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Potato protein isolate-based biopolymers

Yicheng Du,[1] Fangping Chen,[1] Yachuan Zhang,[2] Curtis Rempel,[2,3] Michael R. Thompson,[4] Qiang Liu[1]

[1]Guelph Food Research Centre, Agriculture and Agri-Food Canada, 93 Stone Road West, Guelph Ontario, Canada N1G 5C9

[2]Department of Food Science, University of Manitoba, 250 Ellis Building, Winnipeg Manitoba, Canada R3T 2N2

[3]Canola Council of Canada, 400-167 Lombard Avenue, Winnipeg Manitoba, CanadaR3B 0T6

[4]Department of Chemical Engineering, McMaster University, 1280 Main Street West, Hamilton ON, Canada L8S 4L7

Correspondence to: Q. Liu (E-mail: qiang.liu@agr.gc.ca)

ABSTRACT: Thermal processing of two potato protein isolates (PPIs) with glycerol as a plasticizer was explored in this study. The PPIs were pretreated by alkali or alkali under reducing conditions. The PPIs before and after pretreatment were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, differential scanning calorimetry, and Fourier transform infrared spectroscopy. The effects of plasticizer content and pretreatment on mechanical and thermo-mechanical properties of the compression-molded biopolymers were studied. The highest tensile strengths obtained were 20–25 MPa and the biopolymer can be brittle or ductile depending on the plasticizer contents. The molecular weight and protein structure of the PPIs markedly affected the resultant biopolymers' static and dynamic mechanical properties. The pretreatment of PPIs caused distinctly different changes in the mechanical properties of the two PPIs. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42723.

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INTRODUCTION

The interest in renewable biopolymers as a substitute for non-biodegradable petrochemical-based polymers has increased since the 1980s, and they have been getting more and more attention recently because of environmental benefits such as biodegradability and sustainability. Protein together with polysaccharides and polynucleotides constitute three major classes of biopolymers. A wide variety of protein sources have been studied for biopolymer processing, of which soy protein, wheat gluten, and corn zein are most commonly studied.[1] Several excellent review papers have been published on the structure and properties of protein-based biopolymers.[1–6] In these studies, methods employed to processing biopolymers include wet processing technology, i.e., dissolving protein in aqueous solutions followed by film casting, and dry processing techniques, such as compression molding, extrusion, and injection-molding. The simplicity of dry processing methods makes this technology particularly suitable for mass-production; however, it is difficult to process proteins alone because of the fact that their glass transition temperatures are very close to their thermal degradation temperatures. Thereby, pretreating protein with reducing agents, sodium dodecyl sulfate, or urea, or with additives such as polyol-based plasticizers, are usually used to widen the processing window.

Protein-based biopolymers have relatively low mechanical properties as compared to synthetic polymers, based on a literature review of the mechanical properties of thermally processed biopolymers derived from widely studied protein resources plasticized with approximately 30% polyol (w/w).[7–17] In general, gluten-based biopolymers have the lowest mechanical properties. However, the high strengths of nearly 20 MPa reported for soy protein or zein-based biopolymers show that near comparable values to synthetic polymers can be achieved, although significant variations in mechanical properties were observed among these studies.

Potato protein contains approximately 71% patatin/tuberin, 7.6% glutein, 6.6% albumin, 3% globulin, 1.7% prolamin, and 8.8% other proteins.[18] There have been many fundamental investigations into potato protein undertaken in the Netherlands from the 1970s to early 2000s. The use of potato protein for biopolymer manufacturing, however, has not been studied from the literature. The objective of this paper was to evaluate the feasibility of two different potato proteins as a new renewable resource, to be added into the environmentally friendly and biodegradable protein-derived biopolymer family for packaging applications. Biopolymer test specimens were produced using compression-molding process because of the direct applicability of this technique to industrial manufacturing. Previous research has shown protein is prone to form coagulates at pH values

close to its isoelectric point, while it denatured and unfolded at pH values away from the isoelectric region.[19] This denaturation and unfolding of the protein could extend the protein molecules which in turn would reduce the brittleness of processed polymer. So, aside from producing biopolymer specimens directly from control protein samples, pretreatment of protein at pH values away from the isoelectric point were undertaken. However, only alkali treatment was reported to effectively improve the mechanical properties of protein-based biopolymer.[19,20] Therefore, the effect of alkaline pretreatment prior to thermal processing on the protein's and resultant biopolymer's properties were evaluated.

MATERIALS AND METHODS

Materials

Two potato protein isolates (PPI-A and PPI-B) with different molecular weights were supplied by AVEBE (Veendam, Netherlands). PPI-A protein has a protein concentration of >92%, a molecular weight of >35 kDa, and isoelectric point of <6. The PPI-B has a protein concentration of >95%, a molecular weight between 4 to 35 kDa, and isoelectric point of >6. Glycerol, sodium hydroxide, and sodium sulfite (Na_2SO_3) were purchased from Sigma-Aldrich (St. Louis, MO).

