

Thermal Behavior of Zein Sheets Plasticized with Oleic Acid

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ABSTRACT

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Zein, extracted from underutilized corn gluten meal may serve as an alternative starting material for fabrication of biodegradable packaging. Zein plasticized with oleic acid may be formed into flexible and water-resistant sheets. Our objective was to investigate the effect of plasticization on thermal behavior of zein sheets employing differential scanning calorimetry (DSC). Zein sheets were rolled from a resin prepared by dispersing zein and oleic acid in aqueous alcohol followed by the recovery of the formed moldable compound by precipitation with water.

Sheets were later replasticized with additional oleic acid to increase ductility. DSC thermograms were used to investigate zein-oleic acid interactions in zein sheets. DSC endotherms were attributed to melting of free oleic acid or to the dissociation of zein-oleic acid bonds. Plasticized sheets contained bound and free oleic acid. Further heat-treatment of plasticized sheets apparently resulted in zein absorption of free oleic acid. However, high temperatures were believed to cause dissociation of some zein-oleic acid bonds formed during replasticization.

Interest in nonfood uses of cereal products is increasing, driven by environmental concerns and agricultural economics. Cereal proteins are being considered as agricultural polymers for production of packaging materials (Cuq et al 1998). Zein was investigated for its potential as structural material for flexible biodegradable sheets (Lai and Padua 1997). Utilization of zein in the manufacture of bioplastics could expand coproduct markets for the corn refining industry (Cygnowicz-Provost and Shapouri 1994).

Zein films have been prepared by peeling-off dried aqueous ethanol (Gennadios and Weller 1990) or aqueous acetone zein solutions (Yamada et al 1995) cast on inert, flat surfaces. Protein film formation, in general, is believed to involve the development of hydrophobic associations, hydrogen bonds, and limited disulfide bonds between protein chains in the film matrix (Gennadios et al 1994, Krochta 1997). Zein films, however, are brittle and require plasticizers to induce permanent flexibility (Reiners et al 1973). Plasticizers, including glycerol and polyethylene glycol, were added to zein film casting solutions to improve flexibility (Park et al 1994, Wu 1995). However, hydrophilic plasticizers increased water absorption of films. Gioia et al (1998) studied the effect of hydrophilic plasticizers on thermomechanical properties of corn gluten meal (70% protein). They concluded that the effectiveness of hydrophilic plasticizers, polyethylene glycol, di- and triethanolamine, glycerol, and glucose was affected by miscibility with corn proteins. Polyethylene glycol, which was listed as a good solvent for zein (Reiners et al 1973), was an effective plasticizer. On the contrary, the low efficacy of glucose as plasticizer was attributed to its limited compatibility with corn gluten meal proteins.

Fatty acid plasticization of zein was investigated by Lai et al (1997). They precipitated aqueous ethanol solutions of zein and fatty acids into plastic solids that were suitable for sheet rolling. Preparations containing unsaturated fatty acids, oleic, and linoleic acids produced flexible sheets, whereas addition of saturated fatty acids, stearic, and palmitic acids resulted in stiff sheets. Fatty acid plasticization reduced water absorption of rolled zein sheets. Lai et al (1999) proposed a structural model for zein-oleic acid sheets based on X-ray scattering measurements. They interpreted X-ray spacings in terms of the zein molecular dimensions reported by Tatham et al (1993) and Matsushima et al (1997). The model consisted of an arrangement of staggered zein planes separated by layers of oleic acid. A possible spatial arrangement of the oleic acid in between the zein planes consisted of two layers of oleic acid molecules oriented with their long axis perpendicular to the sheet plane. The model

suggested that plasticization of zein involved binding oleic acid to the zein surface.

Santosa and Padua (1999) investigated tensile and water resistance properties of rolled zein sheets plasticized with oleic or linoleic acids. They observed that sheet properties were affected by the heat treatment applied during preparation and suggested that heating influenced sheet structure by promoting protein-lipid associations. Izzo and Ho (1989) studied lipid-protein interactions during single-screw extrusion of zein and corn oil. They observed a decrease in extractable fatty acids after extrusion of zein with corn oil and attributed their results to zein binding of fatty acids during the extrusion process.

