Effects of the Molecular Weight on the Properties of Thermoplastics Prepared from Soy Protein Isolate

QIANGXIAN WU, LINA ZHANG

Department of Chemistry, Wuhan University, Wuhan 430072, China

ABSTRACT: Commercial soy protein isolate (SPI) was fractionated into four fractions by an acidifying method from pH 5.7 to 4.5 with $2M$ HCl. A mixture of SPI with glycerin (50 g/100 g of dry SPI) was compression-molded to obtain thermoplastic sheets. The weight-average molecular weight (M_w) of the fractions, the structure, and the mechanical properties of the thermoplastic SPI sheets were investigated with light scattering, IR spectroscopy, wide X-ray diffraction patterns, differential scanning calorimetry, ultraviolet spectroscopy, scanning electron microscopy, and tensile testing. After heating compression, the SPI sheets were transparent and exhibited a smooth and homogeneous structure. Moreover, the crystallinity degree of the thermoplastic SPI was obviously higher than that of the premix before compression because of the formation of intermolecular hydrogen bonding. The M_w's of the fractions were 1.17×10^5 to 3.21×10^5, and they increased with increasing pH value in fractionation. The mechanical properties and water resistance (R) of the SPI sheets increased with increasing M_w of the SPI fractions. The tensile strength and breaking elongation of the SPI sheets with an M_w value of 3.21×10^5 were 5.7 MPa and 135%, respectively, and the R value was 0.54 after immersion in water for 15 days. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 82: 3373–3380, 2001

Key words: biodegradable plastic sheet; mechanical properties; water resistance; soy protein isolate; fractionation; compression mold; light scattering

INTRODUCTION

Nondegradable plastics have become a serious threat to the environment and have depleted our limited supplies of petroleum.[1] Therefore, the research and development of biodegradable materials from renewable resources, including cellulose,[2,3] starch,[4,5] and protein,[6,7] have attracted much attention. Protein, as one of the main renewable sources, however, only recently has been studied as a polymer from a materials science perspective.[8,9] Among plant protein sources, soy protein isolate (SPI) is abundant and relatively low-cost. With respect to the manufacture of fully biodegradable SPI materials, a major disadvantage of cast films is the high cost. It is well known that thermomechanical processing techniques are simple, low-cost, and effective ways of making thermoplastics. Processing conditions for thermoplastic SPIs have been reported, including temperature, pressure, and heating time;[9,10] plasticizers such as water[11] and glycerin;[12,13] and blends with starch.[14] Smith and Circle[15] and Ayres et al.[16] indicated that the solubility of SPI in water depended on the pH value, and an in-

crease in solubility occurred from pH 4.5 to 7.0. It can be inferred that at a given pH value, some water-insoluble SPI exists. Therefore, it is possible to prepare a water-resistant material of SPI with the method of acidifying fractionation.

The conformation and molecular weight of protein have been studied with light scattering,[17,18] and proteins such as insulin, myoglobin, and ovalbumin have been proven to be global macromolecules with small radii of 12–23 Å and molecular weights of 5×10^3 to 4.5×10^5. Researchers have scarcely focused on the effects of the second structure of SPI, such as the molecular weight, conformation, and solubility, which may have a remarkable influence on the properties of thermoplastic SPI. An understanding of the relationship between the molecular weight and properties is essential for the successful exploitation of thermoplastic SPI, but such results have been hardly reported.[19] In this work, commercial SPI was fractionated into different fractions, and each fraction, plasticized by glycerin, was compression-molded with a heating press to obtain thermoplastic SPI. The effect of the weight-average molecular weight (M_w) and heating compression on the mechanical properties, water resistance (R), and light transmittance were investigated and discussed.

