

Thermal and Mechanical Properties of Plastics Molded from Urea-Modified Soy Protein Isolates

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ABSTRACT: Soy protein has been considered as a potential alternative of some petroleum polymers in the manufacture of plastics. The purpose of this investigation was to characterize the thermal and mechanical properties of plastics made from urea-modified soy protein. Soy protein isolate was separated from the defatted soy flour, modified with various urea concentrations, and compression-molded into plastics. Differential scanning calorimetry showed that the temperatures of denaturation and the enthalpies of denaturation of the modified soy protein decreased as urea concentrations increased above 1 M. At the same urea concentration, molded plastics made from the modified soy proteins showed a similar temperature of denaturation as the modified soy protein, but a lower enthalpy of denaturation. Tensile strength and Young's modulus of the molded plastics from the modified soy proteins increased as urea concentration increased and reached their maximum values at 8 M urea modification. Both storage modulus and glass transition temperature of the plastics from the modified soy proteins increased as urea concentration increased. The plastics made from the 2 M urea-modified soy proteins showed improvements in elongation, tough fracture behavior, and water resistance. The urea may function as a denaturant, a plasticizer, and a filler. Paper no. J9791 in *JAOCs* 78, 867–872 (August 2001).

KEY WORDS: Denaturation, dynamic mechanical property, mechanical properties, protein modification, soy protein, thermal property.

Environmental pollution caused by petroleum-based plastics is growing worldwide. For example, the U.S. Environmental Protection Agency reported that about 22.4 million tons of plastics were discarded in the municipal solid waste stream in 1998 (1). Efforts to recover these plastics by recycling have not succeeded, and only about 5.4% of the discarded plastics have been recycled (1). Furthermore, petroleum is a limited resource, which will be used up in the future.

In the search for a replacement for petroleum-based plastics, soy protein was identified to be a potential raw material because it is widely available, renewable, and biodegradable (2). Recently, soy protein-based plastic has received great attention. Studies showed that unmodified soy proteins were highly hydrophilic, and plastics made from them were water-sensitive, resulting in poor mechanical properties (2,3). Fur-

thermore, plastics from unmodified soy protein were very brittle (4). Great efforts have been devoted to enhancing the flexibility of soy protein-based plastics (2,5). Various polyhydric alcohols were added to soy proteins and served as plasticizers to improve plastic elasticity. Glycerol, ethylene glycol, and propylene glycol were found to be better plasticizers than propanediol (2). Soy protein also was used as a major resin and cross-linked or blended with other materials. Mechanical strength and water resistance of soy protein could be improved by cross-linking with zinc sulfate (3) or formaldehyde (6), blending with polyphosphate (7) or anhydride group attached polyester (8).

The functional properties of soy protein are highly related to its structure. Protein modification is designed to improve functional properties by tailoring protein structures through physical, chemical, and enzymatic methods. It is well documented that protein modification including denaturation can improve functional properties of food proteins, such as solubility, foaming, emulsifying, gelation, and viscosity (9). Denaturation usually is defined as any modification of the secondary, tertiary, or quaternary structure of protein molecule without breaking covalent bonds. Methods of denaturation of protein include exposure to heat, acid, alkali, detergent, or organic solvent (9). Urea is a commonly used denaturing agent for protein. It can destabilize globular protein by forming strong hydrogen bonds with water molecules that surround the protein and may protect it from denaturation while disrupting protein hydrogen bonds, resulting in partially unfolded protein structures and flexible peptide chains (10). The denatured protein may form more entanglements and cross-linked structures during the molding process, resulting in plastics with a high tensile strength, greater elongation, and reduced water absorption.

No information has been available about the performance of plastics made from urea-modified soy protein. The objective of this study was to explore the effects of urea modification on the denaturation behavior of soy protein and mechanical properties of molded plastics made from the modified protein.

