

Effects of Heating on the Interaction of Lipid and Zein in a Dry Powder System

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The effects of heat treatment on the interaction of lipid and zein in a dry powder system were investigated. Linolenic acid ethyl ester (LAE) was mixed with the zein powder. The glass transition temperature (T_g) for the dry powder zein was shown to be ~ 107 °C by differential scanning calorimetry. The thermogram of the zein–LAE mixed powder showed an exothermic transition near the T_g of zein. Fourier transform infrared (FT-IR) spectroscopy was used for detecting the structural changes in zein by heat treatment, that is, elevating the temperature from 25 to 160 °C. The heat treatment of the powdery zein with and without LAE caused increases in the α -helix, β -turn, and β -sheet, concomitant with decreases in the intermolecular hydrogen-bonded β -sheet and random coil. Such changes in the secondary structure were more drastic for the powder with LAE. The heating of the zein–LAE mixed powder also caused decreases in the peaks originating from LAE in the FT-IR spectra. These results suggest that the heat treatment induced the interaction of the zein and LAE in the powdery system. The influence of heating on the antioxidative activity of dry powder zein was studied by measurements of the peroxide value. When zein–LAE mixed powder was heated before storage, the oxidation of LAE was inhibited for 7 days, whereas LAE was oxidized within 1 day in the absence of heat treatment.

KEYWORDS: Zein; lipid; heating; powder system; interaction; FT-IR spectroscopy; DSC; POV

INTRODUCTION

Cereal proteins impart important properties to the functionality of cooked, baked, and extruded products. One unique property of cereal proteins, especially prolamins, is their antioxidative activity in the powdery system, that is, the ability to inhibit lipid oxidation. It had been believed that such activity was due to the physical shielding of lipid molecules from oxygen by the prolamins matrix. To prove the physical interaction of lipids and zein, which is maize prolamins, we studied the conformational changes in zein by lipids using Fourier transform infrared (FT-IR) spectroscopy in the powdery system (1). The mixing of lipid (linolenic acid ethyl ester, LAE) with zein powder caused decreases in the α -helix and intermolecular hydrogen-bonded β -sheet of zein when the powder was stored in the “humid” state, suggesting the strong interaction of LAE and zein molecules. However, the “dry” condition did not allow such interaction in the powder during storage. It had already been known that zein could inhibit the oxidation of lipids in the humid state but not in the dry state (2–4); therefore, we suggested that the physical interaction of LAE and zein might be responsible for the antioxidative activity of zein in the powder system (1).

Despite the excellent antioxidative effects, the humid state is not desirable for the storage of zein–lipid powder, because the high water activity enhances the deterioration of components via chemical and microbial processes. It is more useful to find an appropriate way to enhance the antioxidative activity of zein even in the dry state. Controlled heating in the dry state of egg white was found to be effective to significantly improve functionalities such as gelling, emulsifying, foaming properties, and water-holding capacity without any loss in solubility (5–13). These results suggest that heating in the dry state can be a new approach to improve the functional properties of food proteins. It seems worthwhile to test the effectiveness of heat treatment as a tool to enhance the antioxidative activity of zein powder in the dry state.

In this study, the effects of heating on the interaction of lipid and zein in the dry powder system were investigated. FT-IR spectroscopy was used to detect subtle conformational changes in zein caused by the interaction with lipids according to the previous paper (1). The heat-induced change in the antioxidative activity of dry powder zein was also assessed by measurement of the peroxide value (POV).

The heating of dry protein powders normally causes a glass transition in the protein matrix from the glassy to a rubbery state. Physicochemical properties such as molecular mobility,

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rate of chemical reactions, gas permeability, and viscosity are completely changed after the glass transition. Although the glass transition behavior was not referred to in the papers about the heat treatments of egg white powders aforementioned (5–13), the changes in functional properties should be closely related to the glass transition of egg white proteins. In this study, therefore, the glass transition behavior of zein was also examined by differential scanning calorimetry (DSC) to understand the important role of the glass transition in changes in the zein-lipid interactions.

MATERIALS AND METHODS

Materials. Zein was purchased from Nacalai Tesque Inc. (Kyoto, Japan). LAE was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Stearic acid ethyl ester (SAE) was purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents of analytical grade were purchased from Nacalai Tesque Inc. or Wako Pure Chemical Industries, Ltd.

