

Improving Wet Strength of Soy Protein Films Using Oxidized Sucrose

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ABSTRACT: The chemical modification of soy protein isolate (SPI) with various amounts of oxidized sucrose was performed in this study. The poor mechanical properties and lack of hydrolysis resistance of SPI have limited its applications in various fields. Although chemical modification proved to be an effective method to enhance the properties of SPI films, current SPI modifiers are either expensive, toxic, or do not impart the satisfiable properties to the modified materials. In this research, the possibility of modification of SPI films using oxidized sucrose to improve their strength and stability was examined. At optimal conditions, oxidized sucrose-modified SPI films showed about 50% higher wet strength than the control films. The melting temperature of modified SPI film was 26°C higher than the unmodified control. © 2014 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2015**, *132*, 41473.

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INTRODUCTION

Because of the increasing concerns in terms of sustainability and pollution, there has been increasing interest in developing renewable and biodegradable packaging materials.¹ As an abundant product of the soybean oil industry, soy protein isolate (SPI) has attracted much attention owing to its good film-forming ability, low cost, availability, and biodegradability.^{1–3} However, SPI is hydrophilic and prone to hydrolysis, leading to poor wet mechanical properties.⁴ Property modifications of SPI-based films have been investigated to solve this problem. Among these are physical modifications such as blending with hydrophobic polymers,^{5–7} or adding fillers^{8–10} and chemical modifications based on crosslinking with glutaraldehyde, formaldehyde, or glyoxal.^{11–15} Chemical modification has proved to be effective.¹⁶ However, the toxic modifiers render the films inedible and inappropriate for use in food or drug packaging.

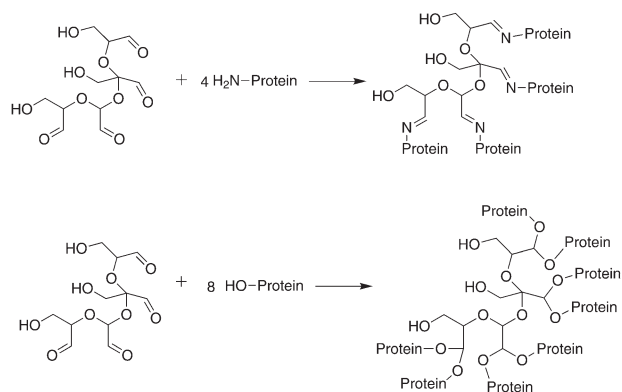
Sucrose is one of the basic food additives in our daily life, which is nontoxic, renewable, and abundant.¹⁷ It has been shown that periodates selectively cleaved the vicinal hydroxyls in carbohydrates to yield aldehyde groups.^{18,19} Nastruzzi and coworkers²⁰ had studied the ability of native and oxidized mono- and disaccharides to induce the modification of gelatin. Their results showed that both native and oxidized sugars were able to reduce the dissolution of gelatin. To our knowledge, sucrose or its derivatives have not been used to modify SPI.

In this research, selective oxidation by periodates was used to produce polyaldehyde derivative of sucrose, which could be used as an effective modifier for SPI films. Enhanced tensile strength and modulus were achieved under both dry and wet conditions with slight decrease for elongation at break. The structure of the modifier was characterized by nuclear magnetic resonance (NMR). The effect of various curing conditions (time and temperature) on mechanical properties of the films had been studied. The thermal stability, morphology, and structure of the films cured with and without the modifier were also investigated.

EXPERIMENTAL

Materials

SPI (PRO-FAM 974 isolated soy protein with 90% protein) was supplied by Archer Daniels Midlands Company, Decatur, IL. Sucrose, glycerol, barium dichloride, sodium periodate, hydrochloric acid, ammonium persulfate, glycine, acrylamide, 1,4-dithiothreitol, bromophenol blue, trimethylaminomethane, *N,N,N,N*-tetramethylethylenediamine, *N,N*-methylene diacrylamide, Coomassie Brilliant Blue G250, sodium dodecyl sulfate acetic acid, and ethanol were purchased from Sinopharm Chemical Reagent Co. Deuterium oxide was purchased from Adamas Reagent Co. Protein standard of 10–250 kDa was purchased from Bio-Rad Chemical Co. All reagents were AR grade and used as received.