MATERIALS AND METHODS

Materials. Defatted soy flour with protein dispersibility index (PDI) of 90 was obtained from Cargill (Cedar Rapids, IA) and used for the preparation of soy protein isolate (SPI). Urea was purchased from Sigma Chemical Co. (St. Louis, MO).

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SPI preparation. The SPI was extracted from the defatted soy flour by isoelectric point precipitation at pH 4.2 (11). The precipitate was freeze-dried (Model 6211-0495 freeze-dryer; The Virtis Co., Inc., Gardiner, NY) and then milled (Cyclone Sample Mill, UDY Corp., Fort Collins, CO) into powder. The freeze-dried SPI had an average protein content of 93% (dry basis).

Protein modification and specimen preparation. The SPI powder was added to solutions of urea (1, 2, 4, 8 M) at a weight ratio of 1:6 and stirred and allowed to react for 6 h. Each modified solution was freeze-dried and milled into powder, as described above. The ratio of SPI to urea in the modified SPI powder with 1, 2, 4, 8 M urea modification was 1:0.33, 1:0.61, 1:1.93, and 1:2.25, respectively. A tensile bar specimen (Type IV) was molded from the modified SPI powder according to ASTM Standard Method ASTM D638-91 (12). Samples (5 g) were placed into a dog-bone-shaped molder and compression-molded using a Hot Press (Model 3890 Auto "M"; Carver Inc., Wabash, IN) at molding pressure of 5.6 MPa at 120°C for 3 min.

Differential scanning calorimetry (DSC) measurements. The thermal denaturation of modified SPI and molded plastics was studied using a differential scanning calorimeter (DSC 7; PerkinElmer, Norwalk, CT), which was calibrated with indium and zinc. About 5 mg freeze-dried SPI or ground plastic powder was weighed into the big DSC pans, about 50 mg distilled water (10 times the amount of SPI powder) was added, and the pans were sealed. The samples were kept at 4°C overnight before measurement at a temperature range from 30 to 140°C and a heating rate of 10°C/min. The denaturation enthalpy was calculated as the sum of the 7S and 11S denaturation enthalpies. The reported values are averages of two replicates.

Tensile testing and fracture morphology. Mechanical properties were measured using an Instron (Model 4465; Canton, MA) according to the Standard Test Method for Tensile Properties of Plastics (ASTM D638-91) (12). The SPI plastics were preconditioned at 23°C and 50% relative humidity for 48 h before testing. The crosshead speed was 5 mm/min. Stress at maximum load, strain (%) at break, and Young's modulus were calculated. The reported results are averages of five samples. The fracture surface of the broken plastic after tensile testing was examined using a scanning electron microscope (SEM) (Hitachi S-3500N; Hitachi Science System, Ltd., Tokyo, Japan). The sample was stuck to an aluminum stub, and the fractured surface was coated with a mixture of 60% gold and 40% palladium particles by a sputter coater (Desk II Sputter/Etch Unit, Moorestown, NJ). Then the microstructure of the plastic was observed using an SEM at 10 kV.

Dynamic mechanical testing. Dynamic mechanical properties of the molded plastics were studied using a dynamic mechanical analyzer (DMA 7e; PerkinElmer) in a three-point bending configuration. The sample size of the SPI plastics was about 13 × 6 × 2 mm. Temperature increased from -25 to 200°C at a rate of 5°C/min with 110 mN static force, 100 mN dynamic force, and 1 Hz frequency. The reported values are means of two replicates.

Water absorption measurement. Water absorption was measured following ASTM standard methods (ASTM D570-81) (13). The SPI plastic samples were preconditioned at 50°C for 24 h and then cooled in a desiccator before being weighed. The preconditioned SPI plastics were submerged in distilled water at 25°C for 2 and 24 h. They were removed and dried with a paper towel before weighing. Water absorption was calculated as a percentage of initial weight. The soluble material loss was checked by weighing the specimens after drying them in an oven at 50°C for another 24 h. The total water absorption for 24 h was calculated including the soluble material loss $\{[(W_2 + W_3) - W_1]/W_1\} \times 100\%$, where W_1 = initial sample weight, W_2 = sample weight after 24 h, and W_3 = soluble material weight).