Protein Pretreatment and Biopolymer Processing

Alkali Treatment. PPI-A or PPI-B powder, 25 g, was dispersed in 500 mL distilled water. The suspension was adjusted to pH 12 with 4*M* NaOH solution, followed by stirring for 2 h at ambient temperature. The PPIs were completely dissolved at these alkaline conditions. Afterwards, the solution was adjusted to pH 4 with hydrochloric acid (37 wt %) to allow for the precipitation of the protein. Another batch of sample PPI-A or PPI-B was treated in 0.1*M* Na_2SO_3 solution to evaluate the effect of the alkaline treatment under reducing conditions. The reaction conditions were kept the same as for the alkali treatment. The pH values were adjusted to pH 12 then pH 4 in a similar manner as previously stated for the dissolution and precipitation of the PPI. The suspension was centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the sediment was collected, washed by distilled water several times, and then air-dried until consistent weights were achieved. The dry protein samples were then ground in a planetary ball mill (Retsch PM 100, Retsch, Haan, Germany) to pass through a 100 μm sieve. The pretreatment caused approximate weight loss of 20% and 60% of the PPI-A and PPI-B samples, respectively.

Biopolymer Processing. The compositions of all samples for processing are summarized in Table I. PPIs or pretreated PPIs were mixed with glycerol manually with a mortar and pestle and the PPI/glycerol mixture was then allowed to condition for 24 h prior to thermal processing. A certain amount of the PPI/glycerol mixture was placed in a mold, which was preheated to 155°C on a Carver press (Carver, Wabash, IN, USA). The mixture was then compression-molded at 7.5–10 MPa for 5 min. The mold containing the formed specimen was removed from the platens after they had cooled to room temperature and the biopolymer samples were subsequently removed from the mold.

Table I. Pretreatment of PPI, Sample Compositions, and T_gs Determined from Tan δ Peak of Resultant PPI Biopolymers

Protein source	Pretreatment	Ingredient (PHR) PPI	Ingredient (PHR) Glycerol	T_g °C
PPI-A	-	100	30	90.5
PPI-A	-	100	40	91.1
PPI-A	-	100	50	84.2
PPI-A	NaOH	100	40	90.3
PPI-A	Na_2SO_3	100	40	88.1
PPI-B	-	100	30	88.8
PPI-B	-	100	40	85.6
PPI-B	-	100	50	79.1
PPI-B	NaOH	100	40	90.2
PPI-B	Na_2SO_3	100	40	88.5

Protein Characterization and Biopolymer Testing

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed according to the method of Laemmli.[21] PPI samples were mixed with sample buffer in the presence of β-mercaptoethanol and SDS, heated for 10 min at 95°C, and loaded onto a 4–10% polyacrylamide gel with SDS. Gels were run at a constant voltage (200 V) for 38 min in a Mini-Protean II electrophoresis cell (Bio-Rad, Hercules, CA, USA). The gels were developed with Bio-Safe™ Coomassie Stain.

Fourier Transform Infrared Spectroscopy. Fourier transform infrared spectroscopy (FTIR) absorption spectra of control PPI-A and PPI-B before and after treatments were recorded on a Digilab FTS 7000 spectrometer (DIGILAB, Randolph, MA) equipped with a Golden Gate diamond single reflection ATR and a deuterated triglycine sulfate detector. The spectrum for each sample was recorded as absorbance data from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1}.

Differential Scanning Calorimetry. The heat denaturation of PPIs, PPI/glycerol mixtures, or treated PPIs were analyzed on a TA Differential Scanning Calorimetry (DSC) Q20 (TA Instruments, New Castle, Delaware) equipped with an RCS90 refrigeration system under nitrogen atmosphere. Samples, ~20 mg, were loaded and sealed in high-volume pans. The samples were equilibrated at 25°C, isothermal for 5 min, and heated to 150°C at 5°C/min. The heat flow data of the samples were collected with reference to an empty pan.

Tensile Testing. Type-V tensile specimens were prepared from the formed samples with a die. The specimens were conditioned in the standard laboratory atmosphere for over 48 h prior to tensile testing. These specimens were tested on a 10 kN benchtop Universal Mechanical Testing System (Model 3366, Instron Corporation, Norwood, MA) with a 0.5 kN load cell in accordance with ASTM Standard D638. A crosshead speed of 1 mm/min was used. The tensile testing was conducted at ambient conditions (23°C, 50% RH). The means and standard deviations reported of all samples were recorded from at least five replicates.

