Plastic Performance of Soybean Protein Components

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ABSTRACT: Soybean proteins recently have been considered as petroleum polymer alternatives in the manufacture of adhesives, plastics, and various binders. The objective of this work was to characterize the plastic performance of soybean protein components during molding processes. Two major soybean protein fractions, 7S-rich globulin (7S-RG) and 11S-rich globulin (11S-RG) were separated from defatted soybean flour, and their purity was examined by sodium dodecyl sulfate-polyacrylamide gel eletrophoresis and high-performance liquid chromatography. The thermal transition properties of the two fractions at 10% moisture content were 137.6°C for 7S and 163°C for 11S, as analyzed using differential scanning calorimetry (DSC). Plastics were prepared using a hot press at various molding temperatures that were selected based on the proteins' thermal transition temperatures obtained by DSC. The plastics were evaluated for mechanical properties, water absorption, and microstructure. The plastics prepared with temperatures at or close to the thermal transition temperature showed a smooth, uniform, and complex structure. Results showed that the plastics made from 11S-RG at its thermal transition temperature were stronger (35 MPa) and had lower water absorption than those made from 7S-RG at 145°C (26 MPa). The plastics made from the 7S- and 11S-RG mixture had the highest tensile strength (39 MPa) and medium water absorption compared to those made from 7S- and 11S-RG alone. These mechanical properties and water absorption behaviors were significantly affected by molding temperatures. The results obtained from this research indicated that interaction between 7S- and 11S-RG could occur during molding and that thermal transition temperature played an important role in thermal processing of soybean proteins.

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KEY WORDS: 7S and 11S globulins, biodegradable plastics, DSC, HPLC, mechanical properties, molding, SDS-PAGE, soy protein, thermal transition, water absorption.

The environmental impact of persistent plastic wastes from disposable items is growing more acute worldwide. Approximately 22 billion pounds of plastic waste were discarded in 1992, and this figure is expected to reach 34 billion pounds by 2002 (1). Alternative disposal methods are insufficient. Incineration can generate toxic air pollution, and satisfactory landfill sites are limited. Since petroleum resources are finite and are becoming limited, the cost of petroleum-based plastics is steadily increasing, and most countries must import these resources.

On the other hand, abundant proteins are available from renewable resources and agricultural processing by-products, such as soybean proteins from oil processing and gluten proteins from corn- or wheat-starch production. For example, soybeans contain about 40% protein, and the United States produces about 52% of the total world soybean crops. Utilizing these protein by-products for biodegradable resins will help alleviate the environmental problems and add value to agricultural by-products.

Research related to using proteins as petroleum polymer alternatives is underway worldwide. Protein polymers have been found to have great potential for many unique industrial, medical, and military applications. Proteins have been considered for environmentally friendly adhesives that could be used for plywood, particle boards, and in packaging and labeling (2–4). Incorporation of protein resins with natural fiber (biodegradable composites) has been found to have many unique industrial and military applications (5). Proteins have been considered for edible coatings and medical capsules (6). Proteins could also be manufactured into many articles and films through extrusion and injection molding (7–9).

A polymer changes to a number of phases during thermal processing, from glassy to rubbery and to liquid flow as temperature increases; this is called thermal transition. After liquid flow thermoset polymers become harder and harder owing to cross-link reactions, which is called the curing process. Different polymer structures have different thermal and flow-curing behaviors, which result in different rheological properties at each thermal state. These characteristics are often used as processing windows to select processing techniques and parameters correctly. Interactions between polymer structure and processing design significantly affect final product quality.

Soy proteins have commonly been used for food and animal feed for many years. However, soy protein is a new polymer for biodegradable resins. Soy protein polymers are macromolecules that contain a number of amino acids and side chains. Major protein components include 2, 7, 11, and 15S fractions, classified by their ultracentrifugal sedimentation rates. The 7S fraction makes up approximately one-third of the total soybean proteins, and its main component is 7S globulin. The 11S fraction is roughly 50% of the soybean proteins, and contains a single component called 11S globulin (10). Physicochemical properties of 7S and 11S globulins have been extensively studied in food applications (11–15).