RESULTS AND DISCUSSION

Protein denaturation. Protein denaturation is accompanied by enthalpy changes that can be monitored by DSC. The peak analysis allows determination of the denaturation temperature (T_d) and enthalpy of the denaturation (ΔH_d) from the maximum peak temperature and the area of the peak, respectively. The thermal denaturation thermogram of the unmodified SPI exhibited two peaks corresponding to the endothermic transitions (denaturation) of 7S globulin (the first peak) and 11S globulin (the second peak) in the soy protein (Fig. 1A). The T_d values for 7S and 11S of the unmodified SPI were 73.3 and 88.2°C, respectively (Table 1), which were in agreement with the T_d values reported by other authors (14).

The T_d and peak area of the modified SPI decreased as urea concentrations increased to above 1 M (Fig. 1A and Table 1), indicating that the higher the urea concentration, the greater the degree of denaturation. Hydrogen bonds are among the major bonds maintaining the stability of protein conformation. At high urea concentrations, more urea was available for breaking the hydrogen bonds, resulting in a higher degree of denaturation, a lower conformation stability, and, consequently, a lower T_d and a lower ΔH_d . No peak was observed for samples with 8 M-urea modification, indicating that the soy protein was denatured completely. This result supported previous studies showing that the complete denaturation of protein could occur at a high urea concentration, such as 8 M (15). The DSC thermogram of the molded plastics made from urea-modified SPI showed a similar trend as urea-modified SPI (Fig. 1B). However, the peaks disappeared for the plastics from 4 M urea-modified SPI, indicating that the soy protein was denatured further during the molding process.

The enthalpies of denaturation of the modified SPI and the molded plastics decreased as urea concentration increased (Table 1). For the 1 M urea-modified SPI, it decreased about 10% compared to the unmodified SPI, and then decreased very rapidly as urea concentrations continued to increase. At the same urea concentration, the modified SPI and the molded plastics had similar denaturation temperatures for 7S and 11S. However, the enthalpy of denaturation for the molded plastics was lower than that for the modified SPI because of