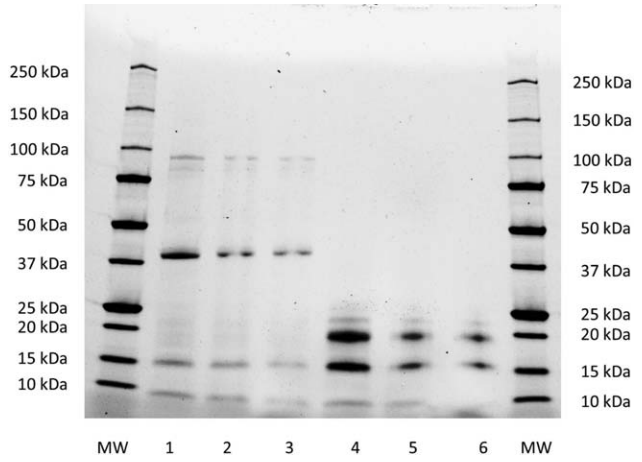

Figure 1. SDS-PAGE gel of two potato protein isolates: PPI-A (1) and PPI-B (4), pretreated PPI-A and PPI-B by alkali (2, 5), and pretreated PPI-A and PPI-B by alkaliunder reducing conditions (3, 6).

Dynamic Mechanical Analysis. Dynamic mechanical properties of the biopolymer samples were measured using a TA Dynamic Mechanical Analysis (DMA) Q800 (TA Instruments, Delaware) operated in a multifrequency strain mode. The specimens were cut into a nominal dimension of 55 mm×10 mm×1.25 mm and were conditioned in the standard laboratory atmosphere for over 48 h prior to tensile testing. Each specimen was mounted on the dual cantilever beam clamp, and an amplitude of 250 μm was applied to the specimen at a frequency of 1 Hz. The storage modulus, loss modulus, and tan δ were recorded as the samples were heated from ambient temperature to 150°C at a heating rate of 5°C /min. Each sample had two replicates.

RESULTS AND DISCUSSION

Molecular Profile of Potato Protein by SDS-PAGE

Figure 1 shows the polypeptide compositions of the two PPIs, before and after pretreatment, analyzed by SDS-PAGE. PPI-A shows a major band at about 40 kDa, three minor bands at 10, 15, and between 75 and 100 kDa. The band at 40 kDa is assigned to patatin. This value is close to 40 or 43 kDa reported by other researchers.[22,23] The two minor bands below 20 kDa are two subclasses of protease inhibitor, and the other minor band between 75 and 100 kDa could be assigned to phosphorylase (80 kDa),[24] or a dimer of patatin.[25] There was no discernible difference in the molecular weight between the control PPI-A and the two PPI-A samples pretreated by alkali or alkali under reducing conditions. The control PPI-B had its major molecular weight band at about 20 kDa, a typical molecular weight range of protease inhibitor as reported by Pots *et al.*[25] Similar to PPI-A, there was no obvious difference in the molecular weight of the control PPI-B and the two PPI-B samples pretreated by alkali or alkali under reducing conditions.

Fourier Transform Infrared Spectroscopy

The vibrational absorbance bands between 1450 and 1700 cm^{-1} of both PPIs and treated PPIs are shown in Figure 2. The absorption band between 1600 and 1700 cm^{-1} [Figure 2(a)] is attributed to the amide I of the protein ascribed to =CO stretching (80%) with minor contribution from C-N stretching,[26] which has been used for assigning protein secondary structure. The absorption band between 1500 and 1550 cm^{-1} was mainly from N-H bending (60%),[26] so a change in this band is an indication of the conformational change of the tertiary structure. The secondary structural content of the native patatin was estimated to contain 45% β-sheet, 33% α-helix, and 15% random-coiled structures.[27] The amide I band of control PPI-A centered at 1641 cm^{-1} was a composite absorption of these three structures, β-sheet, α-helix, and random-coiled structures, which have absorption band at 1625–1640, 1648–1660, and 1640–1648 cm^{-1}, respectively.[26] After the alkali treatment alone or under reducing conditions, the peak of the amide I band slightly shifted to lower frequencies, namely 1634 and 1636 cm^{-1}, respectively. The decrease in the amide I frequency and the change in the shape of amide II band of PPI-A was considered to be a result of extended polypeptide chains in the denatured proteins because of the enhanced intermolecular hydrogen bonds between closely aligned neighboring protein chains.[26]