Scheme 1 Fractionation procedure from SPI by the acidifying method. S and P represent supernatant and precipitate, respectively.
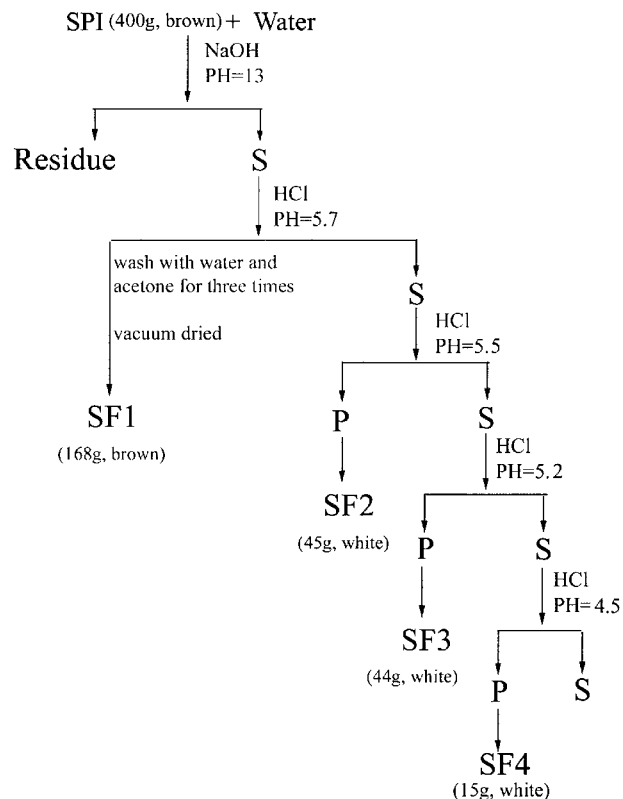
EXPERIMENTAL

Materials

SPI with a protein of about 91% (dry basis) was purchased from Hubei Yunmeng Protein Technology Co. (Yunmeng, Hubei, China) and vacuum-dried at 60°C for 4 h before use. Then, the moisture content of SPI was below 2%. Glycerin (Shanghai Chemical Co., Shanghai, China) was analytical-grade and was used without further treatment.

Fractionation of SPI

Fractions of SPI were prepared according to the acidifying method.[20] SPI (400 g) was dispersed in 6000 mL of distilled water, which was followed by the addition of 2M NaOH until pH 13, and was then stirred for 30 min. After centrifugation at 8000 rpm for 10 min, the supernatant was obtained and adjusted to pH 5.7 with 2M HCl to precipitate the first fraction of protein. After centrifugation at 8000 rpm for 10 min, the obtained

supernatant was stored in a refrigerator at 5°C for the next fractionation. With the same procedure, the other three fractions were isolated at pH 5.5, 5.2, and 4.5. Thus, four fractions were prepared, and they were coded SF1, SF2, SF3, and SF4, respectively. This fractionation procedure is shown in Scheme 1.

Compression Molding

A heat-press device was made in our laboratory, and a sketch map of it is shown in our previous work.[21] The ratio of protein to glycerol was constant at 1:0.5 by weight. SF1, SF2, SF3, SF4, and commercial SPI (CS) were mixed with glycerol. A mixture of SF1 with glycerol was coded GSF1. The mixture was stirred in a 50-mL beaker with a glass bar for 10 min and then pestled in a mortar for 30 min to obtain a homogeneous premix. Finally, the premix was sealed and stored in a refrigerator at 5°C for 2 days.

Each sheet was prepared according to the following procedure. A premix (3 g) was placed into a mold and covered with polished stainless steel

plates on both sides. The mold was placed between steel blocks, whose temperature was kept at 155°C, and then pressure was quickly increased from 0.5 to 15 MPa for 1 min. Mo et al.[10] reported that 155°C and 15 MPa were optimal compression-molding conditions, and our experiment also proved this to be correct. Therefore, the temperature and pressure conditions were applied in this work. After the compression molding, the mold was cooled below 70°C with a fan at a rate of 10°C/min. A slightly yellow and transparent sheet was released from the mold and stored in a desiccator with silica. A series of thermoplastic SPI sheets were prepared and coded TSF1, TSF2, TSF3, and TSF4 from fractions SF1–SF4 and TS (unfractionated SPI), respectively.

Viscosity Measurements

The viscosity of the SPI solutions was measured with an Ubbelohde capillary viscometer at 28 $\pm$ 0.1°C. A 0.1M NaOH aqueous solution containing 0.1M NaCl (0.1M NaOH/0.1M NaCl) was used as a solvent. The intrinsic viscosity ([η]) of the samples was calculated with the Schulz–Blaschke equation[22] as follows:

$$\eta_{sp}/c = [\eta] + K[\eta]\eta_{sp} \qquad (1)$$

where c is the polymer concentration (g/mL), sp the specific viscosity, and K a constant.

