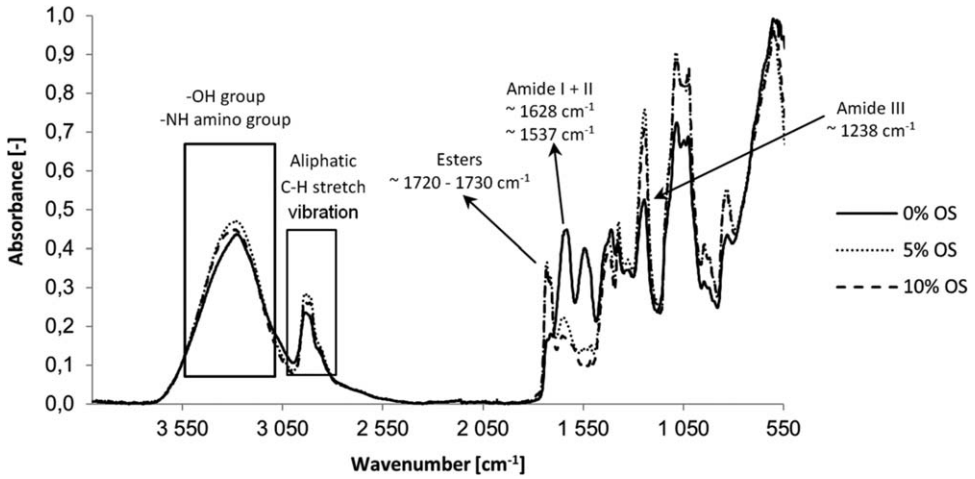

Figure 5. ATR-FTIR spectra of gluten/PSF/PVA nanofiber membranes before and after cross-linking with 5 and 10 wt % of OS.

capable of forming strong intra- and intermolecular hydrogen bonds, in PVA and soy protein as well as with the amino (—NH) groups in protein.[43,44] The absorption band observed between 2820 and 3000 cm^{-1} corresponds to the aliphatic C—H bond (stretching) in PVA as well as protein.[45,46] A typical soy protein spectrum consists of three major peaks, 1628 cm^{-1} corresponding to amide I band (associated with the C—O stretching vibration), 1537 cm^{-1} corresponding to amide II band (N—H deformation) and 1238 cm^{-1} assigned to amide III band (C—N stretching and N—H vibration). ATR-FTIR spectrum of the gluten/PSF/PVA nanofiber membranes without cross-linker in Figure 5 confirms these peaks and agrees well with earlier observations by others.[47,48]

The carboxylic acids formed during sucrose oxidation can also cross-link proteins via formation of anhydride, ester, or amide linkages.[49,50] As can be seen from the spectra shown in Figure 5, absorptions in the range of 1630 and 1530 cm^{-1} shifted down after cross-linking the nanofibers. These strong amide bands present in soy protein disappeared and a new absorption band at about 1720 cm^{-1} appeared after cross-linking assigned to ester peak. These spectral changes indicate cross-linking reaction between the amine groups in the protein and the aldehyde groups in the OS via formation of ester linkages. Because of the large number of amide linkages already present in the PSF, it is not possible to detect the formation of any additional amide bonds. Other formation of imine linkages by the reaction of amine groups with aldehyde is known as Maillard reaction.[30,51] In fact, nucleophilic varieties of primary amines ($—NH_2$) are the main class of compounds that react with aldehydes. Unfortunately, due to the overlap of several peaks in the fingerprint region, it is hard to detect this peak in the ATR-FTIR spectrum. However, the Maillard reaction can be easily confirmed using other characterization techniques including the color change. The Maillard reaction is also known as the nonenzymatic browning reaction and is responsible for the color changes in processed food such as bread, baguette, and most of the bakery products. It is also associated with the color changes that occur during food degradation.[51] The color of the gluten/PSF/PVA nanofiber membranes intensified from pale yellow to brown, as shown in Figure 6, with increasing concentration of OS in gluten/PSF/PVA. Thus, the change in color also indicates, qualitatively, the extent of Maillard reaction. Since this reaction is strongly dependent on reaction conditions such as duration and temperature of reaction, pH and type of sugar present, heating and addition of NaOH to gluten/PSF/PVA solution were carried out to stabilize the conjugation.[33] Addition of NaOH also denatures the protein, that is, opens up the molecules making the Maillard reaction easier.