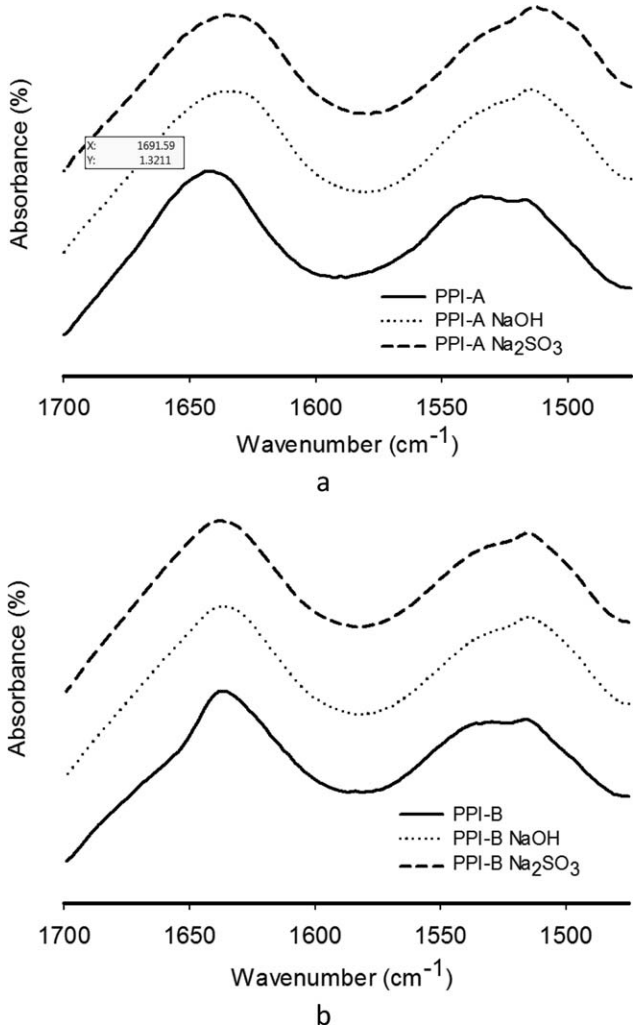

Figure 2. FTIRspectra between 1450 and 1700 cm^{-1} of control and pretreated (a) PPI-A and (b) PPI-B (b) with alkali or alkali under reducing conditions.

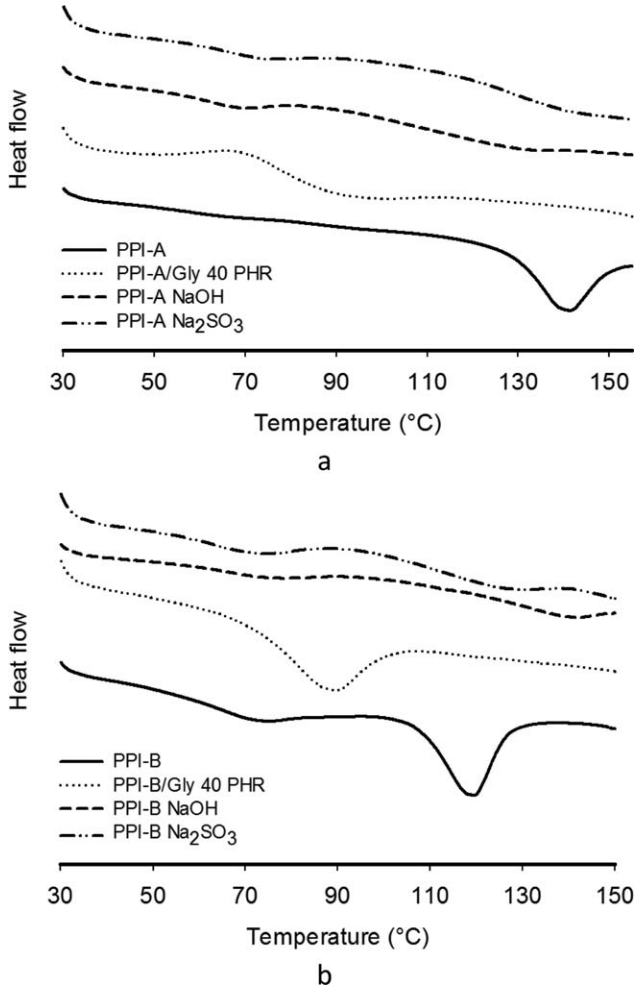

Figure 3. DSC thermograms (exotherm up) of (a) PPI-A (a) and (b) PPI-B, PPIs/glycerol mixtures, and pretreated PPI-A and PPI-B with alkali or alkali under reducing conditions.

PPI-B had the amide I band centered at 1637 cm^{-1} [Figure 2(b)]. In contrast to PPI-A, the amide I absorption band of PPI-B did not shift after being treated by alkali or alkali under reducing conditions. However, there was a slight change in the amide II band shape. So, the secondary structure of PPI-B after the treatments did not change as much as PPI-A did. This is probably because of the smaller protein unit size of PPI-B as compared to that of the PPI-A. The slight change in the shape of the amide II band of PPI-B sample after treatment indicated the PPI-B had been denatured also. There was no significant difference between the spectra of the PPI-A and PPI-B treated by alkali or alkali under reducing conditions.