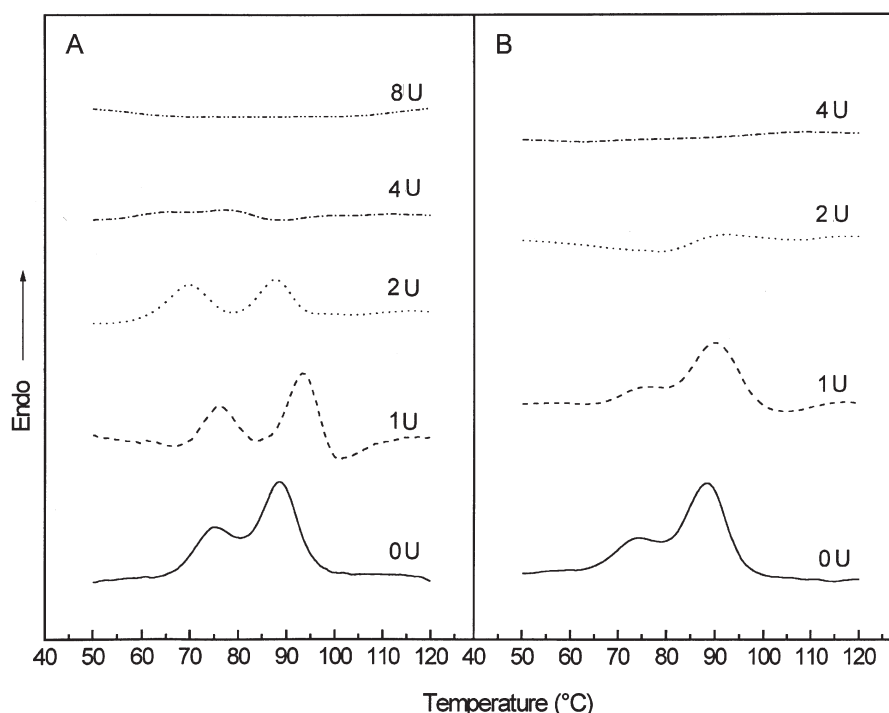


FIG. 1. Differential scanning calorimetry (DSC) thermogram of urea-modified soy protein isolate (SPI) (A) and soy protein plastics (B) molded at 120°C, 5.6 MPa for 3 min for 0U: unmodified SPI; 1U: 1 M urea-modified SPI; 2U: 2 M urea-modified SPI; 4U: 4 M urea-modified SPI; 8U: 8 M urea-modified SPI.

further protein denaturation caused by the heat treatment during the molding process.

Partially denatured protein should have a lower T_d . However, the T_d for the 1 M urea-modified SPI was higher than that for the unmodified SPI, suggesting that the modified SPI had a more orderly and compact protein structure. At a relatively low degree of denaturation, urea might preferentially unfold less stable structures in the proteins, while more stable structures remain undenatured, which might result in a higher T_d . At urea concentrations of 2 M or higher, more extensive denaturation occurred, and protein conformation stability decreased greatly, resulting in a lower T_d .

TABLE 1
Denaturation Temperatures (°C) and Enthalpies (J/g) of Urea-Modified Soy Protein Isolate (SPI) and Molded Plastics from Them^{a,b}

Urea concentration (M)	SPI			Molded SPI plastics		
	T_{d1}	T_{d2}	ΔH_d	T_{d1}	T_{d2}	ΔH_d
0	73.3 ^a	88.2 ^b	11.5 ^a	73.6 ^a	88.8 ^{a,b}	7.1 ^a
1	75.2 ^a	92.9 ^a	10.1 ^b	74.8 ^b	90.3 ^a	5.1 ^b
2	69.7 ^b	86.9 ^b	5.7 ^c	—	87.5 ^b	1.0 ^c
4	65.2 ^b	78.5 ^d	0.7 ^d	—	—	—
8	—	—	—	—	—	—

^aMeans of two replicates, based on least square differences (LSD) procedure at $\alpha = 0.05$ level; means in a column with the same roman superscript letter are not significantly different.

^bDashes in this table indicate that thermal transitions of the sample were not detected from the differential scanning calorimetry (DSC) measurements. ΔH_d , enthalpy of the denaturation; T_d , denaturation temperature.

Tensile testing and fracture morphology. Tensile strength and Young's modulus of the plastics made from modified SPI increased as urea concentration increased and reached their maximum values at 8 M urea modification. The plastic made from 2 M urea-modified SPI had similar tensile strength as the plastic from the unmodified SPI, but about 70 times greater strain at break (Table 2). The mechanical properties of the plastics from SPI are highly related to their structures and components. Soy proteins are composed of 20 amino acids with various side groups. Some side groups, such as amino, carboxy and sulfhydryl groups, are relatively active and may react with each other and form cross-links upon heating. Plastics molded from the modified SPI with a high-degree denaturation would form more entanglements and crosslinks, which would increase mechanical strength. At

TABLE 2
Mechanical Properties of Molded Plastics Made from Urea-Modified SPI^a

Urea concentration (M)	Maximum stress (MPa)	Strain at break (%)	Young's modulus (MPa)
0	13.0 ^c	1.2 ^c	1549 ^b
1	4.0 ^d	13.4 ^b	131 ^e
2	12.5 ^c	71.6 ^a	329 ^d
4	18.3 ^b	1.9 ^c	1404 ^c
8	24.1 ^a	2.1 ^c	2163 ^a

^aMeans of five replicates, based on LSD procedure at $\alpha = 0.05$ level; means in a column with the same roman superscript letter are not significantly different. See Table 1 for abbreviations.