Heat and pressure are two main external factors that influence protein curing behavior and, consequently, the mechani-

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cal properties of soybean protein plastics (16). Heat converts protein from its native state to a denatured state (i.e., unfolds and aggregates the protein molecules); this conversion is irreversible. Those globulins have different thermal transition properties (17,18) and association/dissociation behaviors upon heating (19–21). These findings suggest that plastics made from different soybean globulins might have different properties. The curing behavior of each globulin could be significantly influenced by processing temperatures. The objectives of this study were to determine the functions of 7S and 11S globulins in plastic performance in relation to their thermal transition behaviors and the molding temperature of molded plastics from 7S and 11S globulins, or their mixture.

MATERIALS AND METHODS

Segregation of 7S and 11S globulins. The defatted soybean flour (Cargill, Cedar Rapids, IA) used in this research had a 90% protein dispersibility index according to the specifications. The 7S-rich globulin (7S-RG) and 11S-rich globulin (11S-RG) fractions were separated according to the procedure described by Nagano *et al.* (22). The 11S-RG was the protein precipitated when the protein extract from the defatted soybean flour was adjusted to pH 6.4 and kept at 0–4°C for 12 h. The 7S-RG was the protein precipitated at pH 4.8 after the 11S-RG and other proteins were removed from the protein extracts. The 7S- and 11S-RG separated after centrifugation were redissolved at pH 7.6 and then freeze-dried. The freeze-dried 7S- and 11S-RG powders contained approximately 10% moisture as measured by an air-oven method at 105°C for 4 h (23).

Electrophoresis and high-performance liquid chromatography (HPLC) measurements. The purity of the segregated 7S- and 11S-RG were checked using both electrophoresis and HPLC methods. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method described by Laemli (24). A 14% separating gel and a 3.5% stacking gel in a slab (2 × 5.5 mm) were used. A 2.5 mg protein sample was dissolved in 1 mL sample buffer (pH 6.8) containing 0.063 M Tris-HCl, 2% (wt/vol) SDS, 5% (vol/vol) mercaptoethanol, 20% (vol/vol) glycerol, and 0.01% (wt/vol) bromophenol. After immersion in boiling water for 3 min, the dissolved sample (10 μL) was applied to a slot. The electrophoresis was carried out at a constant voltage of 75 V/gel for 1.5 h. The gel was stained with Coomassie Brilliant Blue R 250 and destained with the mixture of propanol and acetic acid (1:4).

The reversed-phase HPLC (RP-HPLC) method was used to obtain a spectrum for 7S- and 11S-RG. Parameters for the HPLC measurement included buffer (50 mL Tris-HCI at 5% MeSH), column (Jupiter 10-C18), solution (water at 0.1% trifluoroacetic acid and acetonitrile at 0.1% trifluoroacetic acid), elution (linear gradient of 30–70% acetonitrile in 60 min), and detection (214 nm).

Differential scanning calorimetry (DSC) measurements. Thermal transition properties of protein samples were measured with a Perkin Elmer DSC 7 instrument (Perkin-Elmer, Norwalk, CT) that was calibrated with indium and zinc before official measurements. The DSC results were used to determine molding temperatures. Each protein fraction or mixture (1:1) was analyzed at 10% moisture content and in the presence of excess water (sample/water = 1:4). The decomposition temperature (starting at 200°C) of the soy protein polymers was determined using the thermogravimetric analyzer tests (TGA-7; Perkin-Elmer). The DSC temperature scan range was from 30 to 180°C, at a heating rate of 10°C /min.

