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Effects of Enzymatic Deamidation by Protein-Glutaminase on Structure and Functional Properties of α-Zein

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The performance of novel protein-glutaminase (PG) purified from *Chryseobacterium proteolyticum* on α-zein was investigated. Highly insoluble α-zein was able to be deamidated to the extent of deamidation degree 62% by using 50 mM potassium phosphate (pH 8) containing 11.7% ethanol, at 40 °C for 137 h. Analysis by sodium dodecyl sulfate polyacrylamide−gel electrophoresis showed that deamidated and non-deamidated zeins have different mobilities. Results of circular dichroism spectra revealed the decline in α-helix contents of α-zein by deamidation. Besides, Fourier transform infrared spectroscopy analysis demonstrated alterations in the secondary structure of α-zein by deamidation. The assignment of the amide I region showed a remarkable decrease in antiparallel intermolecular β-sheets (around 1690 cm^{-1}) as an indication of the weakening aggregation ability of the deamidated molecules. Solubility and emulsification properties of α-zein, particularly at pH 7, were remarkably improved after the deamidation by PG. Gas chromatography and peroxide value studies pointed out that deamidated α-zein in powder form exhibited an inferior antioxidative property as compared with the non-deamidated one.

KEYWORDS: α-Zein; protein-glutaminase; deamidation; secondary structure; solubility; emulsification; antioxidation

INTRODUCTION

Although maize ranks at the top in cereal harvests (*1*), its proteins, which are obtained numerously as byproducts of starch and oil production from maize seeds (*2*), are not utilized as human foods. Reasons for the hindrance of the utilization of maize proteins as a food ingredient are related to the poor functional properties of the major components of maize proteins, namely, zeins (*3*).

Zein has a high concentration of nonpolar amino acids such as leucine, proline, and alanine (*2*), but it is also characterized by very low concentrations of lysine and tryptophan, which accounts for its low nutritional quality (*4*). Another characteristic is that almost all of the β- and γ-carboxyl residues of glutamic and aspartic acids are amidated to be glutamine (21%) and asparagine (5%), respectively (*5*), with their polar terminal amino group engaging in hydrogen bonding (*3*). These characteristics are responsible for the low solubility of zeins in aqueous solutions. To expand the usage of zein in human food products, solubility in water plays a vital role because protein generally has to be in a solution or in a fine suspension to exert other desired properties (*6*). Besides, maize zein in powder form has also been reported to exhibit an extraordinarily strong antioxidant effect at $A_w = 0.9$ (*7*). This prominent effect was proposed to be due to the binding or physical shielding by zein. In fact, zein has also been utilized as an encapsulant for fish oil (*8*).

It has been believed that deamidation is useful to improve the solubility and other functional properties of cereal prolamins including zeins (*9*, *10*). Chemical methods such as heat treatments in acidic conditions and/or with anions were generally attempted for the deamidation of food proteins (*11*), but the side reactions, for example, peptide bond scission and degradation of other amino acid residues, were inevitable (*12*), consequently causing damage to the functional properties of food proteins.

Therefore, deamidation by enzymatic reaction is preferable for food systems, because the modification is selective and safe (*13*).

Recently, a new enzyme from a bacterium (*Chryseobacterium proteolyticum*), which catalyzes the deamidation of proteins, was found (*14*). The enzyme was named protein-glutaminase (PG), because it attacks only glutamine residues in short peptides or proteins, but not asparaginyl residues or free glutamines (*15*). This enzyme is different from peptidoglutaminase, which were previously isolated from *Bacillus circulans* (*16*), with respect to the activity toward proteins (*17*). Peptidoglutaminase was known to catalyze only the deamidation of glutamine in short

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peptide chains (*17*), but PG preferred proteins such as caseins and whey proteins to short peptides as substrates (*15*).

Due to the high contents of glutamine residues in zeins (*18*), PG could be a useful tool for improving the functional properties of zeins via deamidation reactions. In the present paper, we investigated the efficiency of PG to catalyze the deamidation of α-zein, which is the main group of maize protein (75−85% of the total zein fraction) (*2*, *19*). The conformational changes of this protein after deamidation were examined. Some functional properties of enzymatically modified α-zein were also characterized.