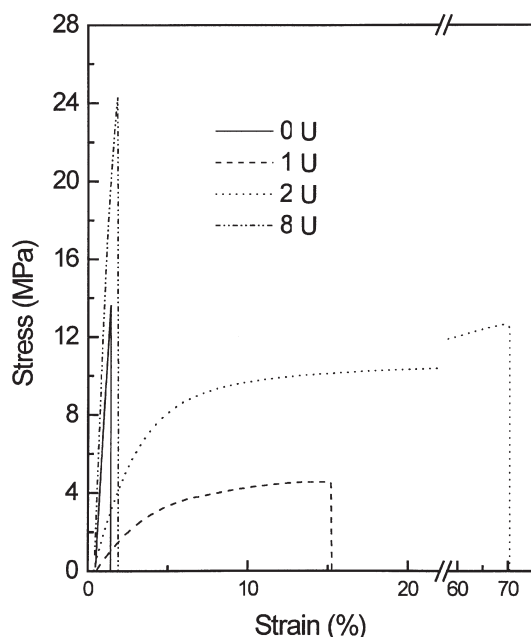


FIG. 2. Mechanical properties of soy protein plastics molded at 120°C, 5.6 MPa for 3 min. See Figure 1 for abbreviations.

relatively low concentrations (1 and 2 M), urea in the plastics may serve mainly as a plasticizer, which increases the plastic strain at break. At high concentrations (4 M or more), urea may serve mainly as a filler, which increases the plastic stiffness.

Nearly linear elastic deformation and brittle fracture behaviors were observed for the unmodified SPI plastics (Fig. 2–0U). The plastics from 1 and 2 M urea-modified SPI showed great deviation from linear deformation (Fig. 2). The fracture surface for the unmodified plastic was relatively smooth with advancing cracks (Fig. 3–0U), indicating a brittle fracture behavior, which was in agreement with the deformation behavior observed in the stress-strain plot (Fig. 2). All plastics from 1, 2, 4 (graph not shown), and 8 M urea-modified SPI displayed rough and fluctuant fracture surfaces (Fig. 3). Therefore, both deformation behavior and fracture surface indicated that the plastics from urea-modified SPI were tougher than that from the unmodified SPI.

Dynamic mechanical properties. The dynamic mechanical properties of plastics were studied by observing the temperature dependence of the storage modulus and the loss modulus (Fig. 4). The definition for glass transition temperature (T_g) as the point where the loss modulus reaches its maximal value and a decrease in the storage modulus occurs was used (16). The storage moduli for all plastics showed a typical decrease in the glass transition zone (Fig. 4A). At 25°C, the storage modulus increased as urea concentration increased from 1 to 8 M, which was in agreement with the trend of Young's modulus observed in the tensile testing. The dependence of loss modulus on temperature showed that significant dissipation energy occurred for the molded plastics (Fig. 4B). The dissipation energy reached its maximum value at the peak. Two peaks were observed for the plastics from the unmodified SPI,

