1 Biodegradable soy protein films with controllable water solubility and enhanced
2 mechanical properties via graft polymerization

3 Yi Zhao^a, Helan Xu^a, Bingnan Mu^a, Lan Xu^b, Yiqi Yang^{*a,c,d}

4 ^a Department of Textiles, Merchandising & Fashion Design, University of
5 Nebraska-Lincoln, Lincoln, NE, 68583-0802, USA

6 ^b Department of Agronomy and Horticulture, University of Nebraska-Lincoln,
7 Lincoln, NE 68583-0915, USA

8 ^c Department of Biological Systems Engineering, University of Nebraska-Lincoln,
9 Lincoln, NE, 68583-0802, USA

10 ^d Nebraska Center for Materials and Nanoscience, University of Nebraska-Lincoln,
11 Lincoln, NE, 68583-0802, USA

12

13 * Corresponding author: Yiqi Yang

14 Phone: 402-472-5197; Fax: 402-472-0640; Email: yyang2@unl.edu

15

16 Abstract

17 Graft polymerization of acrylic acid endowed soy protein films with good tensile
18 properties and water solubility without sacrificing biodegradability. In this research,
19 soy protein was grafted with acrylic acid and cast into biodegradable films as
20 substitutes of non-biodegradable PVA films. The grafted soy protein films had 318%,
21 114%, 60% and 9% higher tensile strength, elongation, dissolving rate and
22 transmittance, compared with ungrafted ones, respectively. Acrylic acid grafting
23 provided soy protein films with biodegradability, flexibility, and adhesion to yarns
24 substantially higher than PVA, while water solubility and abrasion resistance similar
25 to PVA, leading to high potential applications of the grafted soy proteins in the fields
26 of water soluble packaging films and slashing to substitute PVA.

27 *Keywords:* Soy protein, Poly(vinyl alcohol), Water soluble film, Biodegradation, Graft
28 polymerization, Acrylic acid

29

1. Introduction

Using renewable polymers from agricultural byproducts as alternatives to non-biodegradable ones is essential for sustainable industries. Poly(vinyl alcohol) (PVA) figures prominently in the development of water soluble materials, such as fast-dissolving packaging films, oral medicine films and yarns' coating (textile warp sizes), and has a high annual consumption of over 1 million tons worldwide. [1] Due to good mechanical properties and water solubility, PVA films provide anglers' baits [2] or some chemicals, such as laundry detergents, bleach, fertilizers, pesticides, dyes and pigments, with enough protection during storage, and disappear after immersing in water bath for short period of time. [3] PVA coatings also provide warp yarns with protection against external abrasion and tension during high speed weaving, and are easily removed during desizing. [4] However, high amount of desized or released PVA wastewater have led to serious water pollution due to their poor biodegradability. [5] PVA is one of the chemical oxygen demand (COD) contributors to wastewater. In addition, PVA has capability of mobilizing heavy metals from sediments in water body, [6, 7] leading to irreversible accumulation of these metals in organs of marine creatures, and thereby causes related diseases. [8] Therefore, exploitation of biodegradable polymers with good water solubility to replace PVA in industries is imperative.

Substitutes of PVA for packaging films and textile sizes should have good biodegradability, good tensile strength of films with high elongation, and enough water solubility for laundering or desizing. Soy protein has good biodegradability,

52 film formability, and is readily available in large quantities from edible oil and
53 biodiesel production, [9] and thus has high potential to be fabricated into
54 environmentally benign films and textile sizes. [10,11] However, films from soy
55 protein have high brittleness [12] and fail to be directly used in packaging or slashing
56 (textile warp sizing) industries. [13,14] Many physical modifications have been
57 studied to overcome the drawbacks of pure protein films. [15,16] Addition of water
58 soluble plasticizer, such as glycerol, [17,18] hydroamine, [19,20] sucrose, galactose
59 and fructose, [21,22] and sorbitol, [23] could increase flexibility of protein films.
60 However, tensile strength of protein-based films has been substantially decreased with
61 the addition of plasticizers. [24,25] In addition, films from hydrolyzed and plasticized
62 soy protein cannot easily disintegrate or dissolve in water due to the lack of
63 water-soluble groups on molecular chains.

64 Chemical modifications have possibility to improve water solubility of proteins,
65 such as sulfonating hydroxyl groups, transforming amine groups into quaternary
66 ammonium salts, transforming hydroxyl groups into carboxyl groups, and diazotizing
67 amine groups and reacting with compounds with hydrophilic groups. However, most
68 of the above modifications strongly destroy protein molecules due to strong reaction
69 conditions and need toxic chemicals during reaction, leading to poor mechanical
70 properties and toxicity of modified protein films. Chlorosulfonic acid was used to
71 transfer hydroxyl groups on aliphatic chains into sulfo groups using dichloromethane
72 as solvent after overnight reaction. [26-28] Sulfonation of hydroxyls groups on
73 benzene rings occurred only with strong acids, such as toxic chlorosulfonic acid or

sulfur trioxide. [29,30] Sulfonation of benzene rings, which contain hydroxyl, was carried out using 98% sulfuric acid at high temperature. [31] Diazotization of amine groups occurred under strong acid conditions, such as 4 mol/l sulfuric acid. [32] Hydroxyl groups could also react with chloroacetic acid under 40 wt.% alkali condition and form water soluble carboxyl groups by nucleophilic substitution reaction. [33] Also, hydroxyl groups on proteins could react with hydrophilic epoxy compounds to increase their water solubility. However, the reaction only can be carried out under strong alkali conditions. [34] In addition, water solubility of protein has limited improvement via the chemical modifications due to limited reaction sites on proteins. For example, proteins have few tertiary amines, which could be transformed into quaternary ammonium salts.