Scheme 1. Possible reaction among oxidized sucrose and soy protein.

Preparation of the Modifier

A mixture of sucrose (10.26 g), sodium periodate (19.50 g), and deionized water (300 mL) was stirred in a flask under nitrogen atmosphere at room temperature for 26 h.²¹ Barium dichloride (11.20 g) was added and the combined mixture was stirred at 5°C for 1 h to allow complete precipitation. The mixture was filtered, and the filter liquor containing the polyaldehyde derivatives was stored at 5°C for further use.

For ¹H NMR analysis, the solvent was evaporated under approximately 266 Pa at 25°C for 72 h. The resulting sample was dried under vacuum and redissolved in D₂O. Spectra were taken on a Bruker AV-400 FT-NMR spectrometer.

Preparation of SPI Films

The soy protein water solution 4 g/100 mL was adjusted to pH 10.0 with 1.0N NaOH.²² The solution was gradually heated to 90°C for approximately 30 min with vigorous stirring and held at 90°C for 30 min. The heated solution was cooled down to 60°C before a predetermined amount of the modifier solution (0–10%) and glycerols (15–50%) were added. The films were allowed to dry under ambient conditions for 48 h. The dry films were cured at various temperature and time in a hot air oven. The thickness of dry films was ca. 140 μm. A possible mechanism for the reaction was shown in Scheme 1.

Tensile Properties

For dry tensile testing, all samples (using scissors to cut) were conditioned at 21°C and 65% relative humidity for 48 h. Tensile tests were performed according to ASTM D882-02 on a universal material tester (H5K-S, Hounsfield, UK) equipped with a 50-N load cell, at a crosshead speed of 10 mm/min. Wet strength of the films was determined after conditioning the films at 21°C and 90% relative humidity for 48 h. At least eight specimens selected from three separate films were tested for each condition and the average ± one standard deviation was reported.

Film Solubility (Total Soluble Matter)

A method modified from literature²³ was used to determine the solubility of films in acidic (0.01N HCl), alkaline (0.01N NaOH), and neutral water. Films were cut into 20 × 20 mm pieces and dried at 25°C and 13.3 Pa for 24 h. After drying, films were weighed to nearest 0.0001 g to determine the initial dry weights. Films were then placed into 20 mL of the appro-

appropriate solvent in a screw-top test tube. The tubes were capped and placed in a shaking water bath at 25°C for 24 h. The shaking machine was run at 100 rpm. Film pieces were finally taken out, dried at 25°C and 13.3 Pa for 24 h, and weighed to determine the final dry weights. Three replicates of each film were performed. Percent total soluble matter was calculated from the initial and final dry weights of films and reported on initial dry weight basis.

Sodium Dodecyl Sulfate—Polyacrylamide Gel Electrophoresis

Changes in the molecular weight of the SPI due to modification were studied using sodium dodecyl sulfate—polyacrylamide gel electrophoresis (SDS-PAGE). Sample protein powders grinding with agate mortar, 0.1 mg) were dissolved in 100 μL SDS buffer solutions. The samples were heated at 70°C for 10 min and left standing at room temperature for 2 h. The solutions were vortexed before loading. Samples of 10 μL were loaded into individual slots of the gel. After electrophoresis, the gel was stained with Coomassie Brilliant Blue G250 for 2 h at room temperature. The gel was then destained using a 10% acetic acid solution until a clear background was observed. The molecular weights of protein standard mixture ranged from 10 to 250 kDa.

Thermal Analysis

Differential scanning calorimetry (DSC) was performed under nitrogen flow with a DSC 204 F1 from Netzsch. The films were heated from room temperature to 200°C at a rate of 10°C/min.

Thermogravimetric analysis (TGA) was performed with a TG 209 F1 from Netzsch at a heating rate of 10°C/min from 20 to 600°C under nitrogen atmosphere. The weight of the samples was 3–5 mg, and the rate of gas consumption was 40 mL/min.

X-ray Diffraction

The X-ray diffraction analyses were performed on a Rigaku D/max-2550 PC (Japan). CuKα radiation with wavelength λ = 0.1542 nm was used.