Laser Light Scattering Measurement

The light scattering intensities of the samples in 0.1M NaOH/0.1M NaCl aqueous solutions were measured on a multi-angle laser photometer equipped with a He–Ne laser (λ = 633 nm; DAWN-DSP, Wyatt Technology Co., Santa Barbara, CA) at 28°C. A solution of the desired polymer concentration (0.1–1 g/mL) was prepared and filtered first with a 0.45-μm cellulose nitrate membrane filter (Wattman, Inc., NJ) and then with a 0.2-μm filter into a scintillation vial. The scintillation vial was sealed and used for experimentation immediately. The value of the refractive-index increment (dn/dc) of the SPI aqueous solution at 589 nm was 0.181 mL/g.[23] Astra software was used for data acquisition and analysis.

Characterization

Wide X-ray diffraction patterns (WXRDPs) were recorded with an X-ray diffractometer (D/MAX-1200, Rigaku Denki, Tokyo, Japan) and Cu Kα radiation (λ = 1.5405 $\times$ 10^{-10} m) at 40 kV and 30 mA for 2θ = 6–40°. The degree of crystallinity (χ_c) was calculated according to a common method.[24] IR spectra of the sheets on KBr disks were recorded with an FTIR 3000 spectrometer (Shimadzu Co., Kyoto, Japan). The light transmittance of the sheets with a thickness of 0.08 mm was measured in the wavelength range 300–800 nm with a UV-160A spectroscope (Shimadzu). The sections of the sheets were studied with scanning electron microscopy (SEM; S-570 SEM, Hitachi, Tokyo, Japan). The sheets were frozen in liquid nitrogen and snapped immediately, and then the fracture surface was coated with gold for SEM experimentation. A differential scanning calorimetry (DSC) analysis of the samples was performed with a thermal analyzer (DSC-2C, PerkinElmer, Norwalk, CT) under a nitrogen atmosphere from -150 to 100°C at a heating rate of 10°C/min.

Measurements of the Mechanical Properties

The mechanical properties of the sheets were determined with a universal testing machine (CMT6503, Shenzhen SANS Test Machine Co., Ltd., Shenzhen, China) according to ISO6239-1986 (E) with a tensile speed of 100 mm/min to obtain the tensile strength (σ_b) and breaking elongation (ϵ_b). Every sample was measured at least three times to obtain an average value.

The samples were immersed in water at 5°C for 6 h and 15 days and then measured to study their R values.[25] The R values of the sheets were evaluated with $\sigma_{b(\mathrm{dry})}$ in the dry state and $\sigma_{b(\mathrm{wet})}$ in the wet state:

$$R = \sigma_{b(\mathrm{wet})}/\sigma_{b(\mathrm{dry})} \qquad (2)$$

RESULTS AND DISCUSSION
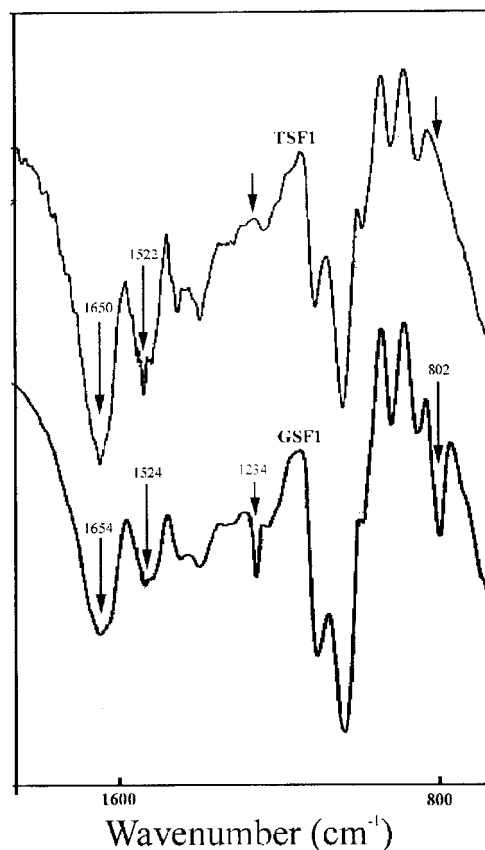
Structure

IR spectra of the samples are shown in Figure 1. Peaks at 3300 and 3400 cm^{-1} were attributed to the absorption of bonded NH groups and free NH groups, respectively.[26] The peak at 3400 cm^{-1} in the fraction significantly decreased with progress in the fractionation of SPI, suggesting that the hydrogen bonding between —NH and —COOH groups in the fractionated protein was destroyed. The absorption from 1700 to 500 cm^{-1} of the fractions did not show an obvious difference, im-
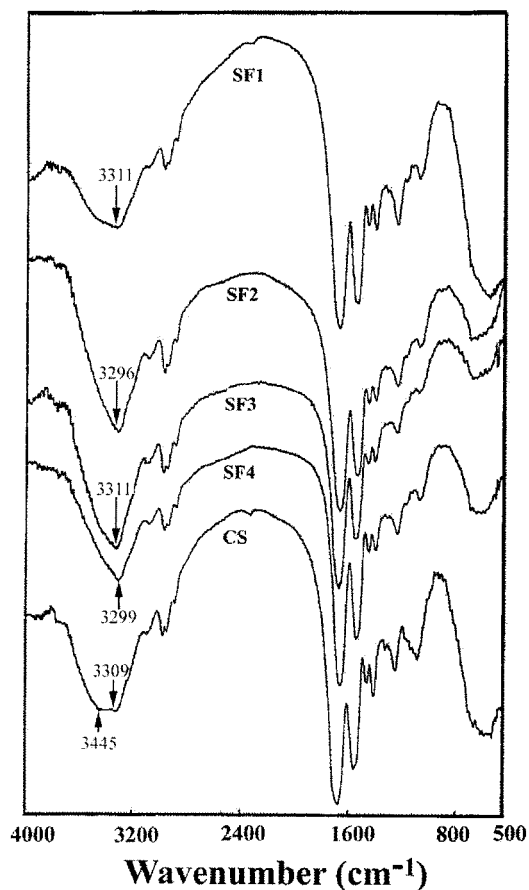
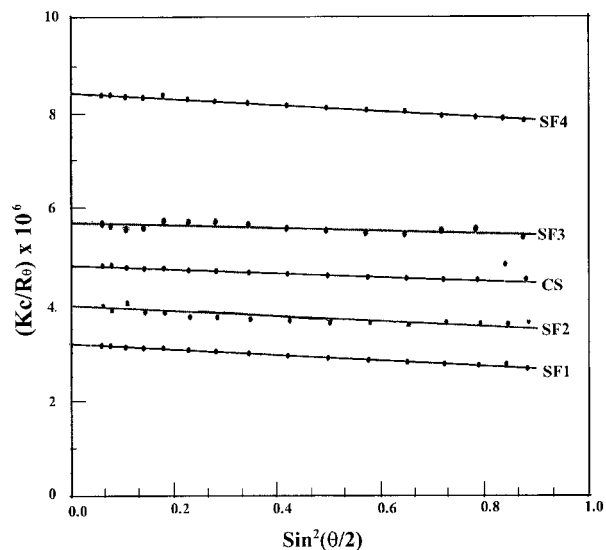**Figure 1** IR spectra of fractions SF1–SF4 and unfractionated SPI (CS).

Figure 2 IR spectra of the TSF1 sheet and GSF1 premix.

plying that the fractions were similar to each other in their chemical structure on the whole. However, different fractions may contain different amino acids with various compositions, such as 2S-, 7S-, 11S-, and 15S-rich globulibin. Therefore, these fractionations showed small differences in the absorption peaks from 700 to 1500 cm^{-1}. The IR spectra of the thermoplastic TSF1 and its premix GSF1 before compression molding are shown in Figure 2. The absorption peaks at 1650 cm^{-1} for the carbonyls groups of TSF1 shifted to a low wave number with a difference of 4 cm^{-1} with respect to GSF1. This indicated that carbonyls groups and N—H groups of SPI formed strong intermolecular hydrogen bonding during heating compression. Two strong absorption peaks at 1234 and 802 cm^{-1} in GSF1 that contributed to the absorption of glycerin disappeared in the spectrum of TSF1, suggesting that a strong intermolecular force occurred between SPI and glycerin because of heating compression.
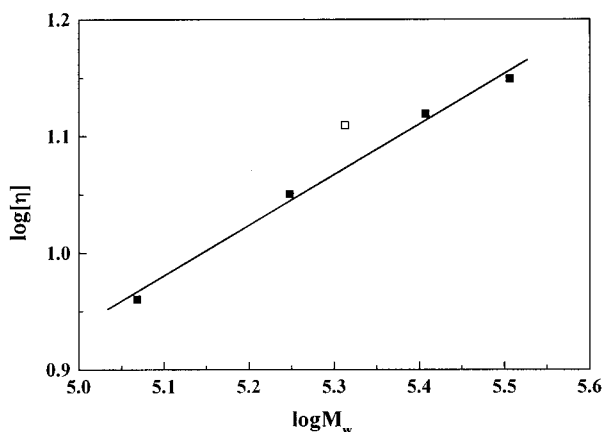

Figure 3 Angular dependencies of $(Kc/R_\theta)_{c=0}$ of the fractions and unfractionated SPI in 0.1M NaOH/0.1M NaCl aqueous solutions.