In this research, soy protein films with good biodegradability, mechanical properties and controllable water solubility have been developed via acrylic-acid graft modification. Under a mild reaction condition, water soluble acrylic acids were grafted onto soy protein, and endowed soy protein with improved film water solubility and mechanical properties without sacrificing biodegradability or destroying protein polymers. Graft polymerization of soy proteins with acrylic acid was characterized. Tensile properties and biodegradability of the grafted soy protein films were studied. Performance properties of the grafted soy proteins, such as effects of grafting ratio on water solubility and transparency of soy protein films, adhesion of grafted soy protein sizes to fibers, and abrasion resistance of the grafted soy protein sized yarns, have been evaluated to explore their potential applications in the field of

96 packaging and textiles.

97 **2. Experimental**

98 *2.1. Materials*

99 Soy protein (PRO-FAM 646) was provided by ADM International, Decatur, IL.
100 PVA was purchased from chemical manufacturers in US. The PVA with a hydrolysis
101 degree of 86–89% and 65 kDa molecular weight was purchased from a commercial
102 sizing agent supplier in USA. Viscosity of the PVA at room temperature was 11.6-15.4
103 mPas at 4% solid content. Other chemicals used in this study were purchased from
104 VWR International.

105 *2.2. Graft modification of soy protein with acrylic acid*

106 Soy protein was grafted with acrylic acid through free radical reaction. Mixture of
107 soy protein and water was deoxygenated by aeration of nitrogen gas for about 20 min.
108 Initiator oxidant (5 wt.% $K_2S_2O_8$, based on the weight of soy protein) and reductant (2
109 wt.% $NaHSO_3$, based on the weight of soy protein) were dissolved in distilled water
110 and added into the soy protein mixture within 10 min in sequence. Then neutral
111 acrylic acid monomer (10-70 wt.%, based on the weight of soy protein) was slowly
112 added into the flask from dropping pipette within around 45 min. Liquor to soy
113 protein ratio was 9:1. The graft polymerization was carried under a nitrogen
114 atmosphere at 70 °C with stirring at 600 rpm for 4 hr. Finally, the graft reaction was
115 terminated by the addition of 0.04 wt.% paradioxybenzene (based on the weight of
116 soy protein). Schematic of the graft polymerization of soy protein with acrylic acid is
117 given in Scheme 1. pH of the reaction products was adjusted to isoelectric point of

soy protein (pH 4.5). [35] The precipitate from centrifugation was thoroughly washed and neutralized to about pH 7 and dried at 105 °C. Except of the addition of acrylic monomers, same operations were carried out to prepare ungrafted soy protein as control.

Scheme 1. Graft polymerization of soy protein with acrylic acid by NaHSO₃/K₂S₂O₈ system

1) Initiation

(H in Soy protein-H: hydrogen atom in -OH, -NH₂, -COOH, -SH)

2) Propagation

.....

3) Termination

2.3. Determination of Grafting Parameters

Total amount of residual acrylic acid was determined by titrating the double bonds of residual monomer in the supernatant. % Monomer conversion was calculated using Equation (1). [36]

$$\% \text{ Monomer conversion} = \frac{W_1 - W_2}{W_1} \times 100 \quad \text{Equation (1)}$$

W_1 and W_2 : weights of the total and the residual monomer, respectively

Percent grafting describes the weight percentage of polyacrylic acid branches grafted onto functional groups on the surfaces of soy protein to the original soy protein and % grafting efficiency describes the weight percentage of polyacrylic acid branches grafted onto functional groups on the surfaces of soy protein to the total polyacrylic acid, including grafted polyacrylic acid and ungrafted homopolymers. % grafting and % grafting efficiency were determined by Equations (2) and (3), respectively. [36]

$$\% \text{ Grafting} = \frac{W_a - W_0}{W_0} \times 100 \quad \text{Equation (2)}$$

$$\% \text{ Grafting efficiency} = \frac{W_a - W_0}{W_1 - W_2} \times 100 \quad \text{Equation (3)}$$

W_a : weight of grafted soy protein after water wash

W_0 : weight of the original soy protein

2.4. Proton Nuclear Magnetic Resonance (^1H NMR)

Ungrafted soy protein and acrylic acid grafted soy protein (AA-g-SP) were characterized by ^1H NMR in deuterated sodium hydroxide solution to confirm the successful grafting using an Avance 600 MHz Digital NMR spectrometer (Bruker Co.

160 Ltd., Switzerland). Sixty-four scans were acquired to obtain an adequate
161 signal-to-noise ratio. The concentration of each sample was about 1 wt.% in alkali
162 solvent.

163 *2.5. Conductometric and potentiometric titration of amine and carboxylic groups*

164 To verify grafting ratio of soy protein, concentrations of carboxylic groups and
165 amine groups were quantified by a pH/conductivity meter (Mettler Toledo Seven
166 Multi TMS47). Thirty grams of grafted and ungrafted soy protein solution with 0.6
167 wt.% concentration of soy protein were prepared, respectively. Standardized 0.024389
168 mol/l HCl was added into the soy protein solution to adjust pH to 3, under which the
169 carboxylic and amine groups in grafted and ungrafted soy protein were protonated.
170 Standardized 0.026112 mol/l NaOH solution was used in titration. Conductivity and
171 pH values were recorded after addition of about 2 ml NaOH solution.

172 *2.6. Molecular weight measurement*

173 Molecular weight of soy protein with different grafting ratio was investigated by
174 sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE). Half mg of dry soy protein
175 with different grafting ratio was dissolved in 1 ml sample buffer and heated at 70 °C
176 for 10 min. Solution of soy protein with different grafting ratio was loaded into each
177 lane (10 µl/lane) of a NuPAGE 4-12% Bis-Tris gel (Invitrogen, Grand Island, NY).
178 After electrophoresis, the gel was fixed by 25 vol.% isopropanol in 10 vol.% acetic
179 acid, stained by the 0.006 wt.% Coomassie Brilliant Blue staining solution in 10 vol.%
180 acetic acid, followed by destaining in 10 vol.% acetic acid for whole night with gentle
181 shaking. [37]