Thermal properties of food proteins are often studied employing differential scanning calorimetry (DSC) (Roos 1995). Protein endotherms are associated with denaturation processes. Arntfield and Murray (1981) observed denaturation endotherms for soybean, field pea, faba bean, and oat proteins. They reported that endothermic enthalpy changes decreased as proteins were subjected to increasing temperatures or pH level and disappeared altogether at extreme values of temperature or pH level. Endotherms were interpreted as indicative of progressive denaturation. Kokini et al (1995) used DSC to investigate the plasticizing effect of water on cereal proteins. They reported that the first few percentages of water resulted in a dramatic decrease in the glass transition temperature (T_g) of zein and gliadin. However, the effect decreased significantly once moisture content increased to $\approx 15\%$. Gioia et al (1999) determined T_g values for corn gluten meal (CGM) and its protein components, zein, and glutelin. The higher T_g value observed for hot-air-dried CGM compared with native CGM was attributed to structural differences and protein-protein interactions. Our objective was to use DSC measurements to investigate the effect of oleic acid plasticization on thermal behavior of rolled zein sheets.

MATERIALS AND METHODS

Materials

Regular grade corn-zein (F4000, Freeman Industries, Tuckahoe, NY) was obtained with a moisture content of 5.2%. Reported protein content of F4000 zein was 94–96% (dry basis). Oleic acid (OA), technical grade, was obtained from Aldrich Chemical Co. (Milwaukee, WI). Oleic acid, analyzed by gas chromatography, contained 92% oleic acid, 5% linoleic, 3% stearic, and 0.5% palmitic acids. Technical grade isopropyl alcohol (Aldrich Chemical) was diluted with distilled water to obtain 80% aqueous alcohol.

Preparation of Zein-Oleic Acid Resins and Sheets

Zein solutions (400 g of zein and 2 L of 80% isopropyl alcohol) were mixed with 280 g of oleic acid and stirred for 10 min at 60°C. Addition of cold water to the zein solutions resulted in the formation of zein-fatty acid precipitates that were recovered as soft solid

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compounds (resin). Precipitates were soaked overnight in cold water (4°C) and subsequently kneaded in a farinograph mixer bowl (type FT3, Brabender, Rochelle Park, NJ) for 20 min to obtain cohesive moldable resins. Resins hydrated at that point were rolled into sheets (≈0.5 mm thick) using a pasta roller (model 150, Atlas, Italy). Resulting sheets were dried at ambient conditions. Oleic acid content of sheets was 40% and moisture content was 4%. Unplasticized zein sheets were prepared using the same method except for the addition of fatty acids.

Replasticization of Zein-Oleic Acid Sheets

Oleic acid plasticized sheets were subjected to replasticization to increase ductility. Replasticization was accomplished by direct absorption of oleic acid by zein sheets prepared as indicated above. Sheets were immersed (tempered) in warm oleic acid for 12 hr at 75°C, 4 hr at 100°C, or 1 hr at 125°C. Samples tempered for 12 hr at 75°C were subsequently oven-heated at higher temperatures. Oven time and temperature treatments were 2 hr at 120°C, 30 min at 140°C, and 10 min at 160°C. Final oleic content of sheets was 45, 52, and 58%, respectively. Alternatively, plasticized sheets were dried in a cabinet dryer at 90°C for 4 hr and ground to a granular solid in a hammer mill. Dried resins were dispersed in warm 80% isopropyl alcohol and added with 10, 20, and 30 g of oleic acid/100 g of dry resin. Dispersions were heated in a microwave oven (model RS458P, 2450 MHz, 1,500W, Amana Refrigeration, Amana, IA) to evaporate the solvent and form a melt. The melted, replasticized resins were rolled to form sheets of ≈0.5 mm in thickness. Oleic acid content of sheets added with 10, 20, and 30 g of oleic acid/100 g of dried resin was 45, 49, and 53%, respectively. Moisture content of replasticized sheets was 3%.

Thermal Properties

Thermal properties of zein sheets were analyzed by DSC (model 9616001, Perkin-Elmer 7 Series Thermal Analysis System, Perkin-Elmer, Norwalk, CT). Samples were stored for at least one week

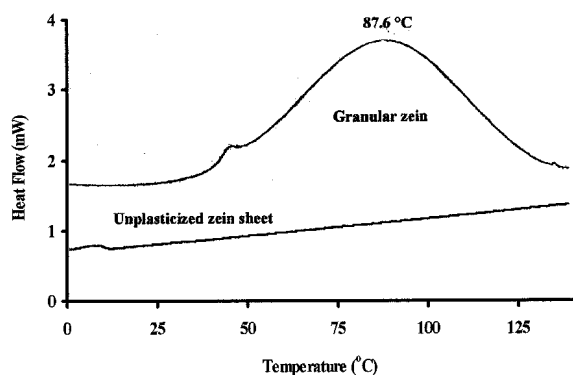


Fig. 1. Differential scanning calorimetry (DSC) thermograms of granular zein and unplasticized zein sheet.