Evaluation of plastic performance. Powders of each protein fraction or their mixture (1:1) at 10% moisture content was molded into a tensile bar specimen (type IV) according to ASTM Standard Method (25). Samples (approximately 5 g) were placed into a dog-bone-shaped molder and compression-molded using the Carver Hot Press (Model 3890 Auto "M"; Carver Inc., Wabash, IN) at 20 MPa for 5 min. The temperature accuracy of this press was ±5°C. In order to investigate the relation between thermal transition and molding temperature, the molding temperatures were chosen according to the DSC thermogram of each sample before, at, and after the DSC transition peak. The molded plastics were equilibrated in a chamber with 60% relative humidity at room temperature for 48 h before measurements.

Mechanical properties, such as tensile strength, modulus and elongation of the molded plastics, were measured using an Instron testing machine (Model 4466, Canton, MA), according to the standard test method for tensile properties of plastics (26) with 5 mm/min of cross-head speed. Water absorption of the plastics was measured using a modified ASTM standard method (27). The molded plastics were preconditioned in an oven for 14 h at 50°C, cooled in a desiccator for a few minutes, and weighed. The conditioned plastics were submerged in distilled water at 25°C for 2 and 26 h. The specimens were removed from the water and dried using a paper towel before weighing. Dry matter from the plastics left in the water during soaking was also included into the water absorption calculation. The microstructure of the fractured surface of the each plastic was observed by scanning electron microscopy (AutoScan, ETEC Corporation, Highlands, TX) at an accelerated voltage of 20 kV.

RESULTS AND DISCUSSION

Characterization of 7S- and 11S-rich globulins. The SDS-PAGE patterns in Figure 1 indicated that the 7S-RG and 11S-RG had highly stained bands for their corresponding subunits, plus some minor bands from contaminations. These results agreed with the previously published results (28,29). Densitometric analysis of the patterns gave an estimation that the 7S- and 11S-RG had purities of about 70 and 90%, respectively. This agreed with results of an analysis by RP–HPLC (Fig. 2).

Thermal transition properties. The DSC thermograms of 7S- and 11S-RG at excess water content (sample/water = 1: 4, w/w) are shown in Figure 3. The 11S-RG exhibited a single endothermic denaturation transition peak at 93.3°C, even though it contained approximately 10% of the 7S globulin.

However, the 7S-RG had two endothermic denaturation transition peaks, a major peak at 77.1°C representing the temperature for 7S-RG, and a minor peak at 93.3°C representing the temperature for 11S globulin. The presence of the minor peak was due to 11S-RG contamination in the 7S-RG, as mentioned previously.

The DSC thermogram of the mixture (1:1) of 7S- and 11S-RG powders at 10% moisture content is shown in Figure 4. The thermal transition temperatures were at 163.4°C for 11S-RG and 137.6°C for 7S-RG. The peak temperature of 11S globulin was about 25.8°C higher than that of 7S globulin; this may be caused by the difference in chemical structures. Previous studies have reported that the thermal transition temperature of 11S globulin was always higher than that of 7S globulin at any water content and that peak temperature decreased as moisture content increases (17,18).

The decomposition temperatures of 7S and 11S and their mixture were all in the 200 to 500°C range, and their weight loss curve patterns were almost identical. Figure 5 shows the weight loss curve of a 7S sample with 10% moisture content. The first weight loss occurred from room temperature to about 100°C. This was mainly caused by the water evaporation. Sample weight remained constant from 100 to 200°C, after which the sample started to lose weight due to decomposition of the components in the 7S sample. Above 500°C, sample weight loss slowed and remained almost constant; the solids left were primarily ashes.