Table I   Experimental Results of M_w and [η] in 0.1M NaOH/0.1M NaCl Aqueous Solution at 28°C for the Fractions and Unfractionated SPI

Code	SF1	SF2	SF3	SF4	CS
$M_w \times 10^{-4}$	32.1	25.5	17.7	11.7	20.5
[η] (cm³/g)	14.1	13.2	11.2	9.1	12.9

Molecular Weight and Conformation of SPI

Figure 3 illustrates the angular dependencies of $(Kc/R_\theta)_{c=0}$ of the fractions and unfractionated SPI, where K is the light scattering constant and R_θ is the reduced Reyleigh ratio at angle θ. The values of second virial coefficients (A_2) of all samples were near zero, resulting in the failure of typical Zimm plots. Therefore, the plots of the angular dependencies of $(Kc/R_\theta)_{c=0}$ were used to calculate M_w and the radius of gyration [$\langle S^2 \rangle^{1/2}$]. The measured values of M_w and [η] for the fractions and unfractionated sample are summarized in Table I. M_w and [η] of the fractions of SPI increased with an increase in the pH value in fractionation. This indicated that the SPI was successfully fractionated in molecular weight order by the acidifying method. The fractions and unfractionated SPI exhibited much lower values of [η] compared with the values of other flexible polymers with the same M_w, such as polystyrene in toluene and tetrahydrofuran[27] and pullulan in water.[28] Generally, the values of [η] and A_2 reflect the extended degree of the molecular chain. This implies that the molecular chains of SPI were relatively compact coils.
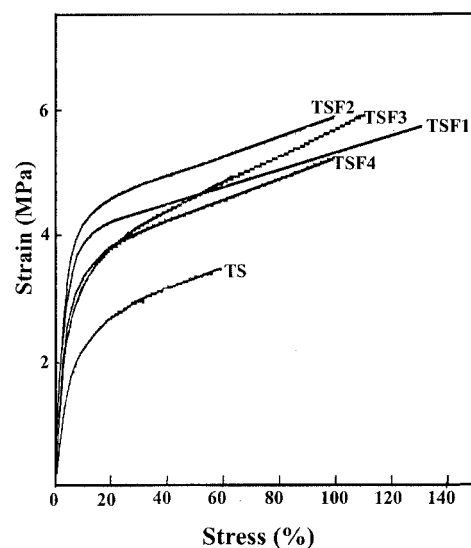
The dependence of M_w on [η] for the SPI samples is shown in Figure 4. The open square represents unfractionated SPI, which deviated from the curve because of the relatively large polydispersity and low molecular weight component. The Mark–Houwink equation was established approximately as follows:
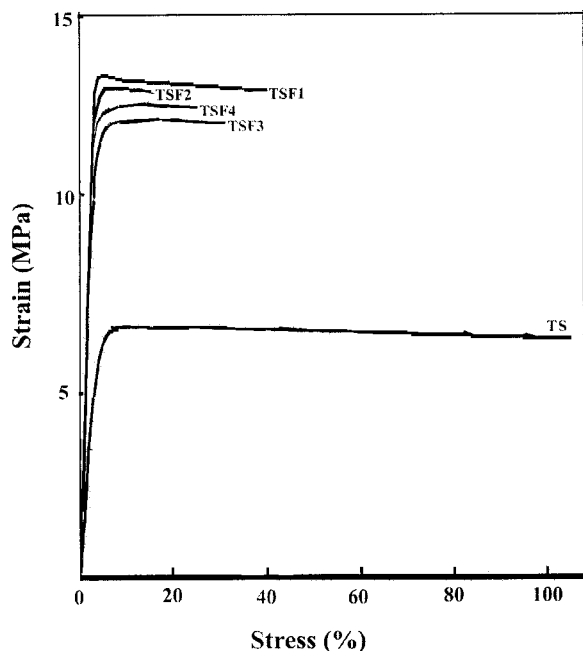
$$[\eta] = 5.8 \times 10^{-2} M_w^{0.44} \qquad (3)$$