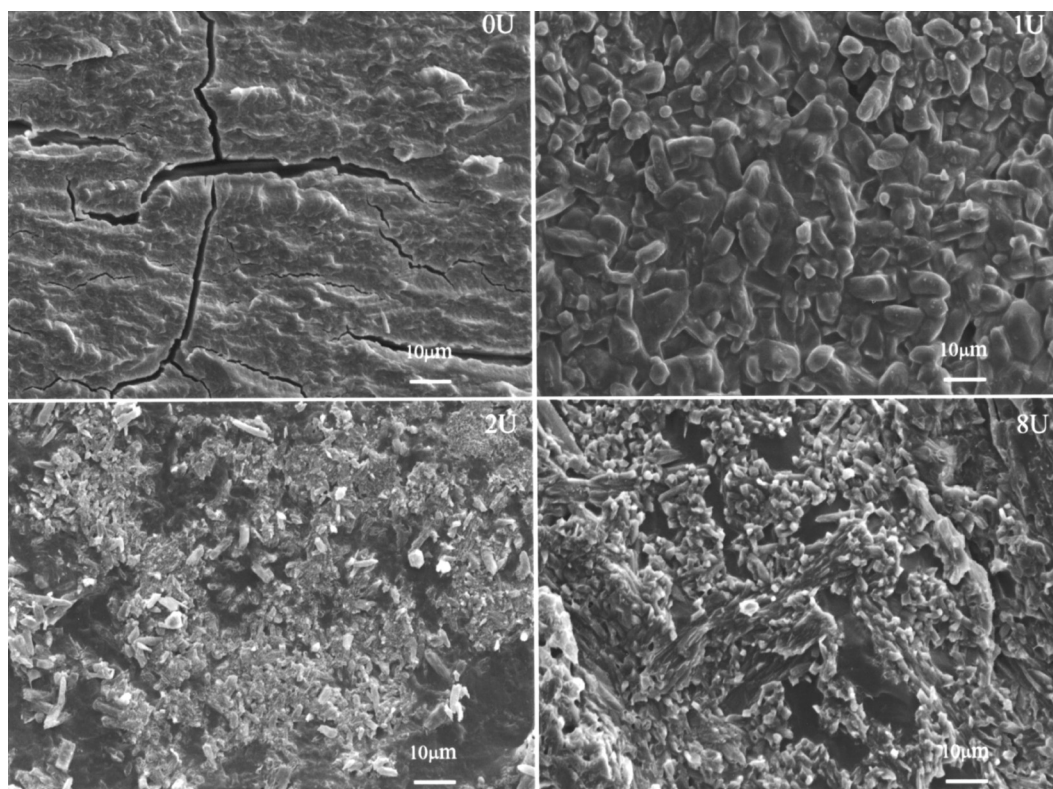


FIG. 3. Surface morphology of soy protein plastics molded at 120°C, 5.6 MPa for 3 min. See Figure 1 for abbreviations.

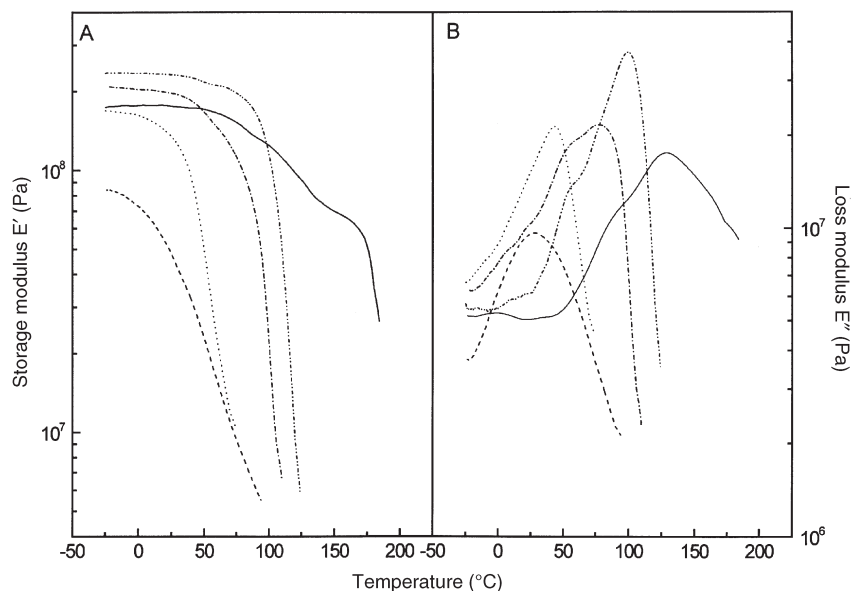


FIG. 4. Dependence of storage modulus (A) and loss modulus (B) on temperatures for soy protein plastics molded at 120°C, 5.6 MPa for 3 min from 0U (—): unmodified SPI; 1U (-----): 1 M urea-modified SPI; 2U (·····): 2 M urea-modified SPI; 4U (-.-.): 4 M urea-modified SPI; 8U (-.-.-.): 8 M urea-modified SPI. See Figure 1 for abbreviations.