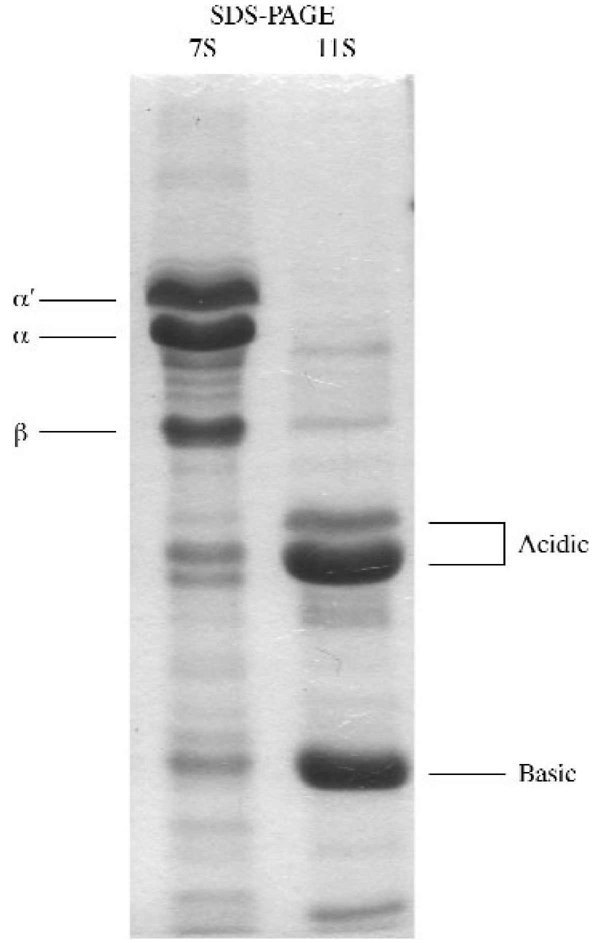

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of separated 7S- and 11S-rich soybean globulins.

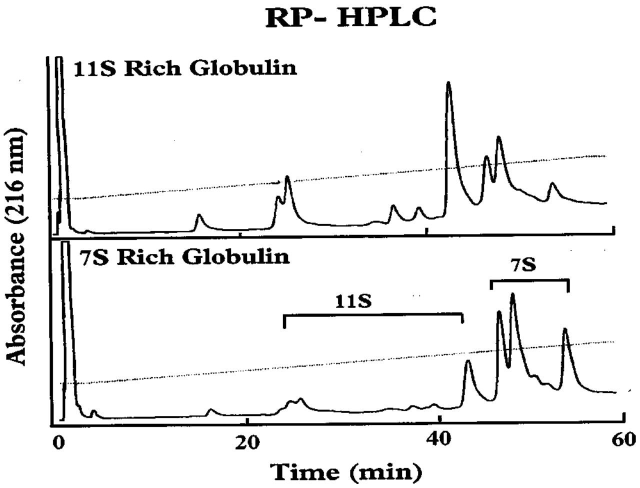

FIG. 2. High-performance liquid chromatography (HPLC) spectrum of 7S- and 11S-rich globulins. 50 mL Tris-HCl (5% MeSH) buffer, Jupiter 10-C18 column, water (0.1% TFA) and ACN (0.1% TFA) solution, linear gradient of 30–70% ACN in 60 min elusion, and 214 nm detection were used.

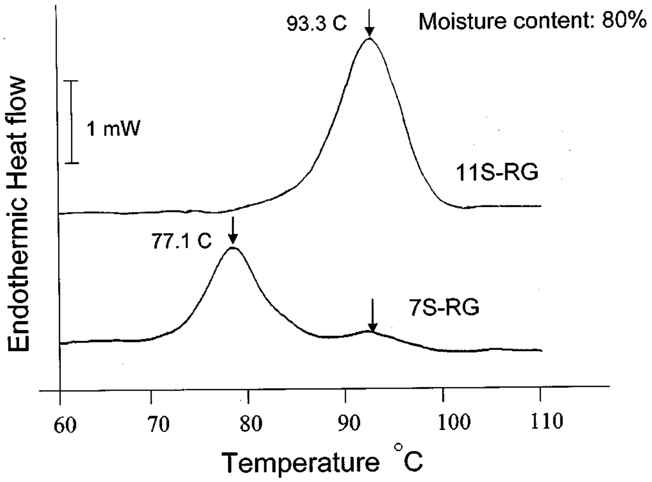

FIG. 3. Differential scanning calorimetry (DSC) thermogram for 7S- and 11S-rich soybean globulins at excess water content (sample/water = 1:4). Large sample pans were used at a temperature scan rate of 10°C/min from 30 to 180°C.