Preparation of Powder. Commercial zein was washed with ethyl acetate to remove potential antioxidants before the preparation (14). LAE or a mixture of LAE and SAE (7:3, w/w) was dissolved in *n*-hexane and added to the zein powder. The mixing ratio was lipid/zein 1:9 or 1:4 (w/w). The sample powders were stored in a desiccator with silica gel for 7 days, which has been shown to include 3% water in our previous paper (1).

DSC. The sample powders of zein and zein-LAE (zein/LAE 4:1, w/w) were prepared as described in the previous section. About 10 mg ($\pm 5\%$) of sample (zein, zein-LAE, or LAE) was transferred to a 70- μ L silver pan, and subsequently the pan was hermetically sealed. DSC measurements were carried out with a DSC 100 (Seiko Instruments Inc., Chiba, Japan) equipped with a thermal analysis data station. The samples were scanned at a rate of 5 °C/min from 25 to 160 °C, using an empty silver pan as a reference. The T_g was determined from the resulting thermograms as the onset temperature of the courses of the shift in the apparent specific heat due to the transition.

FT-IR Measurement. The FT-IR transmission spectra were recorded on an FT-IR-480 Plus spectrophotometer (Jasco Co., Tokyo, Japan). The spectrum of LAE liquids was measured using a cell equipped with two CaF₂ windows. Zein and the zein-lipid powders were measured with KBr. After homogenization of the powders and KBr with an agate mortar and pestle, the mixed powders were pressed into pellets using an MT-1 Micro KBr die kit and MP-1 minipress (Jasco Co.). For each spectrum, a total of 50 scans was collected at 4 cm⁻¹ resolution.

For the secondary structure analysis of the zein, deconvolution of each spectrum was performed using Jasco FT-IR software according to the methods of Fourier self-deconvolution (FSD) (15) and the finite impulse response operator (FIRO) (16). The spectra were analyzed by second derivatization (17, 18) and Gaussian curve fitting (19, 20) in the amide I region (1600–1700 cm⁻¹) using the software. The secondary structural content was calculated from the relative areas of the individual assigned bands in the total area of the amide I region. The assignment of individual components to the secondary structural elements was done according to ref 9. The assignment of the major bands in the lipid region (1000–3100 cm⁻¹) was based on the data from the previous FT-IR analyses of lipid molecules (21, 22).

Heating Treatment on Heating Block. The heating of the samples for peroxide measurement and gas chromatographic analysis was performed using a heating block (Thermo Alumi Bath ALB-121, Iwaki, Asahi Techno Glass Co., Chiba, Japan). The samples were each transferred to a 13-mL glass tube in a nitrogen atmosphere, and the tube was capped immediately and then heated from 25 to 160 °C at a rate of 5 °C/min. Subsequently, aliquots of the sample were stored in a desiccator with silica gel at 40 °C.

Measurement of POV. The POV was measured by using the ferric thiocyanate method (23). A 2-mg portion of LAE was extracted with 1 mL of chloroform/methanol (2:1, v/v). The solution (62.5 μ L) was diluted with 4.7375 mL of the same solvent. A 5-mg portion of each sample powder was extracted as described above and filtered through a Cosmonice filter S, no. 440-85 (Nakarai Chemicals) to remove