Morphological Study

The surface features of the modified SPI film and the control were observed by scanning electron microscopy (Jeol JSM 5600lv, Japan). Films placed on conductive tapes were sputter coated with gold and observed in the microscope at a voltage of 15 kV.

RESULTS AND DISCUSSION

Structure of the Modifier

The sucrose oxidation mechanism by periodate has been studied by Maat and coworkers in 1992.²⁴ The product of the oxidation was expected to be a mixture of isomeric hemiacetals. Proposed structure for one of these hemiacetal derivatives is shown in Figure 1. The ¹H-NMR spectra of sucrose and oxidized sucrose shown in Figure 1 confirmed the formation of these hemiacetals. Because D₂O was used as the solvent, all exchangeable hydroxyl protons did not show up in the spectra. The integrals fit well with the proposed structure featuring one free CHO group (8–8.5 ppm) and four hemiacetal groups (4.5–5 ppm). The total number of unexchangeable protons would have been 13, which was exactly what was observed. These results

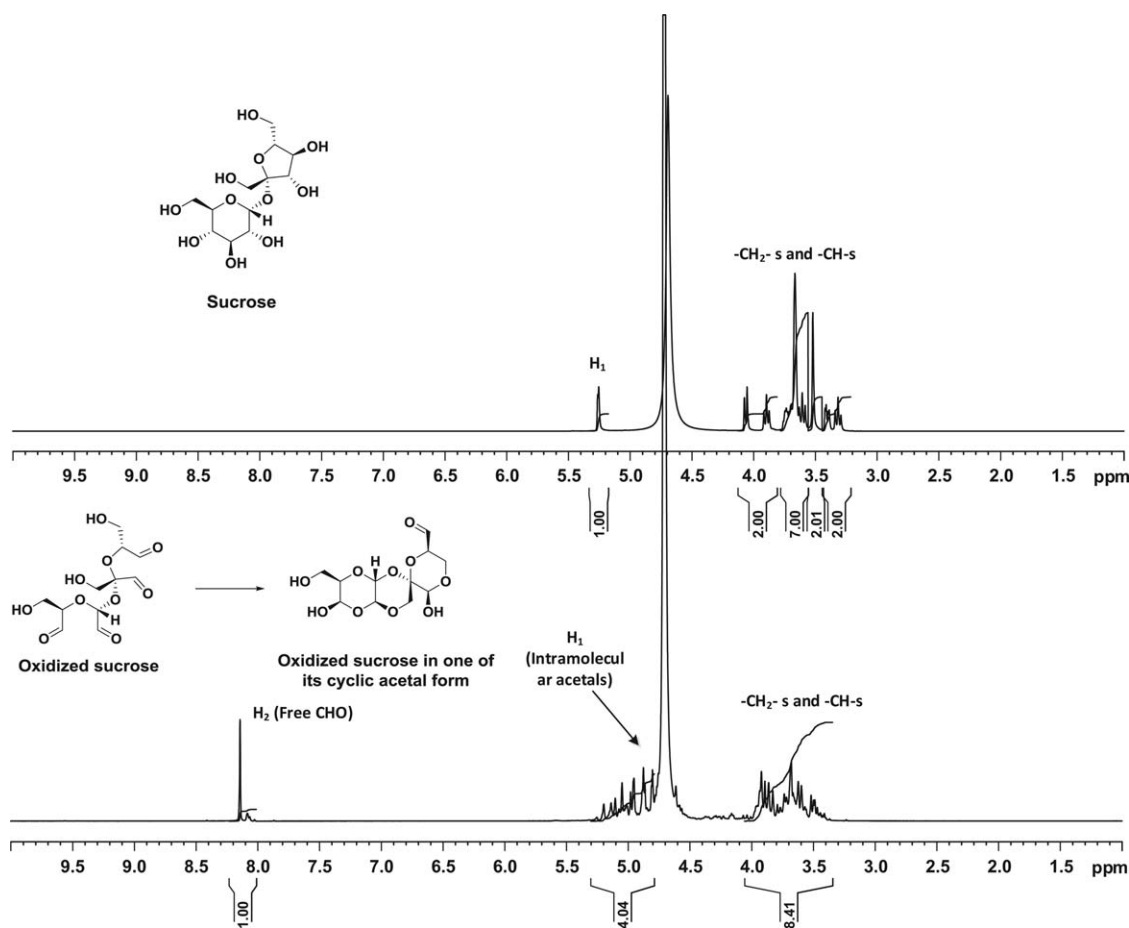


Figure 1. ¹H-NMR spectra of sucrose and oxidized sucrose.

confirmed that sucrose was successfully oxidized to the expected polyaldehyde derivative by using three equivalents of sodium periodate.