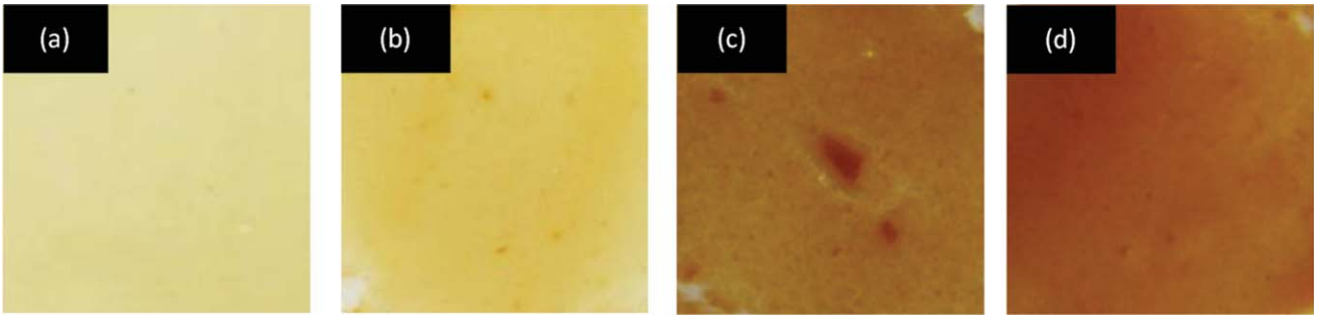

Figure 6. Photographs of nanofiber membranes obtained from gluten/PSF/PVA [30/25/45] with OS: (a) 0 wt %, (b) 5 wt %, (c) 10 wt %, and (d) 15 wt % after cross-linking. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

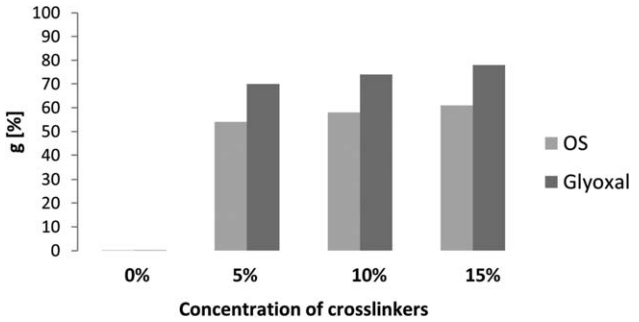

Figure 7. Insoluble content (g) % obtained by the solubility test of gluten/PSF/PVA nanofibers cross-linked by OS and glyoxal.

Solubility of Cross-Linked Protein-Based Nanofibers

The insoluble (cross-linked) content of a given nanofiber membrane was estimated by measuring its insoluble part in dried state after immersion in DI water for, 3 hrs at 60°C, 6 hrs at 80°C, and 1 month at room temperature (21°C). Figure 7 presents the solubility (g [%]) results for nanofibers cross-linked by different cross-linkers: 0, 5, 10, and 15 wt % of glyoxal and OS.

Nanofiber membranes prepared from gluten/PSF/PVA polymer composition without any cross-linkers disintegrated completely within 3 hrs when kept in water at the elevated temperature of 60°C. The results in Figure 7 show that the percentage of insoluble content, g (%), of the cross-linked gluten/PSF/PVA by 5, 10, or 15 wt % of glyoxal reached 70 to 78% depending on the glyoxal concentration. As can be expected, g (%) increased with higher glyoxal content. Since the main reaction occurs between the amine groups in protein and aldehyde groups in glyoxal and that the reaction with the hydroxyl groups in PVA is minimal, the g (%) of 70–78% is considered reasonable. As compared to glyoxal, approximately 54 to 61% of insoluble content was obtained for gluten/PSF/PVA using 5, 10, or 15 wt % of cross-linker OS. Nevertheless, the results of this test demonstrate that the OS did work as a good cross-linker, but the level of cross-linking was slightly lower in comparison to glyoxal. This is believed to be because of the higher number of aldehyde groups present in glyoxal offering more possibilities to link protein macromolecules. All tested nanofiber membranes (cross-linked) did swell when immersed in water, although they remained intact and unbroken as can be seen in Figure 8.

