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THERMAL PROPERTIES OF SEED PROTEINS

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Abstract

Thermal properties of seed proteins, prolamines from rice, wheat and soybean were studied by differential scanning calorimetry (DSC), thermogravimetry (TG), thermal expansion, X-ray diffraction and infrared spectroscopy. Prolamine films cast from aqueous ethanol are amorphous in the random-coil conformation. The glass transition of prolamine is observed at 160, 172 and 150°C for rice, wheat and soybean, respectively. The amorphous prolamine films crystallize at 196, 205 and 199°C for rice, wheat and soybean, respectively. The thermal degradation of prolamine films occurs from 228, 250 and 270°C for rice, wheat and soybean, respectively.

Keywords: crystallization, DSC, glass transition, prolamine, rice, soybean, TG, thermal expansion, wheat

Introduction

The seeds of rice, wheat and soybean contain several kinds of proteins. These proteins are classified into four components according to their solubility [1]; albumin (soluble in water), globulin (soluble in saline), prolamine (soluble in alcohol) and glutelin (soluble in aqueous acid and alkali solution). Very few scientific studies have been reported on seed proteins [2], especially ones directly concerned with the structure and physical properties of these proteins. We have previously studied the glass transition and crystallization of zein, the prolamine of corn [3]. In the present study, the thermal behavior such as glass transition and crystallization of seed prolamine films from rice, wheat and soybean was investigated in detail.

Experimental

Material

The seed prolamines were extracted as zein, the prolamine of corn [3]. The meals of rice, wheat and soybean were prepared by grounding in a mill and then by pestle in a

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mortal. The seed meals were extracted with either 60 or 70% (by mass) aqueous ethanol [4]. The extract was dialyzed against distilled water. Each precipitated protein was centrifuged and lyophilized. The proteins prepared were white yellow granular solids [5]. The proteins were dissolved in 70% ethanol and washed repeatedly with dichloroethane or petroleum ether until all color was removed. The solutions were concentrated by removal of most of ethanol by vacuum distillation and poured into a large volume of 1% sodium chloride solution. The gummy substances obtained were washed with water to remove sodium chloride and then freeze-dried after the remaining ethanol was allowed to dry in the air. The films of each specimen were obtained by casting the protein solutions in aqueous ethanol on a glass plate.

Measurement

Differential scanning calorimetry (DSC) scans were recorded on a Seiko DSC 100 at a heating rate of 10 K min^{-1}. Thermogravimetry (TG) was done with a Rigaku TG-DSC 8085E1. Linear thermal expansion was measured using a Rigaku TMA type CN 8095 by recording the change in length of film specimen under constant tension at a heating rate of 10 K min^{-1}. X-ray diffraction patterns were obtained with a Rigaku D3F X-ray diffraction apparatus. Ni-filtered CuK_α was used at 35 kV and 20 mA. Infrared spectra were recorded on a Nicolet Model 60R infrared spectrometer. The specimen placed between two NaCl plates was heated at 5 K min^{-1} with a Hitachi HPC-300 temperature-programming controller. The temperature was measured by a thermocouple attached to the NaCl window. Band intensity was determined by the base-line method.

Results and discussion

Amino acid composition

The amino acid compositions of prolamines were determined by an amino acid analyzer. The prolamine of rice consists of 16% glutamic acid, 11% glycine, 10% alanine, 8% serine and 55% other amino acids. The prolamine (gliadin) of wheat consists of 40% glutamic acid, 15% proline and 7% serine. The prolamine of soybean consists of 23% glutamic acid, 12% alanine, 7% serine and 7% leucine. The corn prolamine contained 28% glutamic acid, 23% valine, 14% lysine, 11% proline and 9% alanine [3].

Each prolamine was separated into fractions by SDS-PAGE according to molecular mass. The molecular masses of rice prolamine were 28 kD, 16 kD, 14 kD and 13 kD. The molecular masses of wheat prolamine were 45 kD, 40 kD, 36 kD, 25 kD and 23 kD. The molecular masses of soybean prolamine were 45 kD and 21 kD.

Conformation and crystallinity

Figure 1 shows X-ray diffraction patterns of amorphous prolamine films from (1a) rice, (1b) wheat and (1c) soybean and those of heat-treated crystallized prolamine films from (2a) rice, (2b) wheat and (2c) soybean, in which as-cast films were annealed at 210°C for

30 min. An amorphous halo is observed for the as-cast films (Fig. 1 (1a–1c)), whereas diffraction rings appear by annealing films, which were identified to be β-form crystals [3] (Fig. 1 (2a–2c)).