2.7. Biodegradability

Chemical oxygen demand (COD) and 5-day biological oxygen demand (BOD₅) were the major testing indexes of protein-based wastewater. Sludge from local wastewater treatment plant was collected and acclimatized for 2 days under room temperature and pH of the sludge was around 7.5. According to American Public Health Association guidelines in the standard methods for the examination of wastewater and U.S. EPA method 800, grafted soy protein (14% grafting ratio) solution with 0.03% concentration was treated in the activated sludge for 0-5 days. Here, soy protein with 14% grafting ratio was chosen because the grafted soy protein showed good tensile properties of films and water solubility. Supernatant was collected by centrifuging the mixture. COD in sizes solution was measured by digital reactor block DRB 200 (HACH, DE). BOD₅ was tested using optical dissolved oxygen meter HQ440d (HACH, DE). [38] Total nitrogen of the soy protein sizes after activation was measured by UV/vis spectrophotometer DU 720 (Beckman Coulter, USA) and calculated based on the alkaline persulfate oxidation digestion method. Ammonia nitrogen was measured by the same spectrophotometer and calculated using salicylate and hypochlorite in an alkaline phosphate buffer. [38]

2.8. Film preparation and tensile properties

The grafted and ungrafted soy proteins were dispersed in distilled water with pH 8, respectively. Both of the grafted and ungrafted soy protein solutions with 6 wt.% concentration were heated at 90 °C for 30 min. After heating, the solutions were respectively cast onto Teflon coated glass plated and allowed to dry at 20 °C and 65%

204 humidity for almost 40 hr. PVA dispersed in water was also heated at 90 °C for 30 min,
205 and cast into films. To study plasticizer effect on film tensile properties of the grafted
206 soy protein, 5 wt.% - 30 wt.% triethanolamine (TEA) (based on the weight of grafted
207 soy protein) were added into 6 wt.% grafted and ungrafted soy protein dispersed
208 solution. pH of the solutions was adjusted to 8, respectively. The plasticized solutions
209 were heated at 90 °C for 30 min and cast into films.

210 All the films were tested on a MTS tensile tester QTest 10 (MTS systems
211 corporation, USA) according to ASTM Standard 822e to evaluate film elongation and
212 tensile strength. According to the standard, film samples with size of 8 cm x 1.5 cm
213 were prepared and tested using a gauge length of 2 inches and crosshead speed of 10
214 mm/min.

215 2.9. *Morphology of films*

216 Ungrafted and grafted soy protein films were observed using a variable pressure
217 scanning electron microscope (VPSEM) Hitachi S3000N (Hitachi, Tarrytown, NY) at
218 a voltage of 15 KV. Samples were sputter coated with gold palladium before
219 observation under the SEM.

220 2.10. *Water solubility of films*

221 Water solubility of films was tested according to MonoSol Standard Test Method.^[35]
222 A film with size of 2 cm x 1 cm was perpendicularly clamped into 300 ml distilled
223 water, which was stirred at 200 rpm. When half of length of film was dipped into the
224 flow of water, timer was started. Disintegration occurred when the film broke.
225 Dissolution occurred when all film fragments were no longer visible. Time was

recorded to evaluate water solubility of each film. The thickness of films was 0.19 mm $\pm$ 0.04 mm. Temperatures of distilled water were 20 °C and 70 °C.

2.11. Transmittance of films

To determine transmittance of grafted and ungrafted soy protein films under visible light, % transmittance of the films from soy protein with different grafting ratio were measured with a UV – Vis spectrophotometer instrument DU 720 (Beckman Coulter, USA) in the range of 400 to 800 nm at room temperature.

2.12. Adhesion properties

High adhesion between sizes and yarns is one of the major requirements for sizing agents. Adhesion of sizes to cotton, polyester and polyester/cotton were evaluated by testing peel force between two fabrics, which were glued by sizes. A bleached plain-woven fabric with size of 5 cm x 16 cm (width x height) was prepared and cut into two piece of 5 cm x 8 cm fabrics. Two pieces of fabrics were immersed in the 90 °C sizing solution for 5 min. The sized fabrics were stacked face to face and squeezed together in a laboratory padder with 1 lb load to ensure glued fabrics with uniform size pick-up and penetration. Peel force of fabrics was measured according to the method ASTM D1876–08. To obtain different add-on on every two pieces of fabrics, two pieces of same fabrics were dipped in the sizes with different concentration. The sized samples were conditioned at 20 °C and 65% humidity for 34hr. Peel tests were carried out on a MTS tensile tester QTest 10 (MTS systems corporation, USA). On each peel force-strain curve, 20 highest values were collected to calculate average peel force.

2.13. Abrasion resistance

Abrasion resistance of sized yarns was tested on Y731D cohesion tester (Changzhou Huafang Textile Instrument Co., Ltd, China). Sized yarns with known add-on were fasten and rubbed until broken. Abrasion cycles were recorded automatically. Reciprocating stroke is 90 mm and speed of abrasion is 80 cycles per min.

2.14. Statistical analysis

Generated data was analyzed using Tukey's multiple-pairwise comparison using SAS program (SAS Institute, Raleigh, NC). A significance level of $\alpha=0.05$ was considered as statistically significant. Within the level of $\alpha=0.05$, significant difference could be told between two data points. All the experiments were repeated at least three times to report average and standard errors. In each Fig., data points with significant differences were marked with different letters, numbers or symbols.

3. Results and discussion

3.1. Molecular composition analysis of grafted soy protein

Proton NMR spectra of ungrafted soy protein and acrylic acid grafted soy protein (AA-g-SP) are shown in the Fig. 1. Soy protein has the same amount of methyl groups before and after grafting, based on the peaks of methyl appeared at 0.8 ppm. The grafted products were treated to remove homopolymer of acrylic acid (polyacrylic acid). So if peak of methine groups ($>CH-$) was shown in the grafted soy protein spectrum, indicating soy protein was grafted with acrylic acid monomers and formed branched chains of polyacrylic acid as shown in scheme 1. In Fig. 1,

compared to the spectrum of ungrafted soy protein, new chemical linkages were found in that of AA-g-SP. The peaks of methine groups ($>\text{CH}-$) at 2.4 ppm confirmed the grafting of acrylic acid onto the soy protein.