Effect of Curing Temperature

Chemical modification was expected to improve the tensile strength of the films as a result of the formation of extra intra- and intermolecular covalent bonds.²⁵ The effect of curing temperature on the tensile strength and breaking elongation of the oxidized sucrose-modified films is shown in Figure 2. The tensile strength increased with increasing temperature in the temperature range of 140–170°C. Compared with uncapped polyaldehyde modifiers, such as glyoxal, the oxidized sucrose required much higher curing temperature. This was because three of the aldehyde groups were present in the form of cyclic acetal, which was known to be less reactive than free aldehyde.²⁶ Further increase of the curing temperature to 180°C decreased the tensile strength of the films instead. This was most likely because undesirable decomposition of SPI kicked in. Elongation at break of the films continued to decrease with increasing temperature. In the temperature range of 140–170°C, this was mainly caused by modification-induced hindered molecular chain slippage, whereas structure damage was expected to be the main culprit at curing temperatures beyond 170°C. There-

fore, curing temperature was kept at 170°C for the rest of the experiments.

Effect of Curing Time

The effect of curing time on the tensile strength and breaking elongation of the SPI films is shown in Figure 3. As seen from

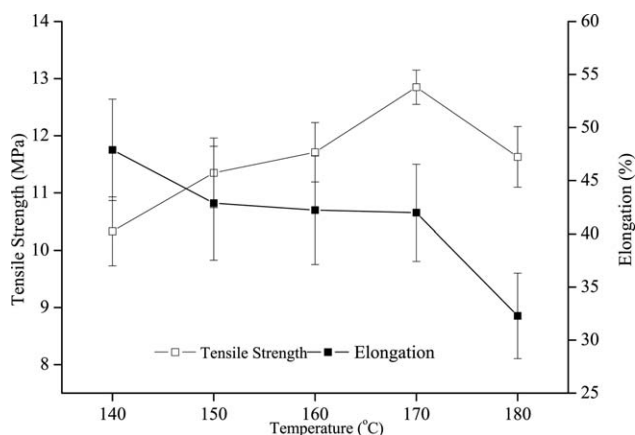


Figure 2. Effect of curing temperature on the strength and elongation of modified SPI films. Films were cast from 4% SPI, 30% (w/w) glycol, and modified using 5% (w/w) modifier for 10 min.

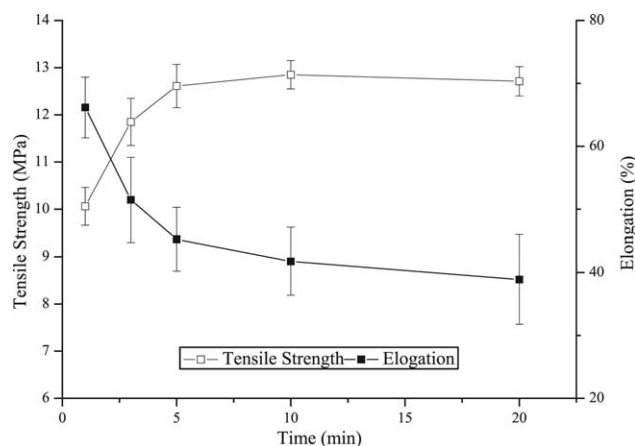


Figure 3. Effect of curing time on the strength and elongation of modified SPI films. Films were cast from 4% SPI, 30% (w/w) glycol, and modified using 5% (w/w) modifier at 170°C.

the figure, the optimum tensile strength was achieved in the narrow range of 3–5 min. Further increase in curing time failed to impart higher tensile strength. Similarly, for breaking elongation, the largest drop appeared in the range of 3–5 min of curing. Further increase in curing time hardly had any effects on breaking elongation. These results indicated that 5 min is appropriate for the reaction between SPI and modifier. From economic and technical point of view, curing at 170°C for 5 min was chosen as the optimum condition for the rest of the experiments. The modification can effectively increase molecular interactions, leading to increase in strength of the modified films.²⁷ Because modification hindered molecular chain slippage, the elongation decreased.