Mechanical properties of the molded plastics. The various molding temperatures were chosen based on the DSC thermograms (Fig. 4), below, at, and over the thermal transition peak temperatures. They were 120, 138, and 145°C for 7S-RG, and 145, 163, and 175°C for 11S-RG.

As shown in Figures 6A and 6B, the tensile strength (TS) and elongation (EL) of the plastics from different protein fractions and the mixture were significantly influenced by molding temperature. Trends of TS and EL changes were similar based on the molding temperatures used. The TS and

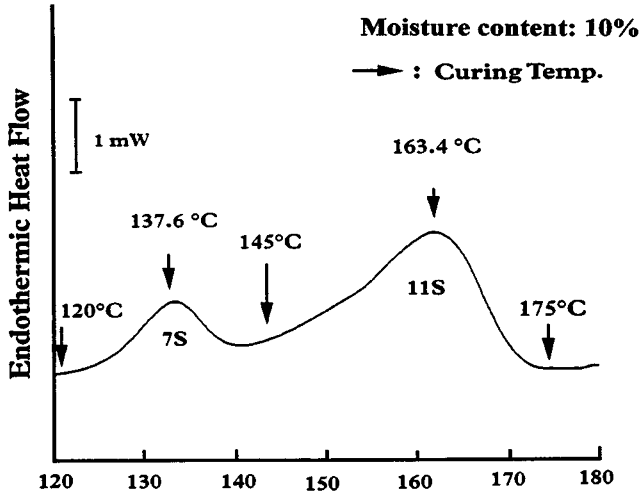

FIG. 4. DSC thermogram for the mixture (1:1) of 7S- and 11S-rich soybean globulins with 10% moisture content. Large DSC sample pans were used at a temperature scan rate of 10°C/min from 30 to 180°C. See Figure 3 for abbreviation.

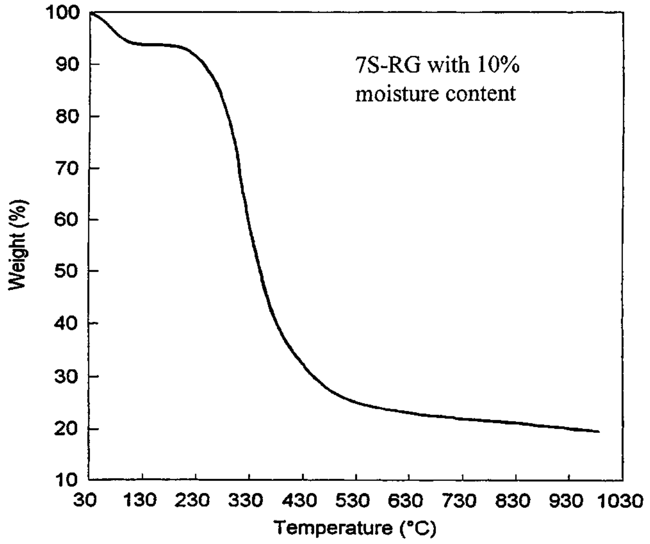

FIG. 5. Thermogravimetric analysis (TGA) weight loss curve of 7S-RG with 10% moisture content. About 5.8 mg sample was used and scan temperature range was from room temperature to 1000°C at 20°C/min increment.