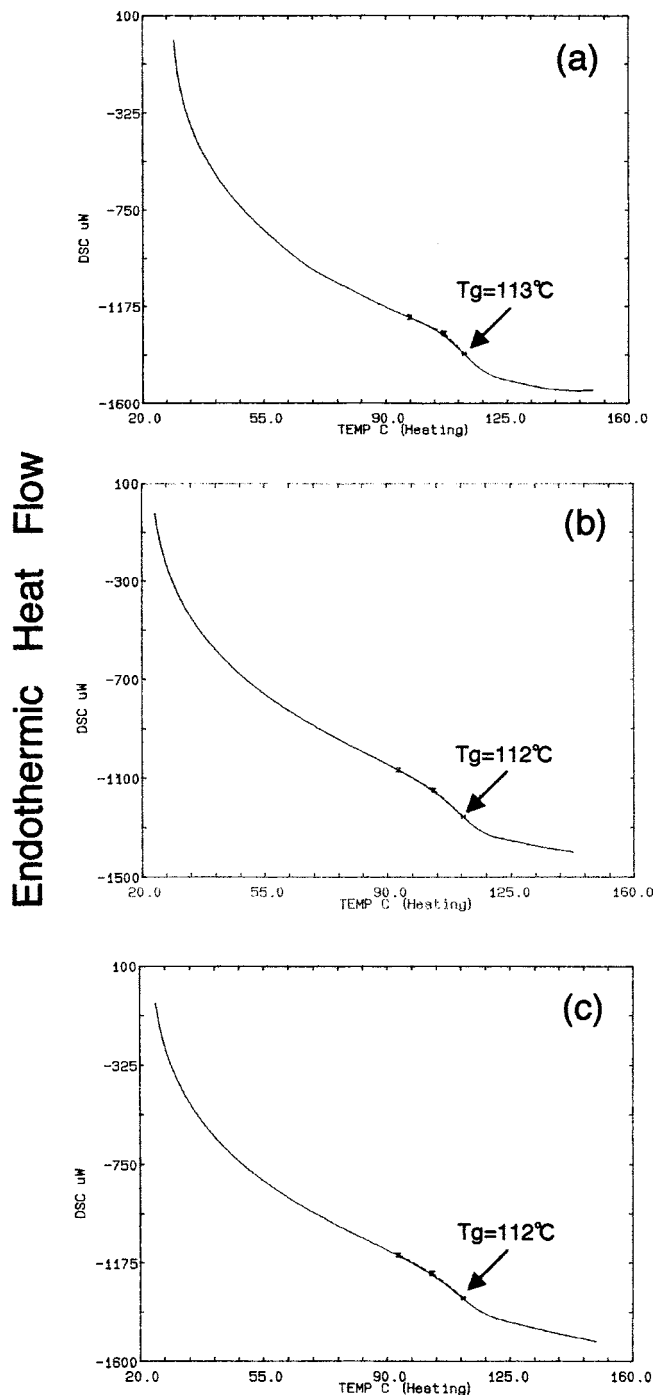


Figure 1. DSC thermogram of zein powder in the dry state recorded at a rate of 5 °C/min: (a) first, (b) second, and (c) third heating trials.

insoluble materials. The filtered solution (0.25 mL) was diluted with 4.55 mL of the solvent; 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of a mixture of equal volumes of 0.02 M ferrous sulfate and 0.02 M barium chloride were added to each diluted sample solution. Absorbance at 500 nm was measured exactly 3 min after the start of reaction. The POV was expressed as the absorbance at 500 nm without calibration.

Gas Chromatography (GC). Zein-LAE-SAE mixed powder (5 mg) was dissolved in 2 mL of 70% ethanol to obtain a transparent solution, and 1 mL of hexane was added and mixed vigorously by vortex. The supernatants were combined by two extractions, evaporated to dryness under a stream of nitrogen, and dissolved in 100 μ L of hexane. About 2 mg of LAE-SAE was dissolved in 1 mL of hexane. An aliquot (1 μ L) was directly injected into the column inlet of a Shimadzu GC-14B gas chromatograph (Shimadzu Co., Kyoto, Japan)