one was at 84.9°C representing the T_g for the low molecular weight component (7S globulin), and the other was at 123.5°C, representing the high molecular weight component (11S globulin) in SPI. The T_g for 7S and 11S globulin of the plastics made from 1 M urea-modified SPI were at 5.2 and 25.3°C, respectively. The T_g value for unmodified SPI was about 10°C higher than the value determined by DSC for water soy protein dough reported by Morales and Kokini (17).

All plastics made from urea-modified SPI had a lower T_g than that from the unmodified SPI (Table 3). However, the T_g of the plastics increased as urea concentration increased. The T_g value for a material is a function of molecular weight and the amount of plasticizer (18). Urea in the plastic from the modified SPI may serve as a plasticizer by introducing free volume and increasing chain flexibility, resulting in a lower T_g . As observed in protein denaturation, a more extensive protein unfolding occurred with higher urea concentration. Consequently, a high molecular weight aggregate/entanglement could be formed from the extensively unfolded protein during the molding process. The increased T_g value of plastics of the modified SPI could be caused by increased plastic molecular weight.

TABLE 3
Glass Transition Temperatures of Molded Plastics Made from Urea-Modified SPI^a

Urea concentration (M)	T_{g1} (°C)	T_{g2} (°C)
0	84.9 ^a	123.5 ^a
1	5.2 ^e	25.3 ^e
2	23.0 ^d	43.3 ^d
4	49.2 ^c	78.2 ^c
8	53.5 ^b	101.4 ^b

^aMeans of two replicates, based on LSD procedure at $\alpha = 0.05$ level; means in a column with the same superscript roman letter are not significantly different. T_g , glass transition temperatures. See Table 1 for other abbreviations.

Water absorption. After 24 h of water soaking, the molded plastics from the 2 M urea-modified SPI gave the lowest water absorption of 404%, compared to 1192% for the plastic from the unmodified SPI. The plastic from 4 M urea modification had greater water absorption than the other plastics from modified SPI after 24 h soaking. A similar trend was observed for the 2-h water absorption test. Loss of soluble materials for the molded plastics increased after 24 h soaking as urea concentration increased (Table 4). In the modified plastics, urea could be the main contributor to the loss of soluble material. Molecular aggregate/entanglements and the amount of urea in the molded plastics would be the two major factors influencing water absorption. Protein with more entanglements would absorb less water. Higher amounts of urea in the plastics would increase water absorption because urea is hydrophilic. The relatively high level of protein entanglements in the plastic of the 2 M urea-modified SPI resulted in the lowest water absorption, whereas the relatively higher level of urea in the plastic from the 4 M urea-modified SPI resulted in the highest water absorption.

TABLE 4
Water Absorption and Soluble Materials Loss of Molded Plastics Made from Urea-Modified SPI^a

Measurements	Urea concentration (M)				
	0	1	2	4	8
2 h Water absorption (%)	179 ^c	133 ^d	156 ^{c,d}	308 ^a	263 ^b
24 h Water absorption (%)	1192 ^a	593 ^b	404 ^d	625 ^b	475 ^c
24 h Soluble material loss (%)	42.3 ^c	26.2 ^d	40.8 ^c	58.2 ^b	69.7 ^a
24 h Total water absorption ^b (%)	1234 ^a	619 ^{b,c}	445 ^d	683 ^b	544 ^c

^aMeans of three replicates, based on LSD procedure at $\alpha = 0.05$ level, means with the same nonitalic letter in row are not significantly different.

^bTotal water absorption % = $\{[(\text{sample weight after 24 h} + \text{soluble material weight}) - \text{initial sample weight}] / \text{initial sample weight}\} \times 100\%$. See Table 1 for abbreviations.

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