EL of the plastic from 11S-RG increased as the molding temperature increased up to its DSC thermal transition temperature (about 163°C). However, the TS and EL of the molded plastic from 7S-RG reached maximum values at 145°C, which was about 7°C higher than its DSC thermal transition (about 138°C). The maximum TS and EL values for 11S-RG were 35 MPa and 3.4%, respectively, and were higher than those for 7S-RG (26 MPa and 2.5%). The decrease in TS and EL at higher molding temperatures could have resulted from thermal degradation of the protein by exposure to a temperature over its denaturation temperature. The color of the molded plastic was white at the molding temperature (before the thermal transition), translucent brown (at the thermal transition), and dark brown (after the thermal transition).

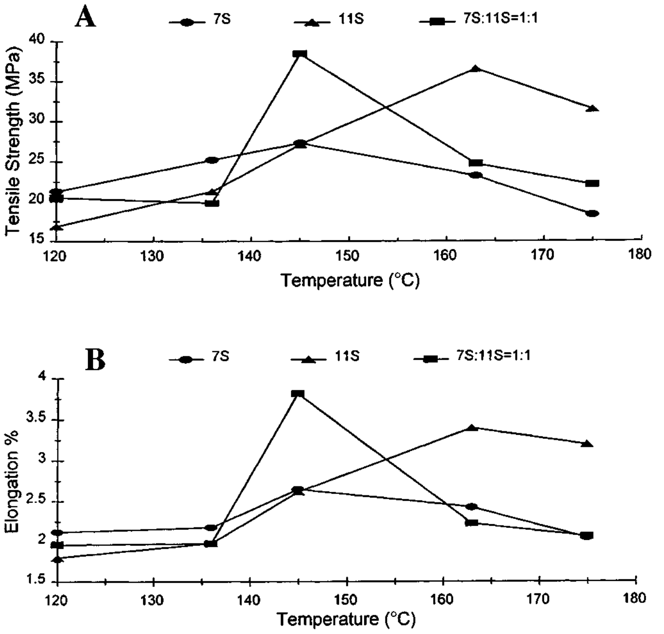

FIG. 6. Effects of molding temperature on tensile strength (A) and elongation (B) of the plastics (about 10% moisture content) made from 7S- and 11S-rich soybean globulins and their mixture (1:1). Each value was the average of three successful measurements with maximum standard deviation ±3.5 MPa for tensile strength and ±0.12% for elongation, respectively.

Interestingly, the plastic from the mixture of 7S- and 11S-RG had its maximum TS and EL values at a molding temperature of 145°C. These TS and EL values were about 39 MPa and 4%, respectively, and were higher than those from either 7S- or 11S-RG alone. This difference suggests that an interaction between 7S and 11S globulins could occur during the molding process.

Evidence for interactions between soybean protein globulins has been observed in gelation behavior by other researchers. Gels from either soybean protein isolates or a mixture (1:1) of 7S and 11S globulins were stronger than those from either 7S or 11S globulins alone (19). Utsumi *et al.* (30) conducted an electrophoretic analysis of a precipitate prepared by heating a (1:1) mixture of 7S and 11S globulins at 0.5% protein concentration at 80°C. The precipitate was found to have a molecular weight of over one million and was composed primarily of the interactions between basic (11S) and β (7S) subunits, and the complex of acidic (11S) and αα′ (7S) subunits located in the supernant, respectively. This result suggested that the interactions between these subunits were predominantly electrostatic in nature and, to some extent, involved the disulfide bonds. Similar results were reported by Yamagishi *et al.* (31).

The temperature at which the interactions occurred in the dilute protein solution (0.5%) was 80°C (30). This temperature was approximately 3°C above the DSC peak temperature for 7S globulins and about 13°C below the DSC peak for 11S globulins in the presence of excess water (Fig. 3). This suggests that interactions between 7S and 11S globulins occurred when the 7S was thermally denatured and 11S was still in its native state. This may explain why the plastic from the mixture showed maximum TS and EL values at 145°C, approximately 7°C over the DSC peak temperature for 7S and roughly 18°C below the DSC peak temperature for 11S globulin at 10% moisture content.