The exponent (α) of 0.44 was lower than that of general polymers in good solvents (0.5–0.8) and even lower than that of polymers as compact coils in θ solvents ($\alpha = 0.5$),[29] indicating that molecular chains of SPI existed as a global conformation in 0.1M NaOH/0.1M NaCl aqueous solutions. This conclusion agrees with the results of Whitaker and Steven.[20]

Effects of the Molecular Weight on the Mechanical Properties

Figure 5 shows the strain–stress curves of the thermoplastic sheets TSF1–TSF4 and TS, all of which were stored in a desiccator at room temperature for a week. The TSF1–TSF4 sheets prepared from the fractions displayed higher σ_b and ϵ_b values than the sheet from TS, and the TSF1 sheet with a relatively high M_w exhibited high values of σ_b (5.7 MPa) and ϵ_b (135%). Interestingly, the strain–stress curves in Figure 5 do not show a yield point, which represents the transition from rubber to plastic, suggesting a rubber-

Figure 4   Dependence of M_w on [η] for SPI in 0.1M NaOH/0.1M NaCl aqueous solutions. The open square represents unfractionated SPI.

**Figure 5**   Strain–stress curves of the TSF and TS sheets.

Figure 6 Strain–stress curves of the TSF and TS vacuum-dried sheets.

like material. However, the strain–stress curves of vacuum-dried TSF sheets show yield points and higher σ_b and lower ϵ_b values than those of TS, as observed in Figure 6. Generally, the higher the molecular weight of a linear polymer is, the better the strength is but the lower the elongation is. The vacuum-dried TS sheet had a higher value of ϵ_b because of the presence of some low molecular weight components, such as plasticizers, which were not removed under vacuum. These results indicated that the fractionation of the SPI could improve the mechanical properties, and the plasticizers played an important role in the enhancement of ϵ_b.

The effects of the molecular weight on R for different immersion times are shown in Figure 7. The R values of the fractions increased with increasing M_w and were higher than those of TS, indicating that R of the fractionated SPI materials was improved. This can be explained as follows: with the progress of fractionation, the ratio of the hydrophobic groups decreased, and the SPI molecular weight increased, suggesting that the fraction fractionated from a high pH aqueous solution may contain less hydrophilic amino acid chains than that from a low pH solution. Therefore, thermoplastic SPI with a high molecular weight showed a high value of R.

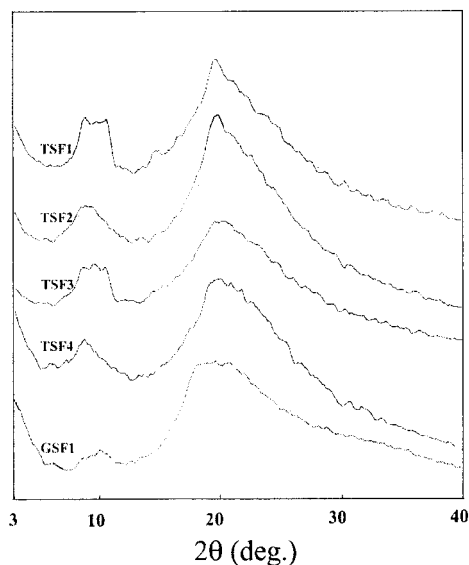Figure 8 shows DSC curves of the TSF1 and TS sheets. TS showed a more obvious thermal tran-
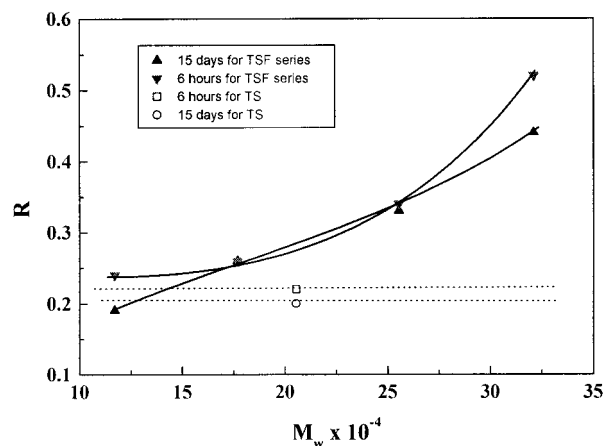
Figure 7 Effect of M_w on R for the TSF and TS sheets immersed in water at 5°C for 6 h and 15 days, respectively.

sition between −80 and −60°C, corresponding to a glass-transition temperature of the materials,[30] than TSF1. This indicated that the molecular chains of SPI in the TS sheet were easier to mobilize than those of TSF1, resulting in good flexibility and relatively weak mechanical properties (Figs. 5 and 6). This is in good agreement with the results from Figure 6, being due to low molecular components that played a role in the enhancement of plastification.