Fig. 1

To further verify grafting of acrylic acid onto soy protein, amounts of amine and carboxylic groups of the soy protein before and after grafting were quantified by conductometric and potentiometric titrations. The whole titration process contains four sections, including neutralization of excessive H^+ from excessive strong acid (HCl), H^+ from weak acid COOH in protein and H^+ from weak acid NH_3^+ and no H^+ section. [39] On both conductometric and potentiometric titration curves, three transition points were observed within the four titration sections. The four sections were simulated using linear regression with r^2 higher than 0.98. Intersections of these regression lines indicated transition from one process to the next.

The first point and the third point at around 10 ml and 40 ml in conductometric curve in Fig. 2a, 10 ml and 50 ml in Fig. 2b, were clearly observed. However, the

second transition point was vague. To determine the second intersection points, potentiometric curves were combined. In the second portion, potentiometric titration curve showed a sharp increase in slope, which could be used to clarify this point. Calculation of the intersection point of conductivity and pH value on the titration curve of soy protein and AA-g-SP is shown as follows.

Soy protein:

$$\text{Point A: } -36.275 X + 1885.7 = 5.823 X + 1422.9$$

$$X = 10.994 \text{ (10.994 ml NaOH)}$$

$$\text{Point B: } 0.3101 X - 2.2053 = 0.1621 X + 2.1512$$

$$X = 29.4358 \text{ (29.4358 ml NaOH)}$$

$$\text{Point C: } 0.2867 X - 1.4287 = 0.0259 X + 9.4704$$

$$X = 41.7910 \text{ (41.7910 ml NaOH)}$$

Therefore, 18.4418 ml and 12.3552 ml NaOH (0.026112 mol/l) were consumed by carboxyl and amino groups in soy protein, respectively.

Grafted soy protein:

$$\text{Point D: } -33.9 X + 2138.3 = 3.765 X + 1737.4$$

$$X = 10.6438 \text{ (10.6438 ml NaOH)}$$

$$\text{Point E: } 0.3099 X - 6.2395 = 0.642 X + 3.9424$$

$$X = 41.4403 \text{ (41.4403 ml NaOH)}$$

$$\text{Point F: } 0.2962 X - 5.577 = 0.0797 X + 5.6241$$

$$X = 51.7372 \text{ (51.7372 ml NaOH)}$$

Therefore, 30.7965 ml and 10.2969 ml NaOH (0.026112 mol/l) were consumed by

308 carboxyl and amino groups in AA-g-SP, respectively.

309 In general, in the first portion, 10.994 ml and 10.6438 ml NaOH were respectively
310 consumed for excessive H^+ in the ungrafted and grafted soy protein solutions. In the
311 second portion, 18.4418 ml and 30.7965 ml NaOH were consumed for carboxyl
312 groups in the ungrafted and grafted soy protein solutions, respectively. In the third
313 portion, 12.3552 ml and 10.2969 ml NaOH were consumed for amine groups in the
314 ungrafted and grafted soy protein solutions, respectively. Differences between NaOH
315 volume consumed by ungrafted and grafted soy protein in the second section indicate
316 NaOH volume react with carboxyl group from grafted acrylic acid. Compared to the
317 ungrafted soy protein, AA-g-SP solution consumed 12.3547 ml NaOH (0.026112
318 mol/l) to react with carboxyl group from the grafted acrylic acid, indicating $3.226E-4$
319 mol NaOH were consumed to react with carboxyl group from the grafted acrylic acid.
320 Therefore, $3.226E-4$ mol (0.0232 g) acrylic acid was grafted onto soy protein. Due to
321 30 grams of 0.6 wt.% grafted soy protein solution were used for titration, the grafting
322 ratio can be calculated by $0.0232/(30 \times 0.6\%) = 12.88\%$. As a result, the grafting ratio
323 calculated by titration method is 12.88%, which is similar to that calculated by weight
324 measurement (14%).

Fig. 2

3.2. Effect of monomer concentration on grafting parameters

As shown in Fig. 3, increasing monomer concentration increased grafting ratio. Increasing concentration of monomer promoted polymerization, including graft polymerization and homopolymerization. Based on the constant amount of soy protein, grafting ratio increased during grafting process. Fig. 3 also showed that grafting efficiency and monomer conversion increased first and then kept unchanged with increasing monomer concentration. Graft polymerization and homopolymerization were competitive with each other during grafting process. At the initial period, graft polymerization took place more easily compared with homopolymerization, because high amount of active sites was formed in soy protein polypeptides. Therefore, grafting efficiency increased firstly. However, the active sites

on soy protein were gradually occupied by monomers of acrylic acid, leading to high possibility of homopolymerization of residual monomers. Therefore, percent grafting efficiency decreased when the monomer concentration was higher than 30%. As to percent monomer conversion, it did not increase when monomer concentration was higher than 50%. As the length of polyacrylic acid branches (including grafted branches and homopolymers) increased, the reaction system became stable gradually. Then, chains of polyacrylic acid were prevented from growing longer, especially when the addition of acrylic acid monomer exceeded 50%. Therefore, percent of monomer conversion remained unchanged when the monomer concentration reached 50%.

Fig. 3

3.3. Effect of grafting ratio on molecular weight of soy protein

Fig. 4 shows that increasing grafting ratio increased molecular weight of soy protein. Compared to the grafted soy protein, the ungrafted soy protein had low

molecular weight, as shown by the band between 17 kDa and 28 kDa. Except the band of small molecular weight, dark shadows dispersed in the lane 1 indicated hydrolysis of soy protein. Grafted soy protein with 4.2% grafting ratio showed 3 major bands at around 62 kDa, 38 kDa and between 17 kDa and 28 kDa. As grafting ratio increased from 4.2% to 20%, grafted soy protein in lane 2 to lane 5 showed major bands moving up gradually, indicating increased molecular weight. Acrylic acid reacted with active groups, such as hydroxyl, carboxyl, sulfhydryl or amine groups, to form grafted branches. Increasing grafting ratio increased length or quantity of grafted branches, leading to increased molecular weight of soy protein.