Water absorption of the molded plastics. Molding temperature had a significant effect on water absorption of all plastics prepared from 7S-, 11S-RG, and their mixture (Tables 1 and 2). Water absorption decreased as molding temperature increased to 163°C; water absorption increased as temperature continued to increase to 175°C except for the sample mixture of 7S and 11S. When exposed to higher temperatures, protein molecules would unfold, resulting in more entanglement (aggregation), and pack more tightly during the molding process possibly inhibiting water absorption. If the molding temperature was too high, for example 175°C, proteins became thermally degraded. The degradation could destroy the protein structure, resulting in smaller peptides that could more easily absorb water. Similar phenomena were observed by several researchers. Heat provided the protein with enough thermal energy to break hydrophobic interactions and dissociate the subunits (32). The dissociation and unfolding exposed the hydrophobic groups that were previously enclosed within the contact area between subunits or on the interior of the folded molecules (33,34). Soy protein dissociated and coagulated at high pressure; this would display large hydrophobic regions and have a high viscosity. The exposure of hydrophobic groups would decrease water absorption (35). Paetau *et al.* (8) cited the statement by McCrum *et al.* (1988) that higher molding temperatures increased the mobility of the polymer chains. Increased mobility would enhance protein molecules flowability and result in improved molecular alignment and interactions, thus decreasing water absorption. Takagi *et al.* (36) found that the number of disulfide (SS) bonds formed in a protein affected water absorption. The protein with higher SS bonds absorbed less water than that with lower SS bonds, and more SS bonds were formed in high heat treatment (37,38).

TABLE 1
Water Absorption (%) of Plastics Made from 7S- and 11S-Rich Globulins and Their Mixture Soaked in Distilled Water at Room Temperature for 2 h[a]

Molding temperature (°C ±5°C)	7S-Rich globulins	11S-Rich globulins	7S/11S (1:1) Mixture
120	335	127	209
138	257	—	133
145	172	57	73
155	102	57	64
163	68	49	54
175	—	58	65

[a]Average of three measurements with maximum standard deviation ±1.5%.

TABLE 2
Water Absorption (%) of Plastics Made from 7S- and 11S-Rich Globulins and Their Mixture Soaked in Distilled Water at Room Temperature for 26 h[a]

Molding temperature (°C ±5°C)	7S-Rich globulins	11S-Rich globulins	7S/11S (1:1) Mixture
120	Dissolved	Dissolved	Dissolved
138	962	—	910
145	889	481	518
155	522	295	401
163	255	268	308
175	—	301	303

[a]Average of three measurements with maximum standard deviation ±5.5%.

Plastics made from 7S-RG absorbed more water than those made from 11S-RG, and the plastics made from the mixture of 7S- and 11S-RG had medium water absorption compared to those made from just 7S- or 11S-RG. Peng *et al.* (39) found that 11S-RG had more SS bonds formed in heating than 7S-RG. When combined with the findings described previously (36–38), it was reasonable that 11S-RG was more hydrophobic than 7S-RG. The water absorption for the mixture sample after water soaking for 26 h (Table 2) at 175°C was 303%, slightly lower than 308%, and was not statistically significant (±5.5%). The number of SS bonds formed during heating was on the order of 11S > mixture > 7S, and the water absorption of these three samples was on the order of 11S < mixture < 7S for water soaking for 2 h and 11S ≤ mixture < 7S for water soaking for 2 h. The exact underlying mechanism of water absorption of a heat-treated protein is still not clear and needs further study.

Structure of the plastics. The microstructures of the plastics prepared from 7S- and 11S-RG or their mixture were significantly affected by molding temperature (Fig. 7). The plastics molded at 120°C before the DSC peak temperature, for example for 7S-RG, had a cracked and nonaggregated or nonagglomerated structure. The plastics molded at a temperature (145°C) close to its DSC peak temperature (137.6°C) showed a smooth and compact structure. The plastics molded at 163°C, about 30°C higher than the DSC peak temperature, had a rough and degraded structure. Similar results also were obtained for 11S-RG. The structures observed by scanning electron microscopy further explained why molding temperature significantly influenced the properties of the soybean-protein plastics.