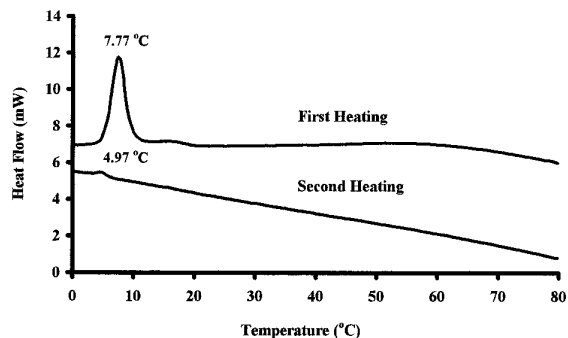


Fig. 2. Two-cycle differential scanning calorimetry (DSC) thermogram of a zein sheet plasticized with oleic acid.

before thermal analysis. Weighed samples (15–20 mg) were placed in 6-mm standard aluminum pans. Samples were cooled down to –10°C with a liquid nitrogen refrigeration system and immediately scanned from –10 to 175°C at the rate of 10°C/min. Double scans were performed immediately, one after the other, with no standing time between them.

RESULTS AND DISCUSSION

Zein

A DSC thermogram of granular zein (Fig. 1) showed a broad endothermic peak centered at ≈87°C. Broad endothermic peaks are usually interpreted as the result of cooperative events occurring within a temperature range, including breakup of hydrogen bonds and other molecular associations. Eliasson and Hegg (1980) observed a DSC endothermic peak for wheat gluten protein at 88°C, which they associated with protein conformational changes occurring during baking. Arntfield and Murray (1981) studied DSC scans of various vegetable proteins and reported endothermic peaks for faba bean protein, soybean protein, and oat protein at 88, 93, and 112°C, respectively. They attributed enthalpy changes to protein unfolding involving the breakup of hydrogen bonds. In contrast, the zein sheet thermogram (Fig. 1) showed no features. Arntfield and Murray (1981) reported that endothermic peaks were observed for faba bean micelle protein and faba bean protein concentrate at similar temperatures, indicating that the concentration process did not affect protein conformation. However, peaks were no longer observed after proteins were heated at 95°C, suggesting that high temperatures induced protein conformational changes. Differences between DSC scans from granular zein and zein sheets were believed to be the effect of thermal treatment applied to zein during sheet preparation. Presumably, the character of zein aggregation changed from granular zein to zein sheets.

Plasticized Sheets

Thermograms of plasticized sheets showed an endothermic peak at ≈7°C (Fig. 2), which was attributed to melting of oleic acid. The melting point of oleic acid was reported at 5–7°C (Budavari 1989).

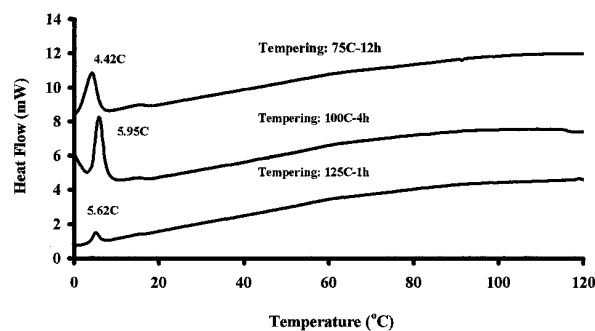


Fig. 3. Differential scanning calorimetry (DSC) thermograms of zein-oleic acid sheets after immersion in oleic acid.

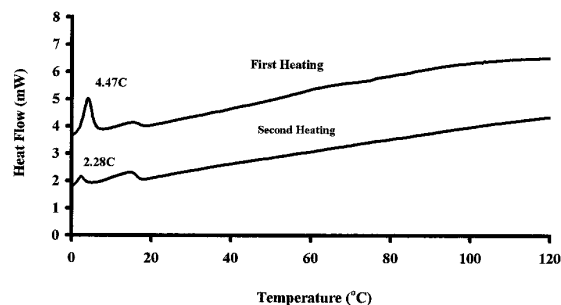


Fig. 4. Two-cycle differential scanning calorimetry (DSC) thermogram of a zein-oleic acid sheet after immersion in oleic acid.

Phase separation of oleic acid in plasticized sheets was thought to result from incomplete miscibility of oleic acid with the zein matrix. The observed peak was possibly due to free oleic acid, present in excess to the binding capacity of the zein surface. Consistently, sheets appeared opaque. Chatfield (1953) explained that resin clarity was indicative of good miscibility among components and suggested that strong interactions existed between them. The peak observed at $\approx 5^{\circ}\text{C}$ in the second cycle of the same thermogram (Fig. 2) was also attributed to melting of oleic acid. However, the enthalpy change in the second cycle was lower, indicating a smaller amount of oleic acid underwent melting. The decrease in enthalpy change suggested that heating decreased the amount of free oleic acid phase, possibly by increasing zein binding of oleic acid. An alternative explanation for the observed smaller peaks could be that oleic acid recrystallizes slowly when distributed within zein, therefore only a fraction of the crystals underwent melting.