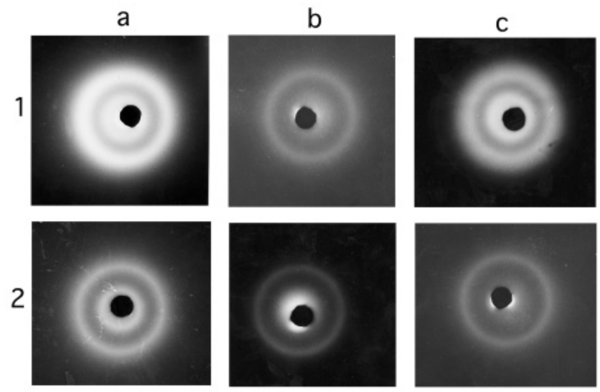

Fig. 1 X-ray diffraction patterns of untreated amorphous prolamine films of 1a – rice, 1b – wheat and 1c – soybean, and of crystallized prolamine films of 2a – rice, 2b – wheat and 2c – soybean, which were annealed at 210°C for 30 min

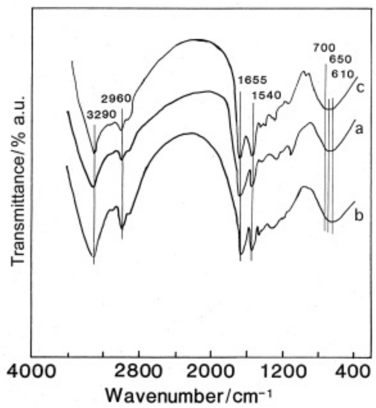

Fig. 2 Infrared spectra of untreated prolamine films; a – rice, b – wheat and c – soybean

Figure 2 shows the infrared spectra of prolamine films from (a) rice, (b) wheat and (c) soybean. Each prolamine film shows absorption bands at 1600, 1540, 1240 and 650 cm^{-1}, which are assigned to amide I, II, III and V bands for the random-coil conformation, respectively [6].

Glass transition and crystallization

Figure 3 shows DSC curves of amorphous prolamine films in the random-coil conformation under nitrogen. A broad endothermic peak appears at about 100°C for each specimen.

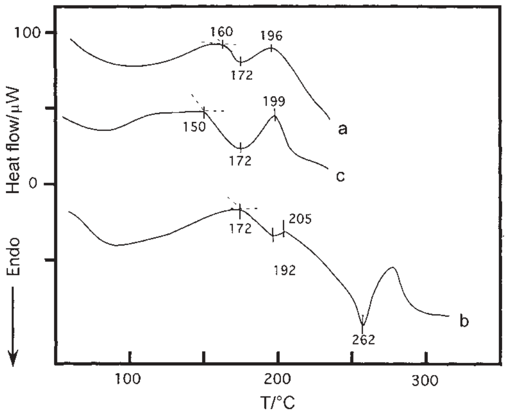

Fig. 3 DSC curves of amorphous prolamine films under nitrogen; a – rice, b – wheat and c – soybean

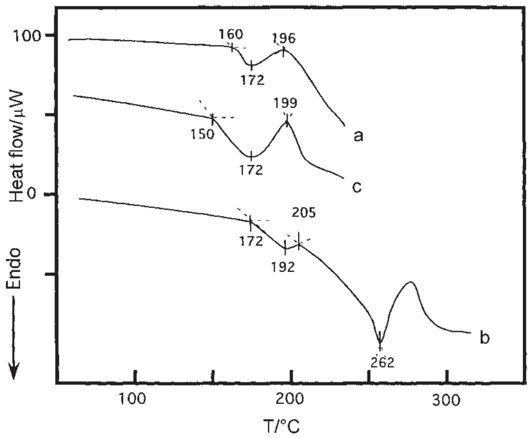

Fig. 4 DSC curves of dried amorphous prolamine films annealed at 102°C for 2 h under nitrogen; a – rice, b – wheat and c – soybean

By annealing each specimen at 102°C for 2 h, the endothermic peak at about 100°C disappears and the baseline becomes straight (Fig. 4). Therefore, the broad endothermic peak at about 100°C is due to evaporation of water absorbed in the specimens.

An endothermic shift and an exothermic peak are observed at 160 and 196°C for rice prolamine, at 172 and 205°C for wheat prolamine and 150 and 199°C for soybean prolamine, respectively. The temperatures of an endothermic shift and an exothermic peak are not affected by the annealing at 102°C (Figs 3 and 4).

Figure 5 shows the linear thermal expansion curves of prolamine films. The as-cast specimens contract initially with increasing temperature, and the length of the specimen begins to increase abruptly at 161°C for rice prolamine, at 172°C for wheat prolamine and 155°C for soybean prolamine. Therefore, the endothermic shift of the

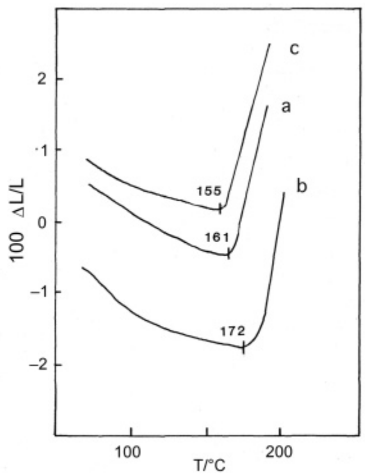

Fig. 5 Linear thermal expansion curves of prolamine films under nitrogen; a – rice, b – wheat and c – soybean