Effect of Glycerol

Glycerol was used as a plasticizer in this study. The effect of glycerol on the tensile strength and elongation of the SPI films was investigated and the results are shown in Figure 4. Because films with less than 15% added glycerol became too brittle after curing due to lack of plasticizing, they were not considered for further studies. A consistent decrease in tensile strength was

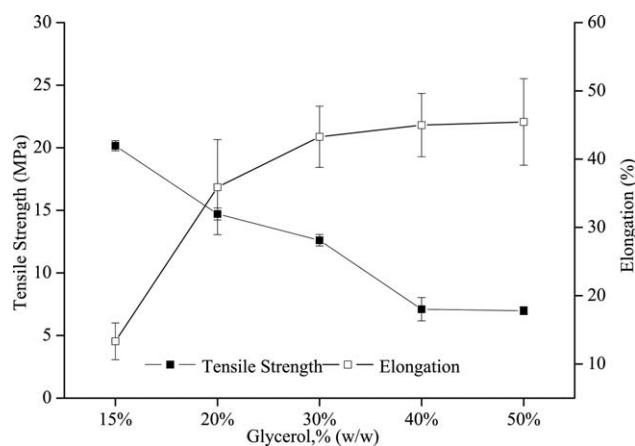


Figure 4. Effect of adding glycerol on the strength and elongation of modified SPI films. Films were cast from 4% SPI and modified using 5% (w/w) oxidized sucrose and cured at 170°C for 5 min.

Table I. Effect of Modifier Concentration on Mechanical Properties of the Films

Modifier (%w/w)	Tensile strength (MPa)	Breaking elongation (%)	Modulus (MPa)
0	10.7 ± 0.3	39 ± 8.9	428 ± 13
1	12.8 ± 0.2	38 ± 6.9	455 ± 14
3	14.5 ± 0.4	36 ± 5.7	511 ± 18
5	14.7 ± 0.5	35 ± 7.5	549 ± 25
10	21.6 ± 0.9	3 ± 0.7	1361 ± 195

Films were cast from 4% SPI with 20% glycerol and cured at 170°C for 5 min.

observed as the concentration of glycerol increased from 15 to 40%, whereas the breaking elongation continued to increase. A similar effect of glycerol on tensile strength and breaking elongation had been reported for SPI films treated with transglutaminase.²⁸ Further increase in glycerol concentration to above 40% had little effect on the breaking elongation or tensile strength of the films. A glycerol concentration of 20% provided the optimum combination of tensile strength and breaking elongation to the films and was used for all further studies.

Effect of Modifier Concentration

As can be seen from the data in Table I, tensile strength of the SPI film increased proportionally with the amount of modifier added. A 102% increase in tensile strength and a 218% increase in modulus were obtained at a modifier concentration of 10%. However, the breaking elongation of the film with this modifier concentration was only 7% of the control. Modified reaction created strong inter- and intramolecular linkages in the protein molecules leading to an increase in the strength and modulus. However, linkages limit the flexibility and therefore the movement of the protein molecules during tensile testing leading to a decrease in breaking elongation.

Wet Strength

Wet tensile properties of the SPI films modified with various amount of oxidized sucrose are summarized in Table II. Films modified with less than 3% of the modifier showed no substantial improvement in wet tensile properties. Adding 5% oxidized sucrose modifier led to improvements of 150 and 500% for the wet tensile strength and modulus, respectively. At the same time, the wet breaking elongation decreased to about 29% of

Table II. Mechanical Property of Unmodified and Modified SPI Films Conditioning at 21°C and 90% Humidity for 48 h