Replasticized Sheets

Thermograms of zein sheets (Fig. 3), taken after tempering by immersion in warm oleic acid and before oven heating, showed melting peaks at $\approx 5^{\circ}\text{C}$. Peaks were attributed to free oleic acid. Smaller peaks obtained for samples treated at higher temperatures suggested that heat treatment reduced oleic acid separation from the zein matrix possibly by promoting zein-oleic acid interactions. A two-cycle thermogram of zein sheets tempered at 75°C for 12 hr (Fig. 4) recorded no endothermic processes at $\approx 5^{\circ}\text{C}$ on the second heating, suggesting that exposure to high temperatures during the first cycle promoted binding of oleic acid to zein surface. Consistently, no endothermic processes at $\approx 5^{\circ}\text{C}$ were observed in samples first tempered at 75°C for 12 hr and subsequently oven-heated at 120°C for 2 hr, or at higher temperatures (Fig. 5), which also suggested increased zein binding of oleic acid upon heating. However, the small peak observed in Fig. 3 for the sample tempered at 125°C for 1 hr suggested that zein binding of oleic acid is affected

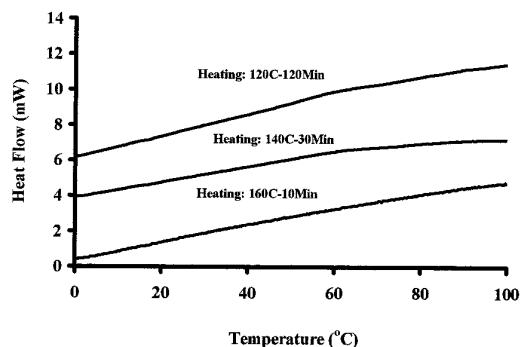


Fig. 5. Differential scanning calorimetry (DSC) thermograms of oven heated zein-oleic acid sheets replasticized by absorption.

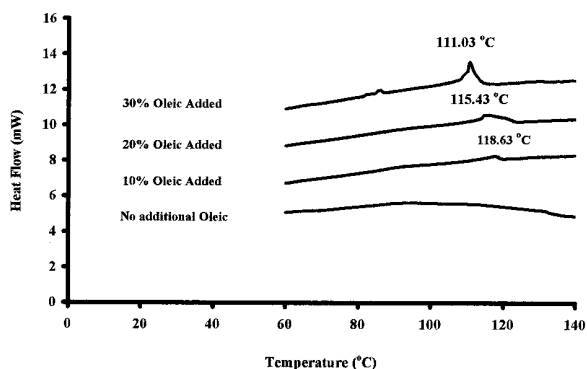


Fig. 6. Differential scanning calorimetry (DSC) thermograms of zein-oleic acid sheets replasticized by redispersion in isopropanol.

by heating time and temperature conditions rather than temperature alone. Izzo and Ho (1989) observed that extrusion cooking of zein and corn oil decreased the amount of extractable fatty acids in the product. They attributed this to the binding of fatty acids to the protein.

Zein binding of oleic acid was thought to occur during resin preparation. Lai et al (1999) proposed a structural model for zein-oleic acid films based on X-ray scattering measurements and on zein molecular shape and dimensions determined by Matsushima et al (1997). In the model, zein prism-like units consisting of ribbons of folded (antiparallel) α -helical segments (Argos et al 1982), formed protein dimers or tetramers. Layers of double-stacked zein units alternated with bilayers of oleic acid, which conferred flexibility to the films. The structural model suggested that plasticization of zein with oleic acid involved the dispersion of zein molecules in aqueous alcohol followed by electrostatic adsorption of fatty acids to polar residues on the zein surface. Addition of cold water during resin precipitation resulted in hydrophobic aggregation of zein-oleic acid units to form sheets. Sheets of molecular dimensions layered atop of each other by hydrophobic aggregation of hydrocarbon ends of oleic acid bound to the zein surface. Sheet formation was facilitated by kneading and rolling actions applied to the resin mass. Endotherms of zein sheets (Figs. 2–4) could be explained according to the model as the result of excess oleic acid present in the resin. Unbound oleic acid formed a separated, discrete, phase that solidified upon cooling and then melted during DSC experiments, generating endothermic peaks at $\approx 6^{\circ}\text{C}$.