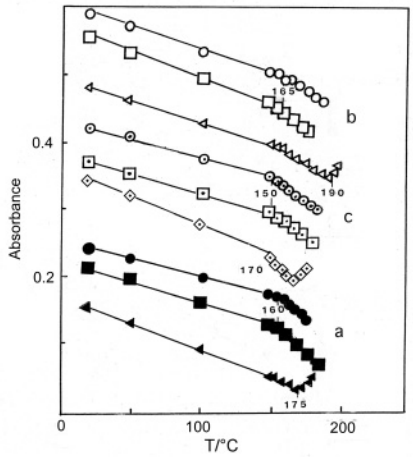

Fig. 6 Temperature dependence of infrared absorption bands of amorphous prolamine films; a – rice, random-coil bands of ● – 1660 cm^{-1} and ■ – 1540 cm^{-1} and β-conformation band of ▲ – 1535 cm^{-1}; b – wheat, random-coil bands of o – 1660 cm^{-1} and □ – 1540 cm^{-1} and β-conformation band of △ – 1535 cm^{-1}; c – soybean, random-coil bands of ⊙ – 1660 cm^{-1} and ⊡ – 1540 cm^{-1} and β-conformation band of ◈ – 1535 cm^{-1}

DSC curves is due to the glass transition. The glass transition temperature of corn prolamine was reported to be 165°C [3].

The exothermic peaks at 196, 205 and 199°C for rice, wheat and soybean prolamines are attributed to the crystallization of amorphous prolamine films. Amorphous halo was obtained in the X-ray diffraction patterns by annealing at 175°C, and the X-ray diffraction patterns of prolamines were observed by annealing at 210°C (Fig. 1 (1a–1c)).

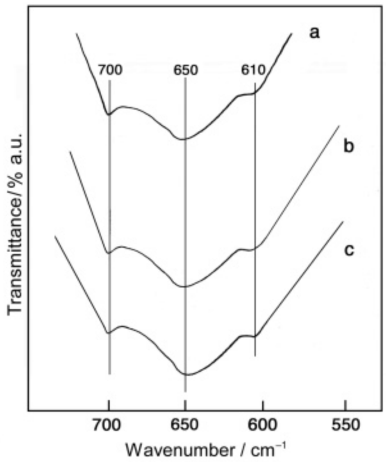

Fig. 7 Infrared spectra of crystallized prolamine films annealed at 200°C for 30 min; a – rice, b – wheat and c – soybean

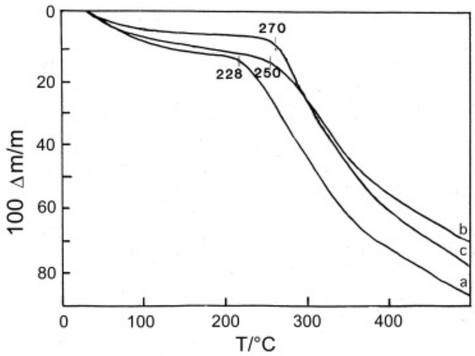

Fig. 8 TG curves of prolamine films under nitrogen; a – rice, b – wheat and c – soybean

Three infrared bands of amorphous prolamine films were measured with stepwise increasing of temperature, as shown in Fig. 6. The absorbance of the bands at 1660 and 1540 cm^{-1}, the amide I and II bands of the random-coil conformation, decreased linearly with increasing temperature, until an abrupt change in slope occurs at about 160, 165 and 150°C for rice, wheat and soybean, respectively.

The absorbance of the amide II band of the β-form at 1535 cm^{-1} also decreases with increasing temperature and begins to increase abruptly at 188°C. Therefore, the conformational change of random-coil to β-form occurs above 175, 190 and 170°C for rice, wheat and soybean, respectively.

The infrared spectra of crystallized prolamines was measured in the range of 550–750 cm^{-1} after annealed at 200°C for 30 min (Fig. 7). In addition to the absorption bands at 700 and 650 cm^{-1} assigned to the amine V for β-form and random-coil, a small band was detected at 600 cm^{-1}, which is assigned to the amide α-helix band. Previously we have reported that corn prolamine crystallizes to β-crystals [3].

Thermal degradation

An abrupt mass loss is observed in the TG curves of prolamine films at 228°C for rice, at 250°C for wheat and 270°C for soybean (Fig. 8). Therefore, the endothermic peak of DSC observed at 262°C (Fig. 3b) is attributed to the thermal degradation of prolamine.

Conclusions

Prolamine films of seed proteins cast from aqueous ethanol are amorphous in the random-coil conformation. Water in the specimen is lost at about 100°C. The glass transition temperature and the crystallization temperature are dependent upon the amino acid compositions of prolamines. The glass transitions of prolamine films are 175°C for rice, 172°C for wheat and 150°C for soybean. The amorphous prolamine films crystallize to β-crystals containing α-helix at about 196°C for rice, 205°C for wheat and 199°C for soybean.

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