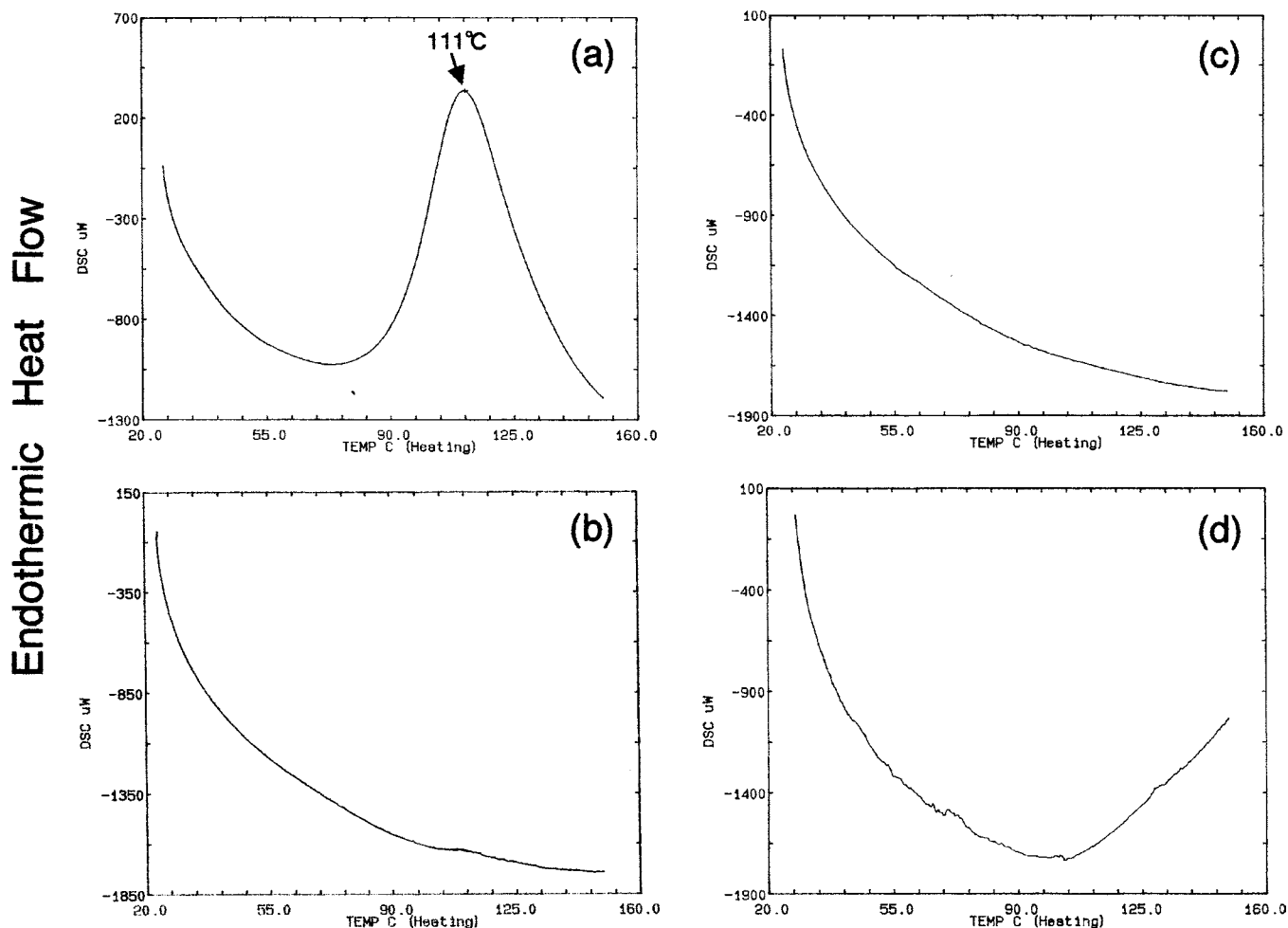


Figure 2. DSC thermogram of zein-LAE (4:1) powder in the dry state recorded at a rate of 5 °C/min: (a) first, (b) second, and (c) third heating trials; (d) DSC thermogram of LAE recorded at a rate of 5 °C/min.

equipped with a hydrogen flame ionization detector. Analytical conditions were as follows: a glass column (0.32 × 210 cm) packed with silar 10C (10%) on Chromosorb W (AW-DMCS, 80–100 mesh); temperature program, 140–240 °C at 4 °C/min; injection and detection temperatures, 260 °C; carrier gas (N₂) at a flow rate of 60 mL/min; flow rate of H₂, 60 mL/min; flow rate of air, 50 mL/min. The ratios of LAE to an internal standard SAE were obtained by calculation from the corresponding recorded peak areas.

RESULTS AND DISCUSSION

Thermotropic Behavior of Zein and Zein-LAE Powders.

DSC measurements were performed on zein and zein-LAE powders in the dry state as described under Materials and Methods.

A thermogram obtained during the first heating of zein is presented in **Figure 1a**. The T_g value of zein in the dry state was ~113 °C, which was detected as the midpoint of the glass transition in the DSC curve. Glass transition was also observed in the second and third heating trials similarly (**Figure 1b,c**). Thus, the glass transition of zein is a reversible phenomenon. Such a reversible glass transition has been reported in the case of wheat proteins (gliadins) (24).

Figure 2a shows a thermogram of zein-LAE (4:1, w/w) dry powder during the first heating. In this thermogram, a large exothermic peak was observed, and the top position was measured to be 111 °C. Because such a peak was not observed in the thermogram of LAE (**Figure 2d**) or in that of zein powder (**Figure 1**), the result of **Figure 2a** indicates the interaction of

LAE and zein in the powdery system. A similar exothermic transition was previously observed in the DSC curve of a gliadin (wheat prolamin) and phospholipid mixture, although both components were mixed in 50% propanol for DSC measurements in this case (25). Irrespective of solution or powdery states, the elevation temperature may induce similar changes in prolamin-lipid mixture systems, which can be detected by an exothermic peak in the DSC curve.