WXRDPs of the TSF sheets and the GSF1 premix are presented in Figure 9. Three kinds of crystals in TSF were determined to be 0.1 at 9.8°, 0.23 at 19.8° and 0.19 at 22.2°, and the degree of total crystallinity (χ_c was 0.53 for TSF1, 0.50 for TSF2, 0.50 for TSF3, and 0.52 for TSF4. However, the χ_c value of GSF1 was 0.35, lower than the values of the thermoplastic sheets. This indicated
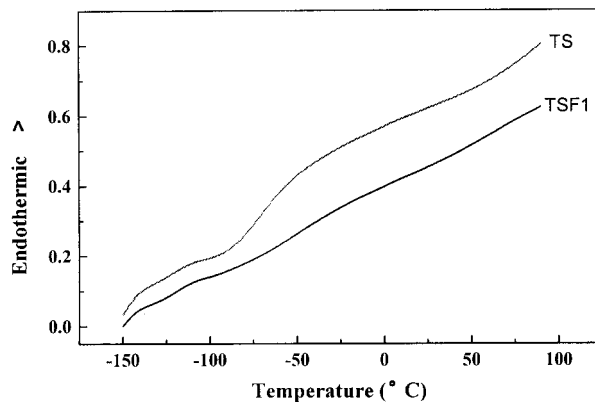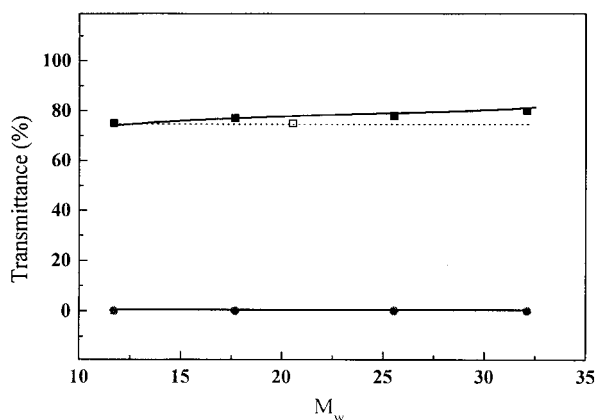

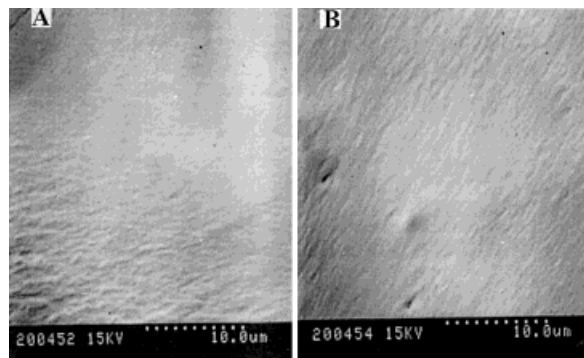**Figure 8** Experimental results of DSC for the TSF1 and TS sheets.

Figure 9 X-ray diffractograms of the TSF sheet and GSF1 premix.

Figure 11 SEM images of sections of the (A) TSF1 and (B) TSF4 sheets.

that χ_c values of the TSF sheets were much higher than those of GSF, suggesting that heating compression played an important role in the promotion of the molecular chain rearrangement of SPI and in the formation of a strong intermolecular force.

The light transmittance of the TSF sheets increased slightly from 78 to 82% at 800 nm with increasing M_w, as shown in Figure 10. The sheet was transparent because global chains of protein were broken to form a novel dense architecture under heating compression. More evidence can be seen in SEM images in Figure 11. The TSF1 and

TSF4 sheets exhibited smooth and homogeneous sections, suggesting the extension and rearrangement of the molecular chains of the SPI under high temperature and high pressure. Interestingly, the TSF and TS sheets can screen ultraviolet, showing near-zero light transmittance from 200 to 300 nm.