Fig. 4

3.4. Biodegradability

COD and BOD₅ of AA-g-SP solutions are shown in Fig. 5. AA-g-SP solution had

COD similar to soy protein-TEA solution, but lower COD than PVA solution during 5 days treatment. After 5 days treatment in activated sludge, COD of PVA solution reduced to 398.3 ± 8.4 mg/l from 520.4 ± 10 mg/l, while COD of AA-g-SP solution decreased to 106.8 ± 4.9 mg/l from 485.0 ± 12.7 mg/l. Grafted polyacrylate branches probably decreased biodegradability of soy protein scarcely, due to good biodegradability of low molecular weight acrylic acid homopolymers. [40] Therefore, AA-g-SP (14% grafting ratio) had slightly higher COD and slightly lower BOD₅ as compared with soy protein-TEA during 5 days treatment in activated sludge. On the contrary, after 5 days treatment in activated sludge, PVA solution showed low BOD₅ and high COD (about 400 mg/l).

Fig. 5

Usually, protein contains certain amount of nitrogen and poses risk to environment by inducing eutrophication in water body. As shown in Fig. 6, the amounts of total and ammonia nitrogen released from the AA-g-SPI desizing effluents during 5 days of treatment in activated sludge were lower than that of soy protein-TEA. In addition, total nitrogen and ammonia nitrogen of AA-g-SPI effluents were 16 mg/l and 0.5 mg/L, respectively. Since The AA-g-SPI desizing effluents shows low levels of total and ammonia nitrogen after 5 days of treatment in activated sludge, AA-g-SPI sizes demonstrates low possibility to arouse water eutrophication and will not affect the operation of the effluent treatment plants.

Fig. 6

3.5. Effect of grafting ratio on tensile strength and elongation of soy protein films

As shown in Fig. 7, increasing grafting ratio from 0% to 14% substantially increased tensile strength of soy protein films. It was probably because that grafting of acrylic acid enhanced the solubility of soy protein in distilled water at pH 8. Dissolved soy protein polypeptides could be highly unfolded and more closely

interacted with each other during film forming. In Fig. 8a and 8b, AA-g-SP films showed higher transparency, less cracks, less holes and smoother surface, comparing to the ungrafted ones. Difference in morphology between the grafted and ungrafted soy protein films proved that grafting of acrylic acid improved solubility of soy protein during film forming.

However, increasing grafting ratio also increased the amount of carboxyl groups in soy protein molecules, leading to enhanced repulsive force between soy proteins. At the same time, grafted branches increased steric hindrance among soy proteins. As a result, soy protein with 20% grafting ratio might have substantially decreased inter-molecular interaction, and thus showed decreased tensile strength of the films.

In terms of film elongation, increasing grafting ratio increased film elongation all the way. Graft modification of acrylic acid increased repulsive force and steric hindrance between soy proteins and also increased moisture absorption of the films, leading to easier slippage between protein chains, and therefore improved film elongation.

Fig. 7

Fig. 8

3.6. Evaluation of performance properties

Water solubility and transmittance of films

As shown in Table 1, dissolving time of soy protein films in 20 °C and 70 °C water bath was decreased as grafting ratio increased. Graft polymerization with acrylic acid introduced water soluble carboxyl groups into soy protein, probably leading to improved water solubility of soy protein films. In warm water (70 °C), dissolving time of soy protein films was substantially shorter than that in cold water (20 °C). Compared to films from soy protein with 14% grafting ratio, PVA films showed about 190% and 55% longer dissolving time ($20'18'' \pm 15''$ and $1'44'' \pm 7''$) in 20 °C and 70 °C water bath, respectively. Thus, AA-g-SP films had good water solubility in both cold water and warm water.

As to film appearance, increasing grafting ratio increased transmittance of soy protein films at the wavelength ranging from 400 nm to 800 nm, especially at wavelength of 600 nm and 800 nm. It was probably because grafting of acrylic acid endowed soy protein with better solubility during film forming. In addition, protein

with enhanced solubility in solvent could form cast films with higher transparency. [41]

Table 1. Dissolving time and transmittance of acrylic acid grafted soy protein films

% grafting	Dissolving time, min		Transmittance at wavelength,%		
	20 °C water bath	70 °C water bath	400 nm	600 nm	800 nm
0	20'44"±16"	3'52"±8"	16.6±0.2	54.2±0.4	67.8±0.4
4.2	20'02"±11"	3'01"±10"	30.3±0.3	62.3±0.4	68.9±0.4
8.5	15'33"±9"	2'25"±5"	30.4±0.2	65.1±0.3	73.3±0.5
14	11'05"±13"	1'40"±12"	17.5±0.1	63.5±0.4	75.3±0.5
20	7'00"±12"	1'07"±9"	27.4±0.3	72.7±0.4	80.9±0.5

Effect of % TEA on tensile strength and elongation of AA-g-SP films

As shown in Fig. 9, increasing plasticizer (triethanolamine) concentration increased elongation but decreased tensile strength of the AA-g-SP films. Compared to ungrafted soy protein, triethanolamine (TEA) endowed the grafted soy protein films with substantially higher film elongation but slightly higher tensile strength. With 0% TEA, ungrafted soy protein films had elongation and tensile strength of around 0.3% and 2.9 MPa, respectively. Meanwhile, the grafted soy protein films had tensile strength and elongation of 115% and 12 MPa, respectively. The difference in solubility could be the reason of different tensile properties of the films. Increasing TEA concentration from 0% to 5% unfolded soy protein molecules, leading to substantial increase in tensile strength and elongation of films. [42] However, as to AA-g-SP, which was unfolded by repulsive force from the grafted branches with

negative charges, addition of TEA probably did not further increase unfolding of soy protein molecules, and thus failed to substantially increase tensile strength and elongation of AA-g-SP films. Further increasing TEA concentration from 20% to 30% slightly decreased tensile strength but increased elongation of both grafted and ungrafted soy protein films. Under the optimal condition, AA-g-SP film showed substantially higher elongation and slightly higher tensile strength than the ungrafted soy protein films.