Heat treatment applied during replasticization increased the clarity and flexibility of zein sheets. The effect was attributed to increased interaction between zein and oleic acid. Heating possibly induced unfolding of the prism-like zein molecule and exposed new sites for binding of oleic acid. Santosa and Padua (1999) reported that excessive heating or mechanical action of the initial zein and oleic acid dispersion caused protein gelation that interfered with resin formation upon addition of cold water. It was hypothesized that zein unfolded under heat and mechanical action promoting zein interchain bonds which resulted in gel formation.

Endothermic peaks centered at $\approx 115^{\circ}\text{C}$ were observed in replasticized sheets prepared by redispersion of dried resins (Fig. 6). Enthalpy changes increased with the amount of oleic acid added on replasticization (0–30 g of oleic/100 g of dried resin). Peaks possibly originated from breakup of zein-oleic associations, which seemingly increased with added oleic acid. A two-cycle thermogram, of a replasticized sheet prepared by redispersion of dried resins (30 g of oleic acid /100 g of resin) is shown in Fig. 7. An endothermic peak at $\approx 106^{\circ}\text{C}$ observed on the first cycle was also attributed to dissociation of zein-oleic acid bonds. However, the peak disappeared on the second heating cycle, while an endothermic peak appeared at $\approx 5^{\circ}\text{C}$. The peak at $\approx 5^{\circ}\text{C}$ was attributed to melting of free oleic acid, which may have resulted from the dissociation of zein-oleic compounds during the first heating cycle. It appeared

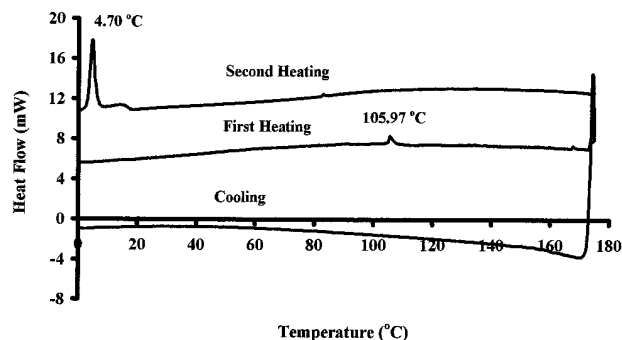


Fig. 7. Two-cycle differential scanning calorimetry (DSC) thermogram of a zein-oleic acid sheet replasticized by redispersion in isopropanol.

that zein binding of oleic acid was affected by the method used to add oleic acid during replasticization. For samples prepared by redispersion of dried resins, temperatures at $\approx 115^{\circ}\text{C}$ were apparently sufficient to break up associations between zein and oleic acid. However, sheets that were immersed in oleic acid baths showed no endothermic transitions up to 175°C . It was speculated that the heat treatment applied to sheets and resins during replasticization affected oleic acid binding. While immersion in oleic acid involved high temperatures for a long time, redispersion and solvent evaporation under microwave heating was accomplished at lower temperatures and short times.

CONCLUSIONS

DSC thermograms were used to investigate zein-oleic acid interactions in zein sheets rolled out of softened, plasticized, zein. Differences between endotherms from granular zein and unplasticized zein sheets were interpreted as changes in molecular aggregation of zein occurring during sheet processing. Thermograms of plasticized zein sheets were taken as evidence of the presence of bound and free oleic acid. Observed melting peaks at $\approx 5^{\circ}\text{C}$ were attributed to free oleic acid, possibly present in excess to the binding capacity of the zein surface. Endothermic peaks attributed to free oleic acid decreased in area or disappeared from the thermograms on subsequent heating of zein sheets, either during second DSC scans or by the replasticization process. Smaller enthalpy changes of oleic acid peaks were interpreted as reductions in free oleic acid due to enhanced zein binding of oleic acid. Zein molecules, originally prism-like units consisting of ribbons of folded (antiparallel) α -helical segments, presumably unfolded at high temperatures, exposed new sites capable of further binding oleic acid. However, zein binding of oleic acid seemed to depend on the thermal treatment applied to the sample. For sheets prepared by redispersion of zein resins, endothermic peaks were observed centered at $\approx 115^{\circ}\text{C}$. Peaks were believed to result from the dissociation of zein-oleic acid bonds formed during replasticization. A second scan of a similar sample yielded a melting peak at $\approx 5^{\circ}\text{C}$. The peak may have resulted from free oleic acid separated from zein during the first heating cycle. Reported dependence of optical and tensile properties of zein sheets on thermal treatment may be related to zein binding of oleic acid.

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