Differential Scanning Calorimetry

DSC thermograms of the two PPIs, PPIs before and after treatment, and PPI/glycerol mixtures, are plotted in Figure 3. PPI-A exhibited an endothermic transition between 130°C and 150°C because of the unfolding (denaturation) of the protein molecules [Figure 3(a)]. The denaturation denotes a change of native conformation, i.e., tertiary structure, of protein without cleavage of covalent bonds (except for disulfide bridges).[28] The peak temperature decreased from 140°C to 93°C with the addition of glycerol. The glycerol acted as a plasticizer to the protein molecules, which decreased the protein denaturation temperature. The endothermic peak was also observed to have flattened, thereby demonstrating lower denaturation enthalpy, suggesting that the PPI-A was partially denatured with the addition of glycerol. An additional broad and shallow exothermic transition centered at approximately 68°C was detected immediately prior to the denaturation event, which may be attributed to protein aggregation.[29]

The control PPI-B has a large endothermic peak spanning from 100°C to 128°C, and a broad, low intensity endothermic peak between 65°C and 83°C, both much lower in temperature than that of PPI-A [Figure 3(b)]. PPI-B may possess two main subclasses of protease inhibitor, which explains the two separate denaturation events. With the addition of glycerol (40 PHR), only one endothermic peak ranging from 65°C to 107°C was present, indicating again significant plasticization occurred.

The addition of plasticizers significantly reduced the denaturation temperature of the two potato protein sources in this study. This is in conformity with previous studies with other protein sources: glycerol with sunflower protein[30] and water with soy protein.[8,31] Unlike PPI-A, the PPI-B/glycerol mixture does not have a protein aggregation exothermic peak, but still possess the same denaturation enthalpy. This suggests that the tertiary structure of PPI-B did not change as extensively as PPI-A with the addition of glycerol.

The endothermic peaks completely disappeared in the thermograms of both PPI-A and PPI-B after alkaline treatment or alkaline treatment under reducing conditions. This demonstrated that these two potato proteins were fully denatured after the treatments, which is in agreement with a previous study on soy protein.[32] There are broad shallow exothermic peaks from 70°C to 130°C on these four treated PPI samples because of the aggregation of unfolded protein.

Tensile Properties

The tensile moduli and strengths at break (brittle specimens) or yield (ductile specimens) of the two PPI/glycerol biopolymers are plotted in Figure 4. The tensile strengths of the biopolymers based on the two PPIs ranged from 10 to 25 MPa, higher than that of the biopolymers from three widely studied protein sources.[7–17] The tensile moduli of the PPI-A/glycerol biopolymer gradually decreased with increasing glycerol content [Figure 4(a)]. Tensile strength of PPI-A/glycerol biopolymer had a slight drop as the glycerol content increased from 30 to 40 PHR, and then significantly decreased as the glycerol content increased from 40 to 50 PHR [Figure 4(b)]. The stress–stain curves of these biopolymer samples during tensile testing are plotted in Figure 5. As shown on the curves [Figure 5(a)], a brittle-to-tough transition occurred between a glycerol content of 40 and 50 PHR for the PPI-A/glycerol biopolymer.

The tensile moduli and strength of the PPI-B/glycerol biopolymer were both significantly lower than that of the PPI-A/glycerol biopolymer [Figure 4(a,b)]. The effect of glycerol content on the tensile properties of the PPI-B/glycerol biopolymer was similar to that of PPI-A. The PPI-B/glycerol biopolymer reached the brittle-