It should be noted that the exothermic peak in **Figure 2a** appeared near the T_g of zein powder shown in **Figure 1**, suggesting the close relationship between the glass transition of zein and the zein-LAE interaction. Below the T_g , LAE permeation into the interior or the core sites of the zein matrix may be restricted because of the extremely low motion of zein molecules. However, the glass transition can probably enhance the molecular mobility, thereby incorporating the lipid molecules into the core sites of the zein matrix. An exothermic reaction normally occurs in the case of the formation of the new ordered structures via non-covalent bondings, such as lipid crystallization (26, 27) and protein-ligand binding (28). In our case, the close contact of hydrophobic core sites of zein with the lipid induced by heat elevation according to the mechanism described above might lead to the formation of a new ordered structure, which was detectable by the exothermic transition.

Neither the glass transition nor the exothermic peak was observed in the second and third heatings of zein-LAE powder (**Figure 2b,c**), indicating the irreversible process of heat-induced

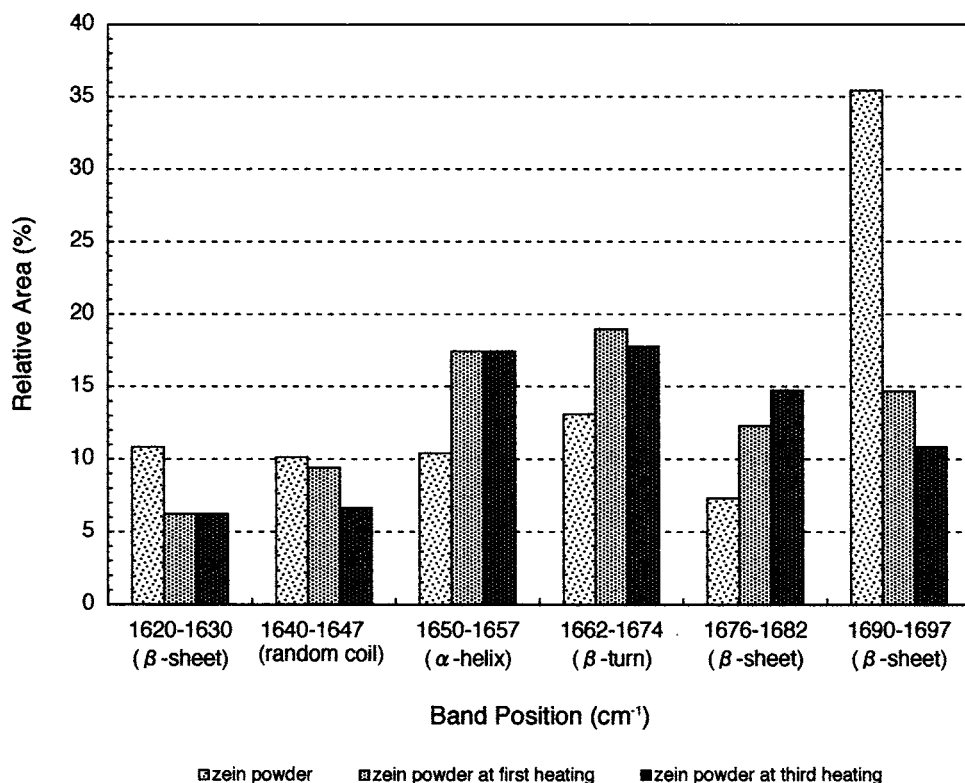


Figure 3. Positions and relative areas of the bands fitted to the Fourier-deconvoluted spectra of zein powder in the dry state. Each band was assigned to the component of the secondary structure according to the previous data.

reorientation of zein and LAE molecules. The interaction of zein and LAE, which was newly formed by the first heating, might be very strong and stable, so that further heating could not induce a new molecular orientation.

Influence of Heat Treatment on Secondary Structure of Zein in Dry State. The effects of heating on the conformation of zein were investigated by FT-IR spectrophotometry (**Figure 3**). The FT-IR spectrum of heated zein powder in the dry state by DSC was measured. The contents of the secondary structural components of the zein were assigned and calculated from the FT-IR spectrum as described in our previous paper (*1*). The content of β -sheet near 1690 cm^{-1} , which involved antiparallel intermolecular hydrogen-bonded β -sheets belonging to protein aggregates, was high in comparison with the α -helix content of zein without heating. These results are consistent with those of previous studies (*1*). The secondary structure of zein in the powder state has been characterized by a high content of the intermolecular hydrogen-bonded β -sheet in common with various proteins (*29*). In such low water environments, zein molecules may be in close contact with neighboring molecules by the formation of intermolecular hydrogen-bonded β -sheets.