Modifier (%w/w)	Tensile strength (MPa)	Breaking elongation (%)	Modulus (MPa)
0	3.0 ± 0.1	94 ± 9.4	16 ± 1.4
1	3.1 ± 0.1	77 ± 11.2	16 ± 1.6
3	3.3 ± 0.1	35 ± 5.7	33 ± 1.8
5	4.7 ± 0.2	27 ± 6.1	81 ± 3.8
10	7.1 ± 0.2	18 ± 3.2	138 ± 8.6

Table III. Total Soluble Matter (%) of Modified SPI Films

	Control	1%	3%	5%	10%
Water	24.5 ± 0.7	22.4 ± 0.5	22.0 ± 0.6	23.1 ± 0.9	17.7 ± 0.4
0.01N HCl	18.6 ± 0.3	18.1 ± 0.8	17.1 ± 0.4	18.4 ± 0.6	15.1 ± 0.7
0.01 N NaOH	29.9 ± 0.5	22.1 ± 0.3	23.0 ± 0.6	23.6 ± 1.0	16.4 ± 0.5

the control. Further increase in oxidized sucrose concentration led to even more severe loss of breaking elongation. From the practical point of view, 5% oxidized sucrose concentration is preferable. The unmodified samples swelled considerably causing poor interaction between the molecules; poor interaction lead to easier movement (sliding) of the molecules during tensile testing and therefore poor strength.²⁹ Modification interconnects the molecules, provides better resistance to swelling and therefore the wet tensile properties of the modified samples are better than that of the unmodified samples.²⁹ Improved wet performance suggested that the films developed in this research

were more stable in water and could be used for various applications involving high humidity.

Water Solubility

Resistance of SPI films to water and to dilute acid and base is desirable if the film is to be used for food packaging.³⁰ Chemical modification was expected to improve the water resistance of the films.³⁰ Total soluble matter of the SPI films modified with various amounts of the sugar-derived modifier was determined and summarized in Table III. The percent soluble matter values were lower in the acidic medium (0.01N HCl) than in water. This could be due to formation of insoluble complexes as reported previously.³¹ In contrast, films dissolved to a greater extent in alkali (0.01N NaOH) than in water. This is because peptides were susceptible to base-catalyzed hydrolysis. Overall, with increasing amount of the modifier, the films became less soluble and more resistant to water. In the case when 10% modifier was used, the total soluble matter decreased by 45% in alkali. This could be due to achieving of high modification degree when 10% modifier was used. To obtain the same total soluble matter, high modification degree films need more time to hydrolysis. In the cases when 1, 3, and 5% modifier were used, the modification degrees' variation cannot influence the mass of the soluble matter, or the variation of the soluble matter cannot be detected under our experimental conditions.

SDS-PAGE

Figure 5 shows the SDS-PAGE image of the SPI films. As seen from the figure, the control cured with no modifier containing protein fragments of molecular weight in the range of 10–250 kDa and over 250 kDa (lane 1), whereas the modified SPI (lane 2) had relatively strong bands near the 250 kDa and above 250