Fig. 9

Adhesion properties

As shown in Fig. 10, AA-g-SPI (grafted soy with TEA) sizes had better adhesion to cotton yarns and polyester/cotton yarns, and similar adhesion to polyester, comparing to PVA. As add-on on cotton and polyester/cotton fabrics increased, AA-grafted soy protein sizes had higher adhesion compared to ungrafted soy protein sizes and PVA sizes. Specifically, AA-grafted soy protein sized polyester/cotton fabrics and cotton fabrics showed 17.6% and 28.6% higher peel resistance, respectively, at 15% add-on,

459 comparing to ungrafted soy protein. It was probably because AA grafting could
460 increase molecular weight of soy protein as compared to the ungrafted soy protein.
461 Also, high content of carboxyl groups were induced into soy protein, leading to the
462 formation of unfolded molecules with increased diameter of micelle. Thus,
463 strengthened Van der Waals' force between soy protein and fibers led to improved
464 adhesion of AA-g-SPI sizes. In addition, AA-g-SPI sizes had more hydrophilic
465 carboxyl groups and thus had comparatively higher adhesion to cotton based fabrics.
466 On the contrary, adhesion of the PVA sizes to cotton based fabrics was poor, although
467 adhesion of PVA sizes to polyester was similar to AA-g-SPI sizes. As shown in Fig.
468 10b and 10c, peel resistances of PVA sizes from cotton and polyester/cotton fabrics
469 were substantially lower than AA-g-SPI sizes.

Fig. 10

Abrasion resistance of AA-g-SPI sized yarns

As shown in Fig. 11, AA-g-SPI sizes had abrasion resistance similar to or higher

474 than the ungrafted soy sizes and PVA sizes on polyester, polyester/cotton and cotton
475 yarns. Both adhesion and film tensile properties could influence abrasion resistance of
476 sizing agents. AA-g-SPI had higher film elongation but lower tensile strength than
477 PVA. In terms of adhesion, AA-g-SPI sizes had substantial higher adhesion to cotton
478 yarns and similar adhesion to polyester and polyester/cotton yarns, as compared with
479 PVA sizes. Therefore, compared to PVA sizes, AA-g-SPI sizes had substantially
480 higher abrasion resistance on cotton and similar abrasion resistance on
481 polyester/cotton and polyester yarns, respectively. This is an improvement comparing
482 to the physically modified soy protein size. [43] In addition, AA-g-SPI sizes had
483 much higher adhesion to yarns and higher film elongation than the ungrafted soy
484 protein sizes. Thus, AA-g-SPI sizes showed higher abrasion resistance on the three
485 types of yarns as compared with the ungrafted soy protein sizes.

Fig. 11

4. Conclusions

The casting films from acrylic acid grafted soy protein had good biodegradability,

water solubility, tensile strength, flexibility, adhesion to fibers and abrasion resistance, indicating high potential as protective coatings for warp yarns and fast-dissolving packaging materials. Acrylic acid grafted soy protein had good biodegradability and could be easily degraded in activated sludge after degradation for 5 days. Grafted branches of polyacrylic acid increased tensile properties, water solubility and transparency of soy protein films. Compared to the ungrafted ones, the grafted soy protein films had 318%, 114%, 60% and 9% higher tensile strength, elongation, dissolving rate and transmittance, respectively. Acrylic acid grafted soy proteins showed higher biodegradability, film flexibility, adhesion properties, and similar water solubility and abrasion resistance, as compared with PVA. Therefore, acrylic acid grafted soy protein have potential to replace water soluble PVA for packaging and slashing applications.

Acknowledgments

This research was financially supported by the United Soybean Board Domestic Programs (1440-512-5296), USDA-National Institute of Food and Agriculture (Multi-State Project S1054 (NEB 37-037) and Hatch Act), and the Agricultural Research Division at the University of Nebraska Lincoln. Yi is grateful to China Scholarship Council and John and Louise Skala Fellowship for their financial support. The authors also thank Madhuri Palakurthi and Wei Li for their help of films testing.

509   **References**

- 510   [1] W. Sun, J. Tian, L. Chen, S. He, J. Wang, Improvement of biodegradability of
511   PVA-containing wastewater by ionizing radiation pretreatment. *Environ. Sci.*
512   *Pollut. Res.* 19 (2012) 3178-3184.
- 513   [2] M. Julinová, J. Kupec, P. Alexy, J. Hoffmann, V. Sedlařík, T. Vojtek, P. Bugaj,
514   Lignin and starch as potential inductors for biodegradation of films based on poly
515   (vinyl alcohol) and protein hydrolysate. *Polym. Degrad. Stab.* 95 (2010) 225-233.
- 516   [3] F. Parvin, M. Rahman, J.M. Islam, M.A. Khan, A.H.M. Saadat, Preparation and
517   characterization of starch/PVA blend for biodegradable packaging material.
518   *Advanced Materials Research* 123 (2010) 351-354.
- 519   [4] Y. Zhao, H. Xu, B. Mu, L. Xu, R. Hogan, Y. Yang, Functions of soymeal
520   compositions in textile sizing. *Ind. Crop. Prod.* 89 (2016) 455-464.
- 521   [5] C.B. Shaw, C.M. Carliell, A.D. Wheatley, Anaerobic/aerobic treatment of coloured
522   textile effluents using sequencing batch reactors. *Water Res.* 36 (2002) 1993-2001.
- 523   [6] L. Lei, X. Hu, P.L. Yue, S.H. Bossmann, S. Göb, A.M. Braun, J. Photochem.
524   Photobiol. *A Chem.* 116 (1998) 159-166.
- 525   [7] Y. Zhao, Y.Z. Zhao, H.L. Xu, Y.Q. Yang, A Sustainable Slashing Industry Using
526   Biodegradable Sizes from Modified Soy Protein to Replace Petro-Based Poly
527   (Vinyl Alcohol). *Environ. Sci. Technol.* 49 (2015) 2391-2397.
- 528   [8] J. Baby, J.S. Raj, E.T. Biby, P. Sankarganesh, M.V. Jeevitha, S.U. Ajisha, S.S.
529   Rajan, Toxic effect of heavy metals on aquatic environment. *International Journal*
530   *of Biological and Chemical Sciences* 4 (2010).