At 10% moisture content, 7S-RG had a thermal transition of 137.6°C, which was lower than that of 11S-RG (163°C). Plastics prepared at or close to the thermal transition temperature showed a smooth, uniform, and complex structure. Plastics molded from 11S-RG at its thermal transition temperature had a tensile strength of 35 MPa, which was stronger than that of 7S-RG (26 MPa) molded at 145°C (about 7°C higher than its thermal transition temperature). The plastics made from the 7S- and 11S-RG mixture had a maximum tensile strength of 39 MPa and was stronger than those of 7S- and 11S-RG alone. The plastics made from 11S-RG had the lowest water absorption at its thermal transition temperature.

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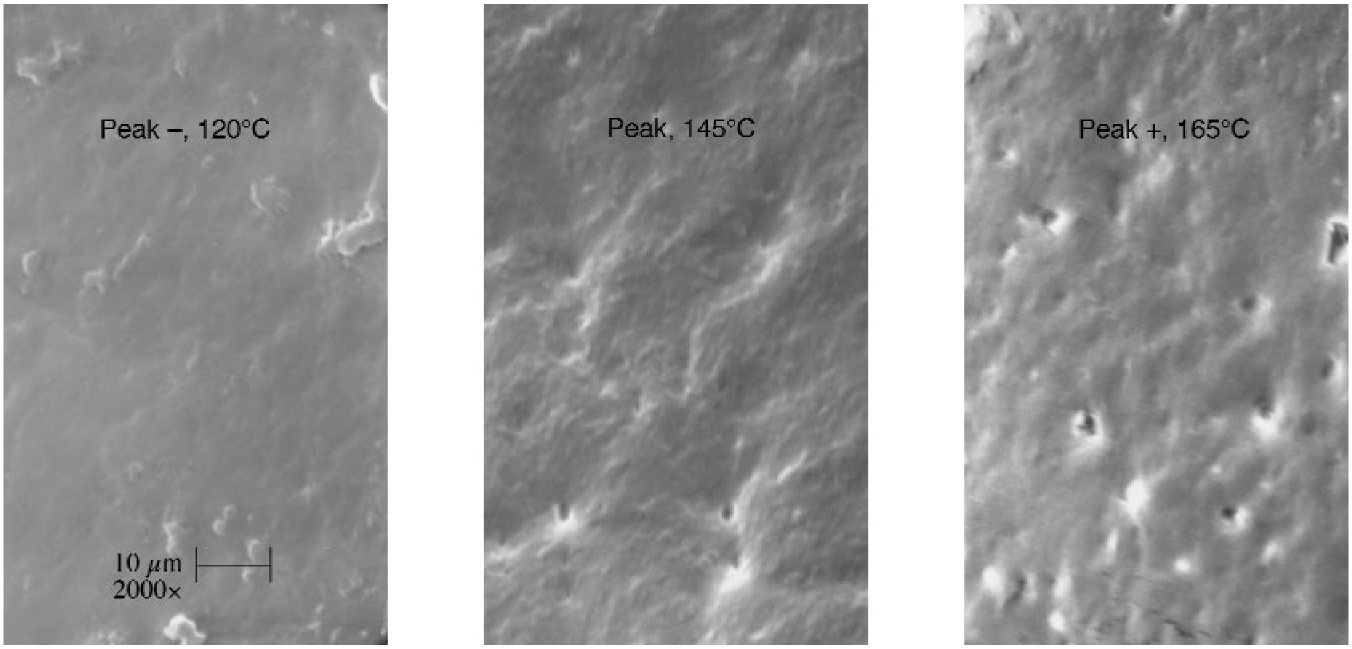

FIG. 7. Scanning electron micrographs of the fractured surface of 7S-rich globulin-based plastics prepared with 10% moisture content at three molding temperatures.

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