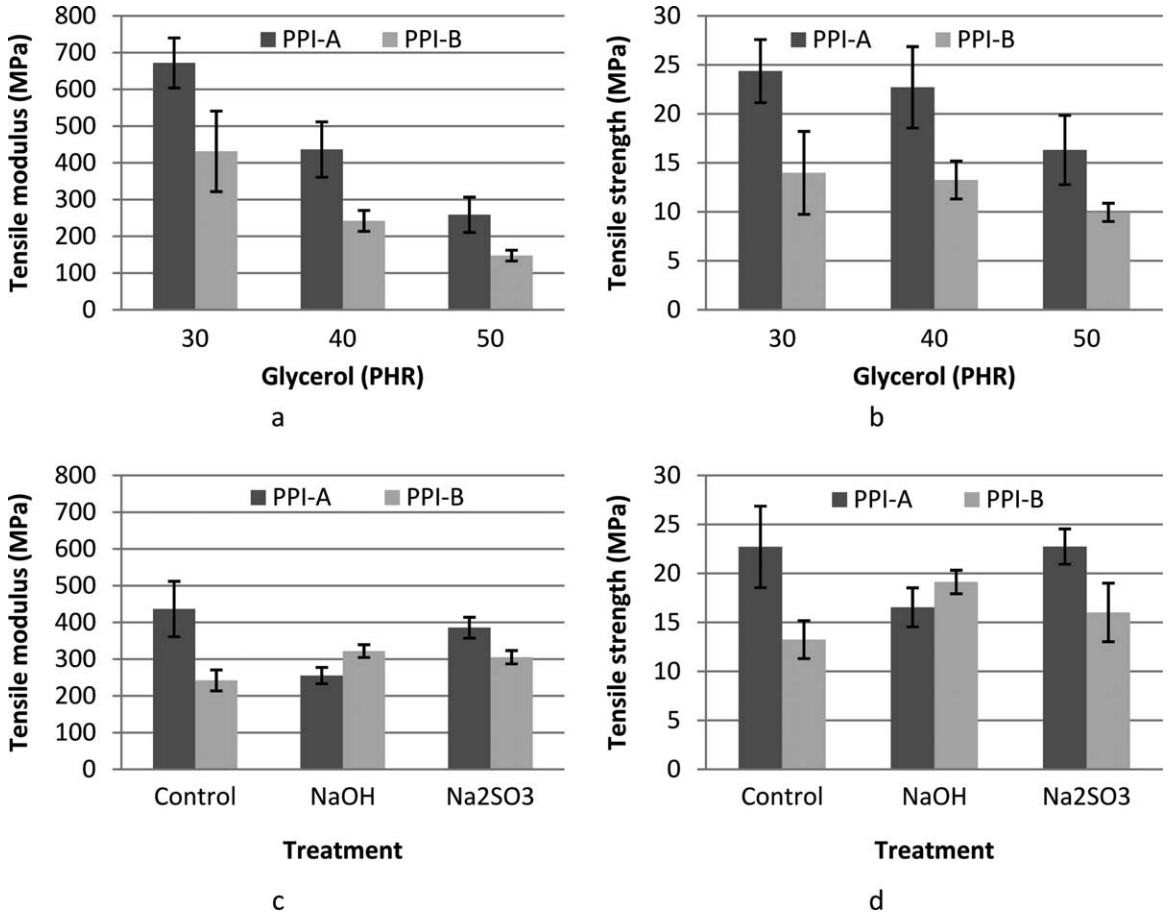

Figure 4. Tensile properties of PPI/glycerol biopolymers: (a) tensile moduli of PPI/glycerol biopolymers vs. glycerol content; (b) tensile strengths of PPI/glycerol biopolymers vs. glycerol content; (c) tensile moduli of PPI/glycerol biopolymers vs. pretreatment (glycerol 40 PHR); (d) tensile strengths of PPI/glycerol biopolymers vs. pretreatment (glycerol 40 PHR).