The changes in the structural components of zein in a powder system induced by the first and third heatings were compared (**Figure 3**). The α -helix (at $1650\text{--}1657\text{ cm}^{-1}$), β -turn (at $1662\text{--}1674\text{ cm}^{-1}$), and β -sheet (at $1676\text{--}1682\text{ cm}^{-1}$) bands of zein increased due to heat treatments. On the other hand, the contents of the intermolecular hydrogen-bonded β -sheets (at $1690\text{--}1691\text{ cm}^{-1}$), the β -sheet (at $1620\text{--}1630\text{ cm}^{-1}$), and random coil (at $1640\text{--}1647\text{ cm}^{-1}$) decreased with heating. A similar phenomenon, that is, an increase in the α -helix concomitant with a decrease in the intermolecular β -sheets, had also been observed when zein powder was stored in the presence of a high water activity (*1*). Therefore, it was shown that both heating and wetting of zein induced a similar rearrangement of the secondary structure. Under high water activity, the T_g is possibly decreased

around room temperature, so that the reorientation of the zein molecules in the rubbery state may occur.

For the zein-LAE (4:1, w/w) dry powder heated by DSC, an FT-IR measurement was performed, and the contents of the secondary structural components of the zein were calculated from the FT-IR spectrum. The effects of heating on the structural components of zein in a zein-LAE mixed powder system are shown in **Figure 4**. In the absence of heating, only small changes in the secondary structure of zein were induced by the addition of LAE (compare the results of **Figures 3** and **4**). The effects of heating on the secondary structure of zein were similar irrespective of the presence or absence of LAE, but increases in α -helix (at $1651\text{--}1658\text{ cm}^{-1}$), β -turn (at $1661\text{--}1669\text{ cm}^{-1}$), and β -sheet (at $1679\text{--}1683\text{ cm}^{-1}$) and a decrease in the intermolecular hydrogen-bonded β -sheets (at $1691\text{--}1696\text{ cm}^{-1}$) on heating were more drastic for a zein-LAE powder system. These results suggest that heat treatments are more effective for modifying the secondary structure of zein when LAE is present in the system.

The effects of heating on the mobility of lipid molecules in the presence and in the absence of the zein are shown (**Figure 5**). In the FT-IR spectrum of nonheated LAE (**Figure 5a**), the bands near 3010 , 2960 , 2930 , 2850 , and 1730 cm^{-1} , which could be assigned to the bands of the asymmetric CH_3 stretching, the antisymmetric CH_2 stretching, the symmetric CH_2 stretching, and the ester $\text{C}=\text{O}$ double-bond stretching, respectively, were observed. These bands appeared in the FT-IR spectrum of heated LAE (**Figure 5b**), indicating that the LAE molecule is scarcely damaged by heating. For the zein-LAE (4:1, w/w) mixed powder without heating (**Figure 5c**), these bands were also observed. However, the heating of the mixed powder induced a decrease in or the disappearance of these peaks, especially the peaks near 3010 and 1730 cm^{-1} (**Figure 5d**). Our previous results showed that these two peaks also disappeared when zein-LAE powder was stored under high water activity (*1*).