- 531 [9] X.S. Sun, Isolation and processing of plant materials. Academic Press, USA 2011.
- 532 [10] R.R. Koshy, S.K. Mary, S. Thomas, L.A. Pothan, Environment friendly green
533 composites based on soy protein isolate—A review. Food Hydrocolloids, 50 (2015)
534 174-192.
- 535 [11] Y. Zou, S. Huda, Y. Yang, Lightweight composites from long wheat straw and
536 polypropylene web. Bioresource Technol. 101 (2010) 2026-2033.
- 537 [12] J.F. Su, X.Y. Yuan, Z. Huang, W.L. Xia, Properties stability and biodegradation
538 behaviors of soy protein isolate/poly (vinyl alcohol) blend films. Polym. Degrad.
539 Stab. 95 (2010) 1226-1237.
- 540 [13] I. Paetau, C.Z. Chen, J.L. Jane, Biodegradable plastic made from soybean
541 products. 1. Effect of preparation and processing on mechanical properties and
542 water absorption. Ind. Eng. Chem. Res. 33 (1994) 1821-1827.
- 543 [14] J.W. Rhim, A. Gennadios, C.L. Weller, M.A. Hanna, Sodium dodecyl sulfate
544 treatment improves properties of cast films from soy protein isolate. Ind. Crop.
545 Prod. 15 (2002) 199-205.
- 546 [15] N. Zhou, X. Geng, M. Ye, L. Yao, Z. Shan, Y. Qiu, Mechanical and sound
547 adsorption properties of cellular poly (lactic acid) matrix composites reinforced
548 with 3D ramie fabrics woven with co-wrapped yarns. Ind. Crop. Prod. 56 (2014)
549 1-8.
- 550 [16] Y.Z. Zhao, Y. Zhao, Y.Q. Yang, Modified soy protein to substitute non-degradable
551 petrochemicals for slashing industry. Ind. Crop. Prod. 67 (2015) 466-474.
- 552 [17] E.M. Ciannamea, P.M. Stefani, R.A. Ruseckaite, Physical and mechanical

553 properties of compression molded and solution casting soybean protein
 554 concentrate based films. Food Hydrocolloids 38 (2014) 193-204.
 555 [18] J. Zhang, L. Jiang, L. Zhu, J.L. Jane, P. Mungara, Morphology and properties of
 556 soy protein and polylactide blends. Biomacromolecules, 7 (2006) 1551-1561.
 557 [19] J. Irissin-Mangata, G. Bauduin, B. Boutevin, N. Gontard, New plasticizers for
 558 wheat gluten films. Eur. Polym. J. 37 (2001) 1533-1541.
 559 [20] H.F. Tian, D.G. Liu, L. Zhang, Structure and Properties of Soy Protein Films
 560 Plasticized with Hydroxyamine. J. Appl. Polym. Sci. 111 (2009) 1549-1556.
 561 [21] B. Cuq, N. Gontard, J.L. Cuq, S. Guilbert, J. Agric. Food Chem. 1997, 45, 622.
 562 [22] M.G.A. Vieira, M.A. da Silva, L.O. dos Santos, M.M. Beppu, Natural-based
 563 plasticizers and biopolymer films: A review. Eur. Polym. J. 47 (2011) 254-263.
 564 [23] S.Y. Cho, C. Rhee, Sorption characteristics of soy protein films and their relation
 565 to mechanical properties. LWT-Food Sci. Technol. 35 (2002) 151-157.
 566 [24] N. Laohakunjit, A., Noomhorm, Effect of plasticizers on mechanical and barrier
 567 properties of rice starch film. Starch - Stärke 56 (2004) 348-356.
 568 [25] P. Lodha, A.N. Netravali, Effect of soy protein isolate resin modifications on
 569 their biodegradation in a compost medium. Polym. Degrad. Stab. 87 (2005)
 570 465-477.
 571 [26] K. Pradhan, P. Bhattacharyya, S. Paul, A.R. Das, Synthesis of 3,
 572 4-dihydropyridin-2-one derivatives in convergent mode applying bio catalyst
 573 vitamin B 1 and polymer supported catalyst PEG-SO₃H from two different sets
 574 of building blocks. Tetrahedron Lett. 53 (2012) 5840-5844.

- 575 [27] M.A. Nasser, S. A. Alavi, B. Zakeri Nasab. PEG–SO₃H as a mild, efficient and
576 green catalytic system for the synthesis of pyrazole derivatives in aqueous
577 medium. *Journal of the Iranian Chemical Society* 10 (2013) 213-219.
- 578 [28] S. Badvel, R.R. Gopireddy, T.B. Shaik, S. Hasti, V.R. Tummaluru, N.R.
579 Chamarthi, An efficient one-pot three-component synthesis of pyrimido [4, 5-d]
580 pyrimidine derivatives in aqueous medium. *Chem. Heterocycl. Compd.* 51 (2015)
581 749-753.
- 582 [29] G. Strandlund, P. Lagerstrom, Sulfonation of 2-methylphenol and
583 3-methylphenol. *ACTA Chem. Scand. B* 33 (1979) 261-264.
- 584 [30] H. Cerfontain, A. Koeberg-Telder, H.J. Lambrechts, P. De Wit, Aromatic
585 sulfonation. 90. Sulfonation of three symmetrical 2, 6-dialkylphenols, 2,
586 6-dimethylanisole. Sulfation and sulfonation product distributions and
587 mechanisms. *The Journal of Organic Chemistry* 49 (1984) 4917-4923.
- 588 [31] A.V. Pisareva, G.V. Shilov, A.I. Karelin, Y.A. Dobrovolsky, R.V. Pisarev,
589 2-Hydroxy-4-methylbenzenesulfonic acid dihydrate: Crystal structure, vibrational
590 spectra, proton conductivity, and thermal stability. *Russ. J. Phys. Chem. A* 84
591 (2010) 444-459.
- 592 [32] D.M. Zurcher, Y.J. Adhia, J.D. Romero, A.J. McNeil, Modifying a known gelator
593 scaffold for nitrite detection. *Chem. Commun.* 50 (2014) 7813-7816.
- 594 [33] X.Q. Li, H.Y. Wen, H.Q. Dong, W.M. Xue, G.M. Pauletti, X.J. Cai, Y.Y. Li,
595 Self-assembling nanomicelles of a novel camptothecin prodrug engineered with a
596 redox-responsive release mechanism. *Chem. Commun.* 47 (2011) 8647-8649.