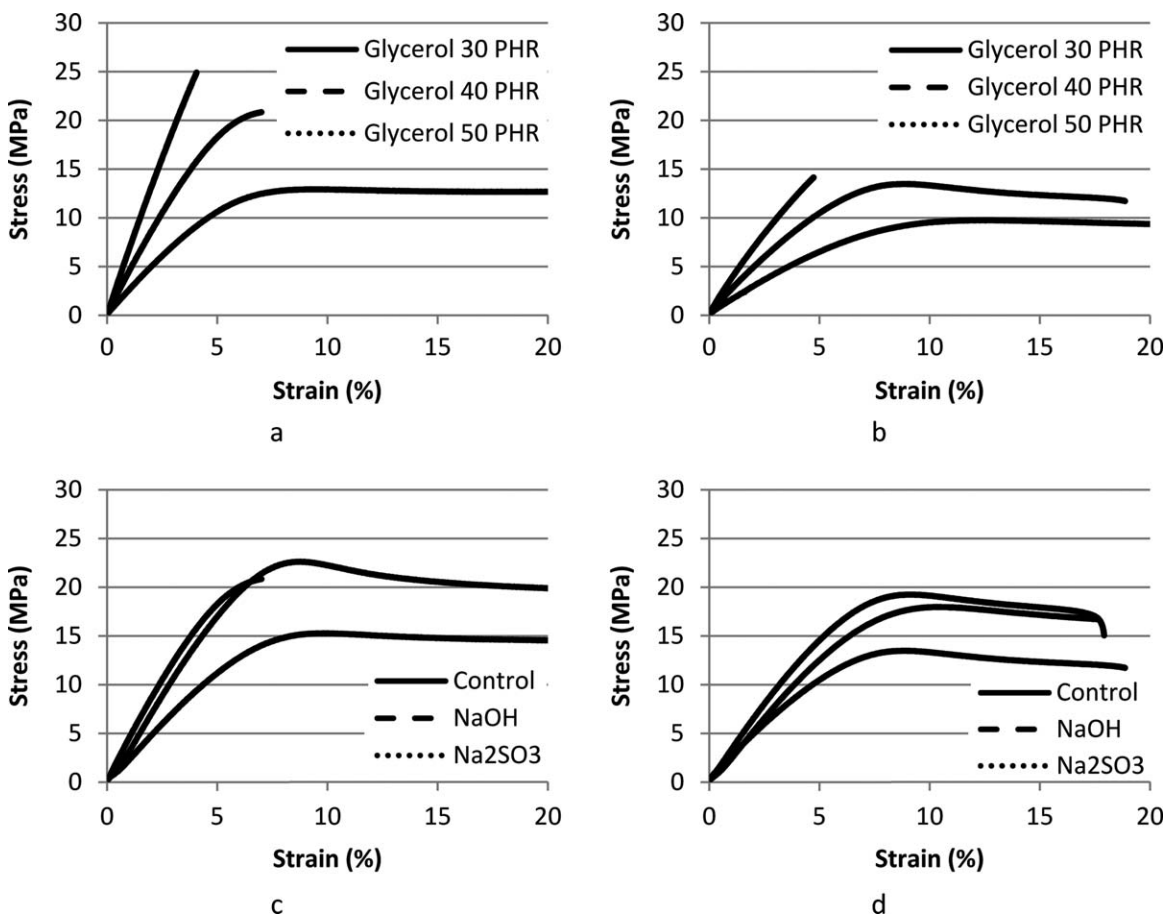

Figure 5. Stress–strain curves of PPI/glycerol biopolymer: (a) PPI-A with 30, 40, and 50 PHR glycerol; (b) PPI-B with 30, 40, and 50 PHR glycerol; (c) control or pretreated PPI-A/glycerol biopolymer (40 PHR); (d) control or pretreated PPI-B/glycerol biopolymer (40 PHR).

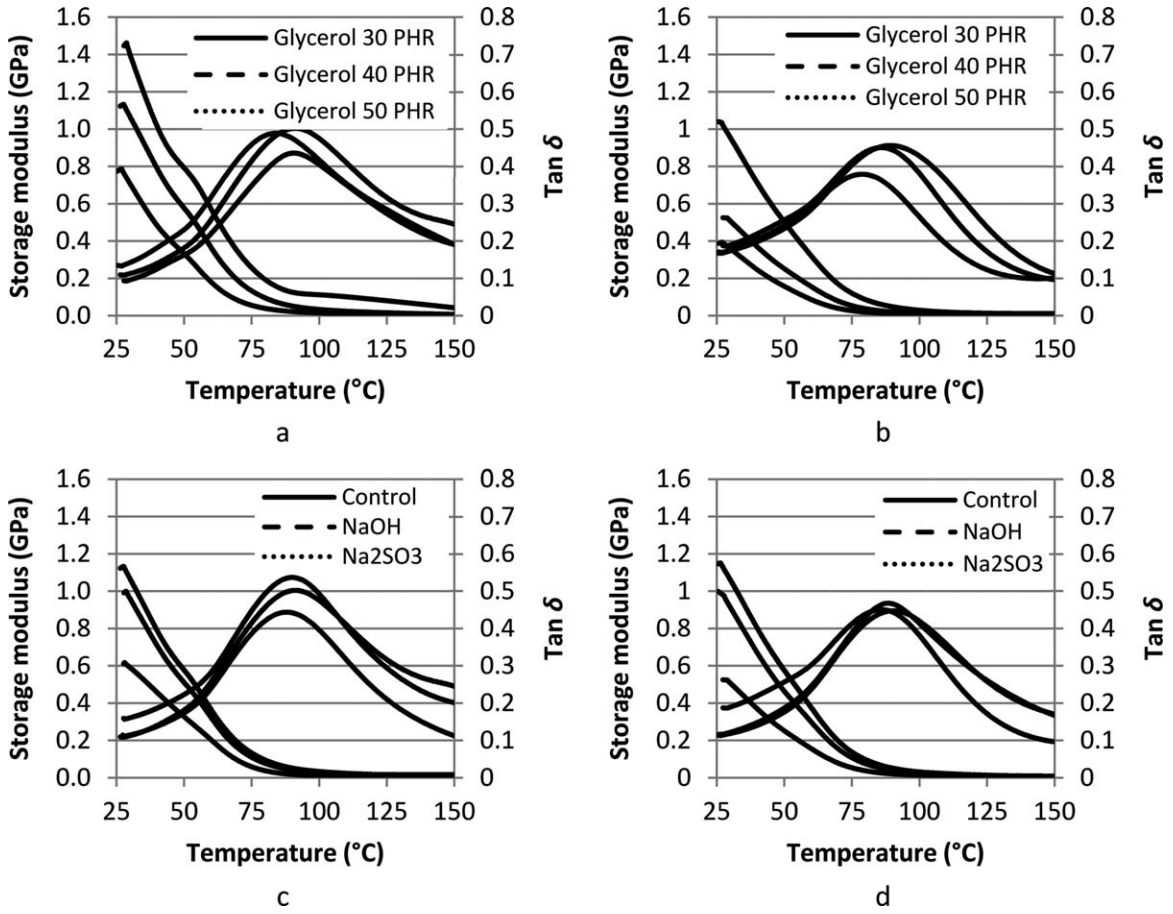

Figure 6. Storage modulus and tan δ curves of PPI/glycerol bio-polymer: (a) PPI-A with 30, 40, and 50 PHR glycerol; (b) PPI-B with 30, 40, and 50 PHR glycerol; (c) control or pretreated PPI-A with 40 PHR glycerol; (d) control or pretreated PPI-B with 40 PHR glycerol.

to-tough transition at a lower glycerol content (30–40 PHR) as compared to PPI-A [Figure 5(b)].