None of the nanofibers prepared from gluten/PSF/PVA composition cross-linked with glyoxal dissolved after any of the testing conditions of 3 hrs at 60°C, 6 hrs at 80°C or 1 month at room temperature. Nanofibers prepared from gluten/PSF/PVA composition with 5 wt % of OS did not dissolve in water after 3 hrs at the temperature 60°C as well. However, after 6 hrs at 80°C, they seem to begin to disintegrate. In contrast, nanofibers cross-linked by 10 and 15 wt % of OS did not dissolve when treated under the same conditions or after 1 month of water immersion at room temperature. Structures of nanofiber membranes immersed in water after 1 month at the room temperature are shown in Figure 8.

Typical SEM images presented in Figure 9 show changes in the nanofiber membrane morphology of gluten/PSF/PVA after cross-linking with 15 wt % of OS and water treatment for 6 hrs and 1 day at room temperature, respectively. Nanofibers can be clearly seen to have swollen after water immersion for 6 hrs compared to unsoaked nanofibers shown in Figures 2 and 3. However, the nanofiber membranes retained the fiber structure even after 1 day of water immersion test, although the pores almost disappared due to swelling.

To compare the water resistance of the plant protein-based green nanofiber membranes with the petroleum-based ones, it is important to realize that both water-resistant and water-soluble polymers made of petroleum are available and that the nanofiber membranes could be fabricated using both of them. This fact plays an important role for final applications. For example, water-resistant nanofibers are desirable in water filtration, whereas water-soluble nanofibers could play an important role in drug delivery systems.

Water-resistant petroleum-based nanofiber membranes with high mechanical properties and good water permeability have contributed in a major way in the water treatment.[52,53] In the past decade, numerous journal articles have documented nanofiber membranes for water treatment applications made from poly(vinyldifluoride),[54] poly(amide),[55] poly(ethersulfone),[56,57] poly(acrylonitrile),[58] etc. In these cases, petroleum-based polymers

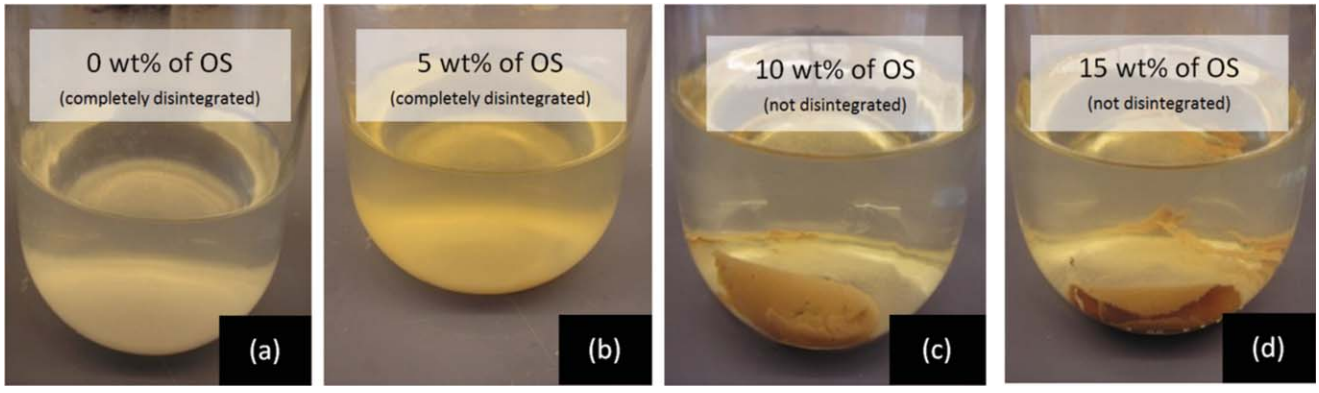

Figure 8. Cross-linked nanofiber membranes of gluten/PSF/PVA immersed in DI water for 1 month: (a) without cross-linker (control), (b) with 5 wt %, (c) with 10 wt %, and (d) with 15 wt % of OS. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