- 597 [34] T.S. Gandhi, M.R. Patel, B.Z. Dholakiya, Synthesis and characterization of
 598 different types of epoxide-based Mannich polyols from low-cost cashew nut shell
 599 liquid. *Res. Chem. Intermediat.* 40 (2014) 1223-1232.
- 600 [35] A.H. Brandenburg, C.L. Weller, R.F. Testin, Edible films and coatings from soy
 601 protein. *J. Food Sci.* 58 (1993) 1086-1089.
- 602 [36] E. Jin, N. Reddy, Z. Zhu, Y. Yang, Graft polymerization of native chicken
 603 feathers for thermoplastic applications. *J. Agric. Food Chem.* 59 (2011)
 604 1729-1738.
- 605 [37] Y. Zhao, Q. Jiang, H. Xu, N. Reddy, L. Xu, Y. Yang, Cytocompatible and
 606 water-stable camelina protein films for tissue engineering. *J. Biomed. Mater.*
 607 *Res. B Appl Biomater.* 102 (2014) 729-736.
- 608 [38] N. Reddy, Y. Zhang, Y.Q. Yang, Corn distillers dried grains as sustainable and
 609 environmentally friendly warp sizing agents. *ACS Sustain. Chem. Eng.* 1 (2013)
 610 1564-1571.
- 611 [39] L. Shen, H. Xu, L. Kong, Y. Yang, Non-Toxic Crosslinking of Starch Using
 612 Polycarboxylic Acids: Kinetic Study and Quantitative Correlation of Mechanical
 613 Properties and Crosslinking Degrees. *J. Polym. Environ.* 23 (2015) 588-594.
- 614 [40] R.J. Larson, E.A. Bookland, R.T. Williams, K.M. Yocom, D.A. Saucy, M. B.
 615 Freeman, G. Swift, Biodegradation of acrylic acid polymers and oligomers by
 616 mixed microbial communities in activated sludge. *J. Environ. Polym.*
 617 *Degr.* 5(1997) 41-48.
- 618 [41] Z. Shi, N. Reddy, L. Shen, X. Hou, Y. Yang, Grafting soyprotein isolates with

619 various methacrylates for thermoplastic applications. *Ind. Crop. Prod.* 60 (2014)
620 168-176.
621 [42] H.F. Tian, D.G. Liu, L. Zhang, Structure and Properties of Soy Protein Films
622 Plasticized with Hydroxyamine. *J. Appl. Polym. Sci.* 111 (2009) 1549-1556.
623 [43] H. Xu, M. Yang, X. Hou, W. Li, X. Su, Y. Yang, Industrial trial of high-quality
624 all green sizes composed of soy-derived protein and glycerol. *J. Cleaner Prod.* 135
625 (2016) 1-8.

Figure captions

Fig. 1. Molecular composition (^1H NMR) of soy protein with or without grafting.

Fig. 2. Conductometric and potentiometric titration of amine and carboxylic groups in ungrafted and grafted soy protein solution. Grafting ratio of soy protein was 14%.

Fig. 3. Effect of monomer concentration on grafting parameters. The graft polymerization was carried out at 70 °C for 4 h. Data points with different numbers, letters, or symbols indicate statistically significant difference.

Fig. 4. SDS-PAGE image of prestained standard protein (Lane 0), ungrafted soy protein (Lane 1), soy protein with grafting ratio of 4.2%, 8.5%, 14% and 20%, respectively (Lane 2-5).

Fig. 5. Changes in a) chemical oxygen demand (COD); b) Five-day biochemical oxygen demand (BOD_5) of acrylic acid grafted soy protein solution. Grafting ratio of soy protein was 14%. Weight percent of triethanolamine was 20% (based on the weight of soy protein). Data points with different numbers or letters indicate statistically significant difference.

Fig. 6. Changes in total nitrogen and ammonia nitrogen of acrylic acid grafted soy protein solutions. Grafting ratio of soy protein was 14%. Weight percent of triethanolamine was 20% (based on the weight of soy protein).

Fig. 7. Effect of grafting ratio on tensile strength and elongation of soy protein films. Grafting ratio of soy protein was from 1.2% to 20%. Data points with different letters indicate statistically significant difference.

Fig. 8. a) Digital images and b) Scanning electron micrograph of the soy protein films

with different grafting ratio. Grafting ratio of soy protein was 0%, 8.5% and 20%.

Fig. 9. Effect of % triethanolamine on tensile strength and elongation of acrylic acid grafted soy protein films. Grafting ratio of soy protein was 14%. Data points with different numbers, letters, or symbols indicate statistically significant difference.

Fig. 10. Adhesion of sizing agents to a) polyester fabrics; b) cotton fabrics; c) polyester/cotton (50/50) fabrics. AA-grafted SPI: acrylic-acid-grafted soy protein with grafting ratio of 14% and 20% TEA. SPI-TEA: soy protein isolates with 20 wt. % triethanolamine. Data points with the same letters indicate no statistically significant difference.

Fig. 11. Abrasion resistance of sized a) polyester yarns; b) cotton yarns; c) polyester/cotton (50/50) yarns. AA-grafted SPI: soy protein isolates grafted with acrylic acid with grafting ratio of 14% and 20% TEA. SPI-TEA: soy protein isolates with 20 wt. % triethanolamine. Data points with the same letters indicate no statistically significant difference.