The pretreatment of the PPI with alkali or alkali under reducing conditions significantly changed the tensile properties of both the PPI-A/glycerol and the PPI-B/glycerol biopolymers [Figure 4(c,d)]. However, the effects of pretreatment on the tensile properties of two PPI/glycerol biopolymers were distinctly different. The alkaline treatment significantly reduced the tensile modulus and strength of the PPI-A biopolymer and the alkali treatment under reducing conditions caused only a slight reduction in the modulus and strength of the PPI-A biopolymer. Both pretreatments increased the elongation at break of the PPI-A biopolymer by altering the failure mode of the biopolymer from brittle to ductile at a glycerol content of 40 PHR [Figure 5(c)]. As for the PPI-B/glycerol biopolymer, both pretreatments significantly increased the modulus and strength [Figure 4(c,d)], with the alkali pretreatment being the more effective of the two pretreatments.

The mechanical properties of polymers can be markedly affected by two factors: molecular weight and polymer's secondary bonds.[33] It was observed in this study that potato protein-based biopolymers strictly followed this rule. The dependency of mechanical properties on molecular weight explained that (1) the tensile modulus and strength of PPI-A/glycerol biopolymer was higher than that of PPI-B/glycerol biopolymer; and (2) the increment in percentage of high-molecular weight of pretreated of PPI-B caused a significant increase in both modulus and strength of the PPI-B/glycerol biopolymer, as there was an approximately 60% weight loss of low-molecular weight PPI during alkaline treatment. The difference in tensile properties of PPI-A caused by pretreatment was a result of unfolding of original protein in crystalline form during denaturation and partial secondary structure changes before thermal processing.

Dynamic Mechanical Analysis

The dependency of storage modulus (E') and tan δ for each PPI biopolymer on temperature are plotted in Figure 6. The glass transition temperatures (T_gs) from the peak of tan δ are summarized in Table I. The increase of glycerol content markedly reduced the E' of both PPIs/glycerol biopolymers before a rubbery plateau was reached as shown in Figure 6(a,b), which was similar to the static tensile modulus. The T_gs of both the PPI-A and PPI-B biopolymers did not change appreciably as the glycerol content increased from 30 to 40 PHR, however, there was a sudden drop in T_gs when the glycerol content further increased to 50 PHR. The T_gs of the PPI-A biopolymers were 1.7–5.5°C higher than that of the PPI-B biopolymers within the studied glycerol content range.

The effect of pretreatment on the two PPIs with alkali or alkali/sodium sulfite on their respective biopolymers' E's and T_gs was similar to the effect of pretreatment on their static tensile

moduli. Before the rubbery plateau was reached, the *E*'s of the PPI-A/glycerol biopolymer decreased if the protein was pretreated, while the results were the opposite for the PPI-B/glycerol biopolymer. There was little change in the T_gs of the PPI-A/glycerol biopolymer after the pretreatment of protein with alkali or alkali under reducing conditions. Conversely, the T_gs of the PPI-B/glycerol biopolymer increased after the pretreatment. The difference in T_gs of pretreated PPIs can be a combined effect from changes in three factors occurring in this study: (1) average molecular weight increment because of the weight loss of low-molecular weight component during pretreatment, (2) enhanced hydrogen bonding because of protein denaturation, and (3) the conformational destruction of the protein in crystalline form. The first two factors would cause an increase in T_gs, while the third one would cause a decrease in T_gs. The increase in the T_g of PPI-B could be a result of the significant weight loss of low-molecular weight protein component during pretreatment. Further investigation to quantify the effect of these three factors is needed.

CONCLUSIONS

Biopolymers with two PPIs and glycerol as a plasticizer were prepared. The highest tensile strengths obtained were 20–25 MPa of the PPI-A/glycerol (30–40 PHR) biopolymer. The biopolymer can be designed to be brittle or highly ductile. The brittle-to-tough failure transition of the PPI-A and PPI-B biopolymers was in a plasticizer content range of 40–50 PHR, and 30–40 PHR, respectively. The alkali treatment changed the PPI's tertiary and secondary structure and/or molecular weight, and these changes in turn led to the change in static tensile properties and dynamic mechanical properties of the PPI/glycerol biopolymers.

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