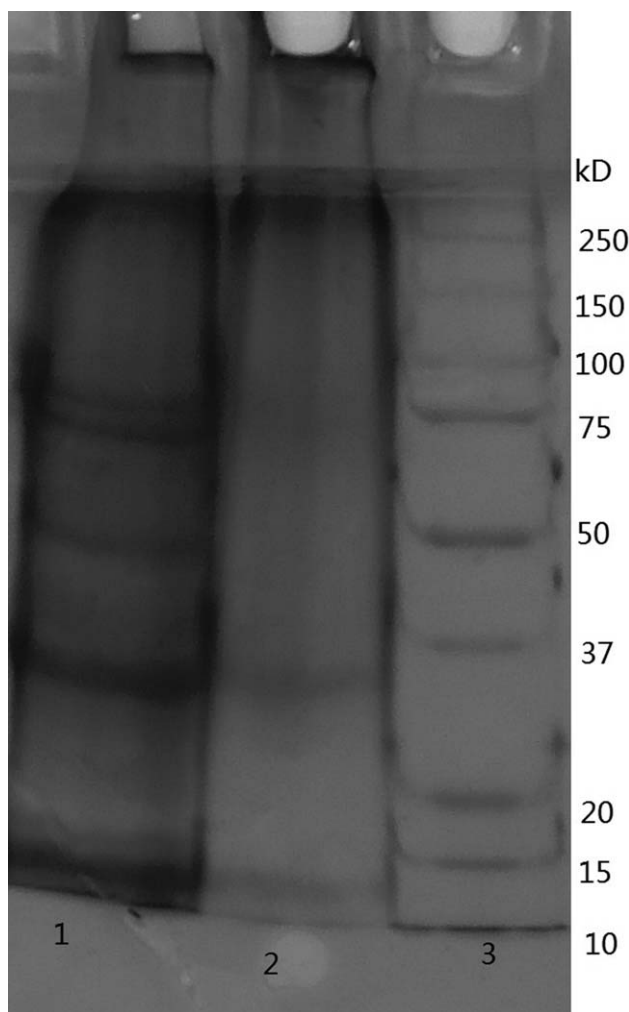


Figure 5. SDS-PAGE image of unmodified (lane 1) and modified (lane 2) SPI compared with the molecular weight standards (lane 3).

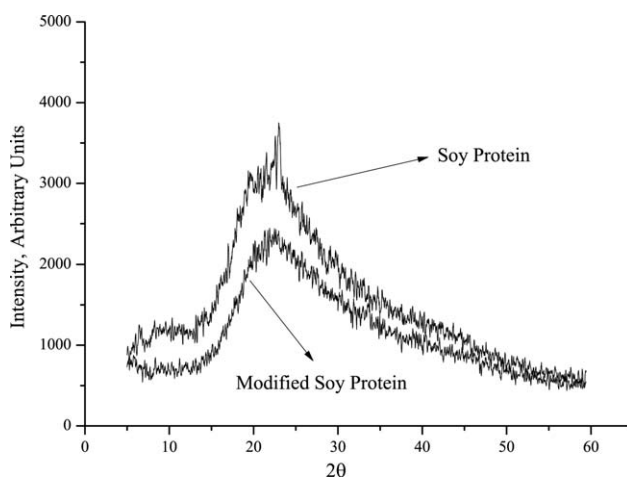


Figure 6. Diffraction patterns of modified and unmodified SPI films.

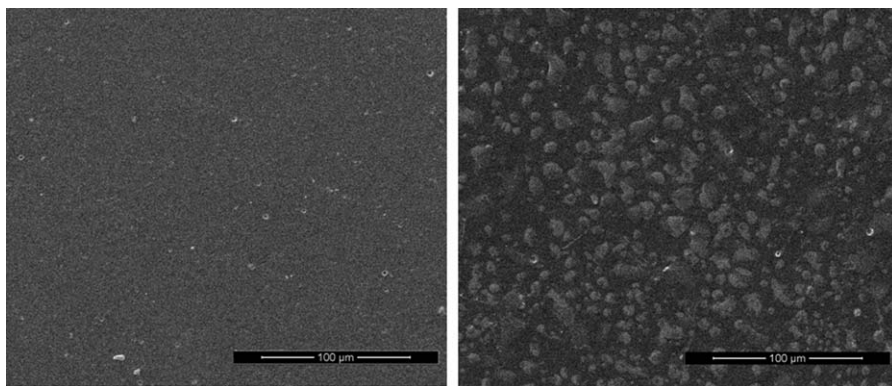


Figure 7. SEM pictures of soy protein films (a) and oxidized sucrose-modified soy protein films (b).

kDa region and no strong bands in the 10–250 kDa region. The observed increase in molecular weight supported the effective modification of SPI by oxidized sucrose under the curing conditions.

Physical Structure

X-ray diffraction patterns of the SPI film modified with oxidized sucrose were similar to that of the control, with one prominent peak at about 22° (Figure 6). Both films had similar percentage crystallinity of about 25 and 19%, respectively. The results indicated that the crystalline structure of SPI was only slightly destroyed by modification with oxidized sucrose. The SPI chain molecules packed more closely in crystalline area than in noncrystalline, perhaps leading to modification reaction mainly occurred in the noncrystalline.