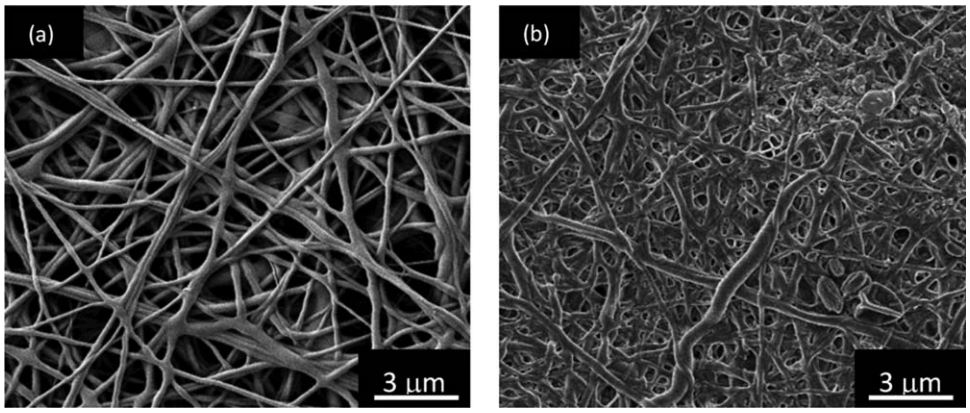

Figure 9. Cross-linked nanofiber membranes of gluten/PSF/PVA with 15 wt % of OS after: (a) 6 hrs of water immersion test and (b) 1-day water immersion test at room temperature.

are water-resistant and do not need any cross-linking. The nanofiber membranes made of these polymers did not change their morphology after immersion in DI water as can be expected.

On the other hand, there are hydrogels or water-soluble nanofiber membranes made from poly(ethylene-glycol), poly(vinyl-pyrrolidone), poly(vinyl-alcohol), poly(ester), poly(acrylic acid) which have been reported as well.[59–62] These polymers are not water-resistant. In these cases, the large surface area of nanofibers can be effective for controlled release of antibiotics or growth factors into wound while the high porosity of nanofiber mats allows rapid diffusion and absorption of body fluids and waste. Such types of petroleum-based nanofiber membranes are not or partially water-resistant which is desirable for this type of application.

In the present study, water-resistant inexpensive plant protein-based nanofiber membranes were prepared by cross-linking them with green cross-linker (OS). By controlling the cross-link density, it should be possible to control either the release rate and/or the nanofiber membrane degradation rate in water.

CONCLUSIONS

The present research discusses fabrication of novel and inexpensive plant protein-based nanofiber membranes prepared by electrospinning process. The SF was successfully purified using an acid-wash process to increase the protein content and used as the major constituent material along with gluten for electrospinning process and successfully spun into nanofiber membranes with no polymer beads or other defects. PVA, a linear polymer, was used as the "helper material" for easier electrospinning. The higher stability of nanofiber membrane in water was achieved by cross-linking reaction without the use of any toxic cross-linkers. OS, prepared by a benign H_2O_2 oxidizing process, was confirmed as a green cross-linker for protein-based nanofiber membranes by ATR-FTIR analysis and solubility test. The stability of protein-based nanofiber membranes in water was confirmed by water immersion test. However, if kept away from water, such nanofiber structures can last for a long time. Also, while the cross-linking achieved by OS was slightly lower than that achieved by glyoxal, the nanofibers do show good resistance to water.

The use of such nanofiber-based membranes for filtering fine dust, bacteria, and possibly, viruses is very promising.[34] These protein-based nanofiber membranes may also be used with other natural resins to develop composite materials with higher value-added products. Finally, some biotechnology applications also seem to be very interesting and promising. To outline future prospects, products based on this type of nanofiber membranes could be promising for tissue engineering, wound healing, or biosensors as well.

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