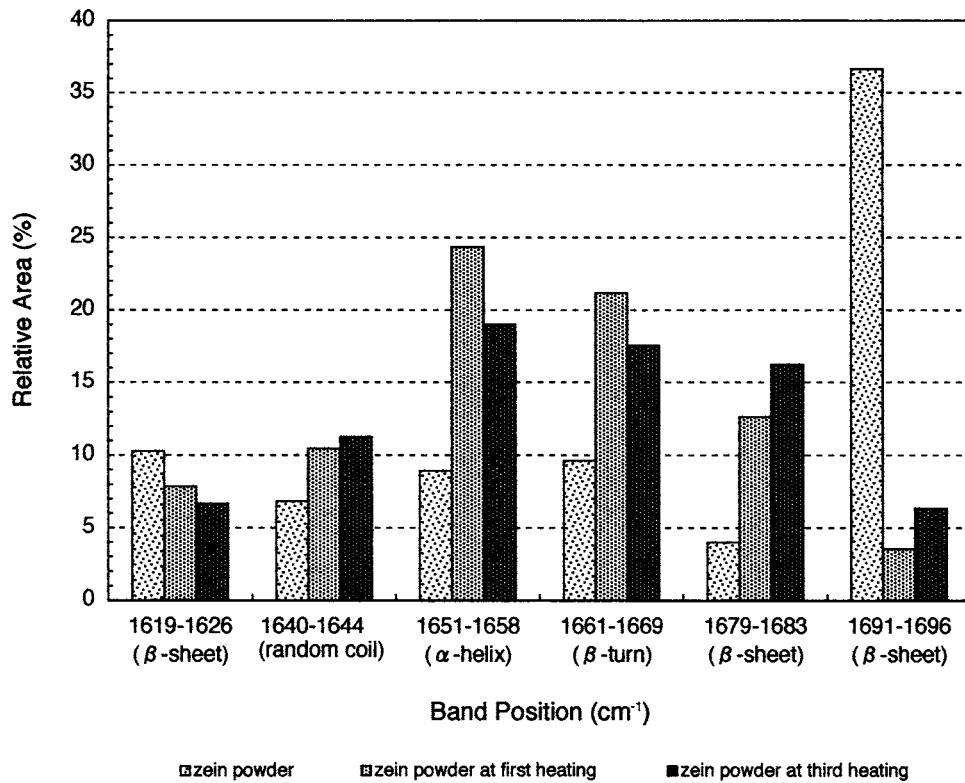


Figure 4. Positions and relative areas of the bands fitted to the Fourier-deconvoluted spectra of zein-LAE (4:1) powder in the dry state. Each band was assigned to the component of the secondary structure according to the previous data.

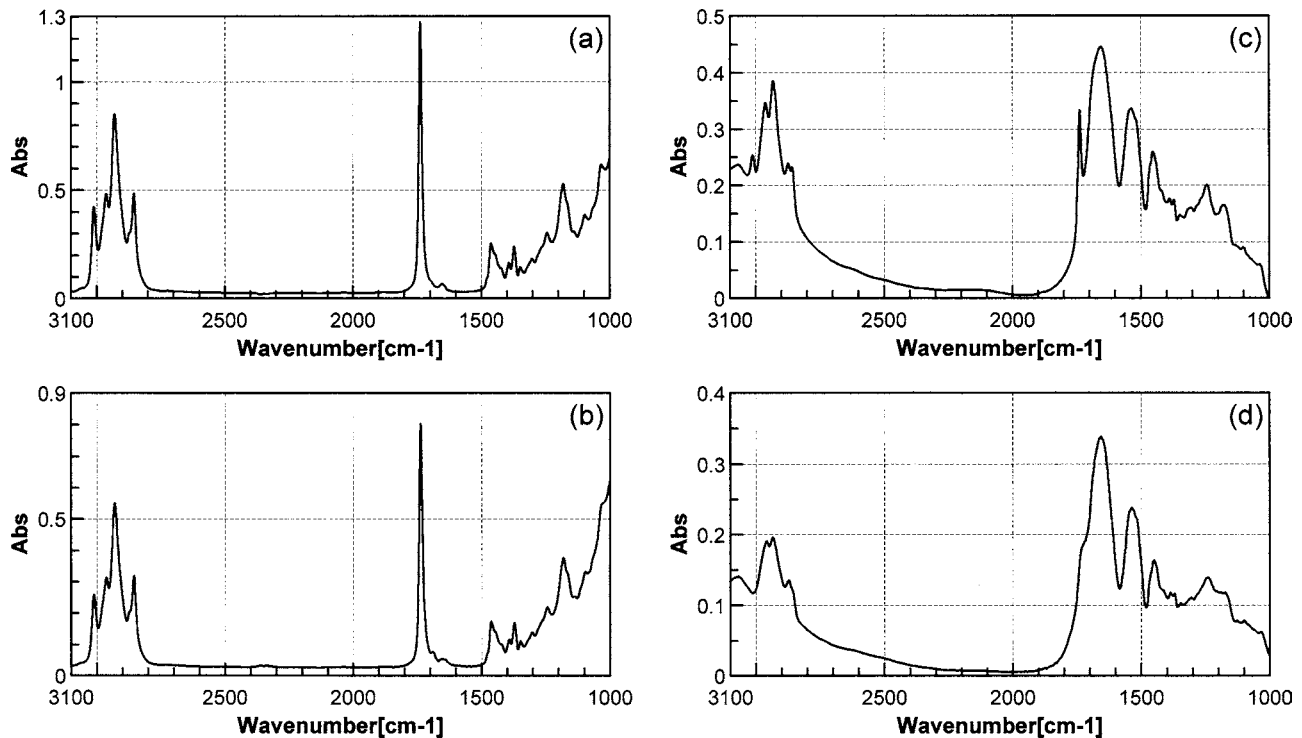


Figure 5. FT-IR spectra of LAE and zein-LAE (4:1) powder in the dry state: (a) LAE (- heating); (b) LAE (+ heating); (c) zein-LAE powder (- heating); (d) zein-LAE powder (+ heating). Abs, absorbance.