Morphological Structure

Figure 7(a,b) shows the surface morphologies of the SPI films observed with SEM. It was clear that the control featured a homogenous surface. The oxidized sucrose-modified SPI film had a rougher surface. This could be resulting from the formation of aggregates due to the presence of major modification. A similar consequence was observed for genipin-crosslinked SPI films.³²

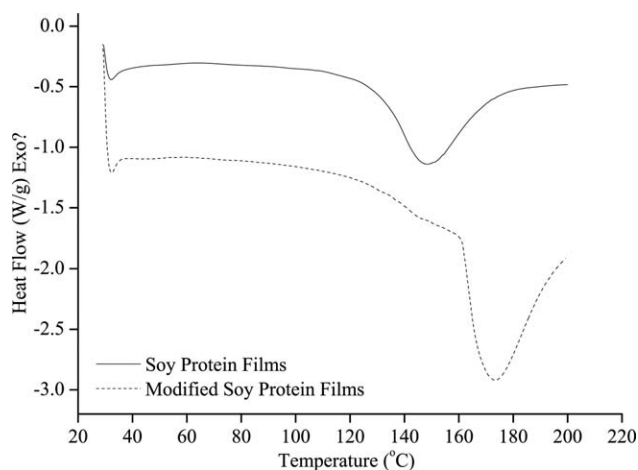


Figure 8. DSC thermograms of the SPI films cured with and without 5% oxidized sucrose modifier.

Thermal Analysis

Thermal behaviors of the SPI films cured with and without the modifier are shown in Figures 8 and 9, respectively. As seen from Figure 8, the melting peak for the control appeared at 148°C with a melting enthalpy of 104 J/g. Addition of oxidized sucrose caused a shifting of melting peak to 174°C and an increase of melting enthalpy to 177 J/g. Adding a modifier resulted in the formation of more intra- and intermolecular covalent bonding in addition to self-reaction, leading to increased melting enthalpy and melting temperature.³³ The TGA curves in Figure 9 showed that both samples lost about 10% weight under 100°C owing to moisture releasing. However, the residual percent weight of the modified SPI was higher than the control, indicating improvement in thermal stability.

CONCLUSIONS

In summary, in contrast to unmodified SPI films, a 102% increase in tensile strength was obtained for modified SPI films at a modifier concentration of 10% conditioning at 21°C and 65% humidity for 48 h. A 150% improvement of tensile strength for modified SPI films when adding 5% oxidized sucrose modifier conditioning at 21°C and 90% humidity for 48 h. This research demonstrated that SPI could be made into

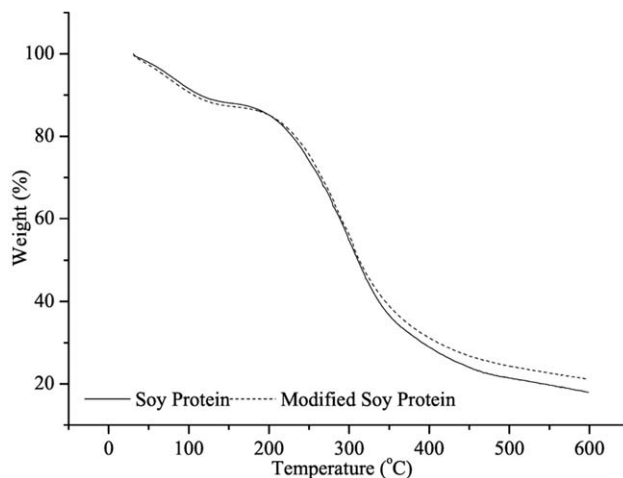


Figure 9. Thermogravimetric thermograms of the SPI films cured with and without 5% oxidized sucrose modifier.

films with good dry and wet strengths by curing with a bio-based modifier derived from sucrose. Because of the formation of hemiacetal functionalities by self-condensation, the modifier required higher curing temperature (170°C) compared with common aldehyde modifiers. Upon adding 10% of the modifier, the resulting SPI film had tensile strength of 21.6 MPa, breaking elongation of 3%, and modulus of 1361 MPa, respectively. Wet tensile strength and modulus were also improved appreciably. A moderate modifier concentration of 5% was needed to achieve good dry and wet mechanical properties. Modified films also showed improved thermal stability and melting temperature. The fact that the modifier could be prepared from table sugar by a simple one-step procedure made it an interesting potential choice for fabricating renewable films of regenerated proteins for food packaging and other applications.

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