CONCLUSION

Commercial SPI was successfully fractionated into four fractions with changes in the pH value of the solution in the fractionation. M_w of the fractions decreased with fractionation progress, that is, with decreasing pH. Thermoplastic sheets were prepared from the fractions and unfractionated SPI with glycerin (50 g/100 g of dry SPI) by compression molding. σ_b of the sheets from fractionated SPI was obviously higher than the value from unfractionated SPI. With increasing M_w, the mechanical properties and R of thermoplastic SPI significantly increased. χ_c of heating-compressed SPI was much higher than that of the original SPI, suggesting a molecular rearrangement in this case. Thermoplastic SPI was transparent and had a homogeneous, dense structure resulting from the destruction of global chains and the formation of a novel architecture sustained by strong intermolecular interactions under heating and compression.

Figure 10 Effect of M_w on the light transmittance of the TSF and TS sheets at (●) 300 and (■) 800 nm. The open square represents the TS sheet at 800 nm.

REFERENCES

1. Huang, S. J. J Macromol Sci Pure Appl Chem 1995, 32, 593.

2. Zhang, L.; Zhou, Q. J Polym Sci Part B: Polym Phys 1999, 37, 1623.

3. Zhang, L.; Zhou, J.; Huang, J.; Gong, P.; Zhou, Q.; Zhang, L.; Du, Y. Ind Eng Chem Res 1999, 38, 4284.

4. VanSoest, J. J. G.; Kortleve, P. M. J Appl Polym Sci 1999, 74, 2207.

5. VanSoest, J. J. G.; Benes, K.; De Wit, D. Polymer 1996, 37, 3543.

6. Otaigbe, J. U.; Goel, H.; Babcock, T.; Jane, J. J Elast Plast 1999, 31, 56.

7. Cuq, B.; Gontard, N.; Guilbert, S. Polymer 1997, 38, 2399.

8. Lin, Y.; Hsieh, F.; Huff, H. E. Cereal Chem 1996, 73, 189.

9. Paetau, I.; Chen, C.-Z.; Jane, J.-I. Ind Eng Chem Res 1994, 33, 1821.

10. Mo, X.; Sun, X. S.; Wang, Y. J Appl Polym Sci 1999, 73, 2595.

11. Liang, F.; Wang, Y.; Sun, X. S. J Polym Eng 1999, 19, 383.

12. Wang, S.; Sue, H.-J.; Jane, J. J Macromol Sci Pure Appl Chem 1996, 33, 557.

13. Wang, S.; Zhang, S.; Jane, J.; Sue, H.-J. Polym Mater Sci Eng 1995, 72, 88.

14. Zasypkin, D. V.; Yuryev, V. P.; Alexeyev, V. V.; Tolstoguzov, V. B. Carbohydr Polym 1994, 18, 119.

15. Smith, A. K.; Circle, S. J Ind Eng Chem 1938, 30, 1414.

16. Ayres, J. L.; Branscomb, L. L.; Rogers, G. M. J Am Oil Chem Soc 1974, 51, 133.

17. Chirico, G.; Beretta, S.; Baldini, G. J Chem Phys 1999, 110, 2297.

18. Liu, Y.; Ma, C. Q.; Lie, K. A.; Tong, S. Y. Anal Chim Acta 1999, 379, 39.

19. Huang, H. C.; Hammond, E. G.; Reitmeier, C. A.; Myers, D. J Am Oil Chem Soc 1995, 72, 1453.

20. Whitaker, J. R.; Steven, R. T. Food Proteins; Avi Publishing: Westport, CT, 1977; p 300.

21. Wu, Q.; Zhang, L. Ind Eng Chem Res 2001, 40, 558.

22. Schhlz, G. V.; Blaschke, F. J Prakt Chem 1941, 158, 130.

23. Huglin, M. B. Light Scattering from Polymer Solutions; Academic: London, 1972; p 225.

24. Rabek, J. F. Experimental Methods in Polymer Chemistry: Applications of Wide-Angle X-Ray Diffraction (WAXS) to the Study of the Structure of Polymers; Wiley Interscience: Chichester, England, 1980; p 505.

25. Gong, P.; Zhang, L. J Appl Polym Sci 1998, 68, 1313

26. Skrovanek, D. J.; Howe, S. E.; Painter, P. C.; Coleman, M. M. Macromolecules 1985, 18, 1676.

27. Hiromi, Y.; Motoharu, F. Macromolecules 1974, 7, 128.

28. Kato, T.; Okamoto, T.; Tokuya, T.; Takahashi, A. Biopolymers 1982, 21, 1623.

29. Einaga, Y.; Miyaki, Y.; Fujita, H. J Polym Sci Polym Phys Ed 1979, 17, 2103.

30. Zhang, J.; Mungara, P.; Jane, J. Polym Prepr 1998, 39(2), 162.