We speculate that this change in the FT-IR pattern is due to the peak shifts to the overlapping region, and such peak shifts are indicative of the strong interaction of the zein and LAE, that is, incorporation of LAE molecules into the zein matrix by the dissociation of zein aggregates (*1*).

Influence of Heating on the Antioxidant Effect of Zein in the Dry Powder System. As mentioned in the Introduction,

the antioxidative effect of zein was not observed when the powdery lipids were stored with a silica gel. The results from DSC and FT-IR spectroscopy suggest the possibility that heating affects the antioxidant activity of dry zein powder by modifying the interaction of zein and LAE. **Figure 6** shows the time course of hydroperoxide formation of LAE and zein-LAE (9:1, w/w) mixed powder during storage at 40 °C in the dry state. The

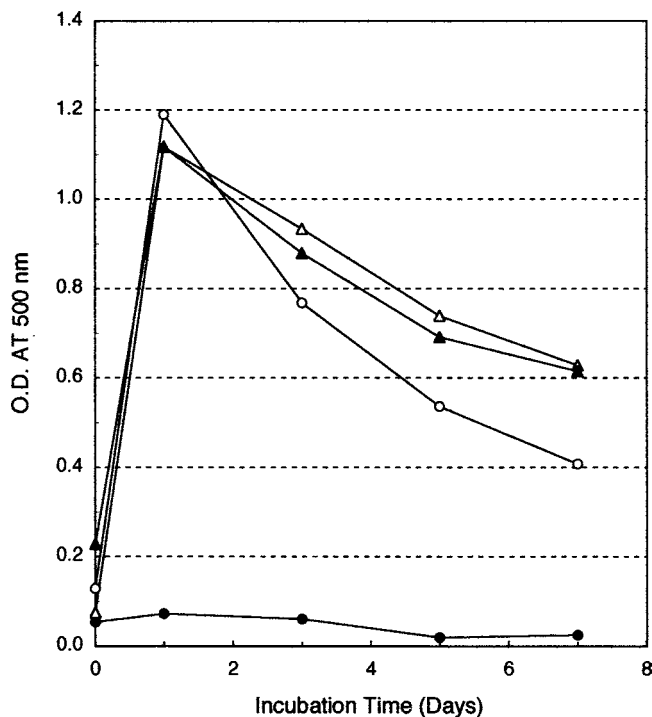


Figure 6. Time-dependent changes of POV in LAE and zein-LAE (9:1) powder stored in the dry state at 40 °C: (△) LAE (- heating); (○) zein-LAE powder (- heating); (▲) LAE (+ heating); (●) zein-LAE powder (+ heating).

samples were not heated or heated on a heating block as mentioned under Materials and Methods. In the case of nonheated and heated LAE, the POV was drastically elevated 1 day after the beginning of the experiment. A similar rise in the POV was observed for nonheated zein-LAE mixed powder. On the other hand, for heated zein-LAE powder, the level of POV was very low over 7 days.

GC measurement confirms no breakdown of LAE in the mixed powder after heat treatment (data not shown). These results, therefore, indicate that heating of zein-LAE mixed powder is effective in elevating the antioxidant activity of zein in the dry state. It is known that the oxidation reaction rate decreases with the reduction of the water activity, reaching a minimum and increasing in low water activity values (30). In our case, the water content of the sample powders was controlled to be very low (only 3%) during measuring. Such restricted water content can explain the low antioxidative effect of zein without heating, but not the excellent stability of heated zein-LAE powder. Probably, the modification of zein-LAE interaction is responsible for the improvement of antioxidative activity of zein by heating.

As described in the Introduction, it was previously shown that zein has antioxidative activity in a powdery system under high water activity conditions, but not under low water activity conditions, which was disadvantageous for the commercialization of zein-lipid powder as a food ingredient. Our results suggest that heat treatment of zein in the dry state gives rise to the beneficial effects with respect to the antioxidative activity of the zein powder. Instead of increasing the water activity, the elevation of temperature might enable the rearrangement of the secondary structure and the strong interaction of zein with lipid molecules, thereby improving the antioxidative activity of zein in the powdery system. It is likely that the glass transition behavior plays important roles in all of these processes. If we make good use of the glass transition behavior, it may be

possible to improve the functional properties of various food proteins by heating in the powdery state as suggested by previous studies (5-13).

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