MATERIALS AND METHODS

Materials. PG derived from *C. proteolyticum* strain 9670 was purified according to the method of Yamaguchi et al. (*15*). The purified enzyme was judged to be homogeneous by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) (*15*). Specific activity was 160 units/mL. The standard method for PG assay using benzyloxylcarbonyl (Cbz)-Gln-Gly (Peptide Laboratory, Osaka, Japan) as a substrate and the definition of the enzyme unit were described previously (*14*). The α-zein from maize (catalog no. 368-17) was purchased from Nacalai Tesque (Kyoto, Japan). Other chemicals were purchased from Wako Chemical (Osaka, Japan), Nacalai Tesque, and Sigma Chemical Co. (St. Louis, MO) as analytical reagent grade.

Deamidation of α-Zein by PG. Deamidation of α-zein was carried out in 50 mM potassium phosphate (KP_i) buffer (pH 8.0) containing 11.7% ethanol, 10 mg/mL α-zein, and 0.13 unit/mL PG. Such ethanol concentration was determined by the preliminary experiments based on the efficiency of enzymatic reaction, that is, a compromise between ethanol-induced damage of PG and zein solubility. The enzymatic reaction was conducted at 40 °C for various periods of time (0−137 h). A control sample for α-zein was treated in the same condition without PG for 20 h. Amounts of ammonia released from deamidated glutamine residues were determined according to a method described in the following section. Degree of deamidation (DD) was expressed as the ratio of the amount of released ammonia by PG reaction and the total glutamine residues of the proteins. The number of total glutamine residues was assessed by measuring the released ammonia when the proteins were treated with 3 N sulfuric acid. The resultant solution and precipitation were dialyzed toward 0.1 M acetic acid and lyophilized.

Determination of Ammonia. After incubation with the enzyme, aliquots of the reaction mixture (200 μL) were placed in microcentrifuge tubes, followed by the addition of 800 μL of protein removing reagent (sodium tungstate containing phosphate) (reagent in Ammonia Test Kit from Wako Chemical) to stop the enzymatic reaction. After the filled tubes were centrifuged at 10 °C at 12000 rpm for 10 min, the ammonia in the supernatant (400 μL) was determined by using an Ammonia Test Kit according to the manufacturer's instruction (Wako Chemical).

SDS-PAGE was performed on 12.5% slab gel for α-zeins by using the procedure of Laemmli (*20*). The gels were stained with Coomassie brilliant blue R250 (Fluka Chemika, Buchs, Switzerland).

Measurement of Circular Dichroism (CD). Two milligrams of lyophilized non-deamidated and deamidated α-zein was dissolved in 4 mL of 70% ethanol, respectively. The measurement of far-UV CD was conducted at room temperature using a Jasco J-720 spectropolarimeter (Jasco Co., Tokyo, Japan) equipped with a data processor (model DP-501). The far-UV spectra were measured using a cuvette with a 0.1 cm light path over the wavelength range of 185−250 nm. The CD data were expressed as mean residue ellipticity (degree cm^2/dmol).

Measurement of Fourier Transform Infrared Spectroscopy (FT-IR). KBr pellets were prepared by admixing ∼1 mg of α-zein samples, respectively, with ∼100 mg of spectroscopy-grade KBr and pressing the mixture into a 3-mm-diameter disk with a minipress (Jasco MP-1). Infrared spectra of each pellet were recorded in a Jasco FT/IR-480 Plus (Jasco Co., Tokyo, Japan) equipped with a deuterated L-alanine triglycine sulfate (DLATGS) detector. Each sample was held in an IR cell, and the scans were performed at 4 cm^{-1} resolution with 50 scans. Deconvolution and curve fitting of the infrared spectra were performed using Jasco Spectra Manager software (Window 95/NT version) and according to the method of Kauppinen et al. (*21*). The band assignment to secondary structural components was carried out for amide region I (1600−1700 cm^{-1}).

Determination of Solubility. The lyophilized α-zein samples (1 mg) in microcentrifuge tubes (1.5 mL) were dissolved or dispersed, respectively, in 1 mL of buffer solutions of various pH values (10 mM acetate buffer for pH 3 and 5, 10 mM phosphate buffer for pH 7). Each solution was kept overnight at 20 °C and vortexed. After vortexing vigorously, the solutions were centrifuged at 3000 rpm for 10 min at 10 °C, and the soluble fractions were collected. Then, the protein content in the supernatants was determined according to the method of Lowry et al. (*22*).

Evaluation of Emulsifying Properties. The lyophilized α-zein samples were dissolved or dispersed respectively in buffer solutions with different pH values (10 mM acetate buffer for pH 3 and 5, 10 mM phosphate buffer for pH 7). Then, corn oil was mixed into each of the solutions or dispersions; the final oil and protein concentrations were 10 and 0.09% (w/w), respectively. The mixtures were then homogenized with Physcotron NS-50 equipment (Nichion Ltd., Chiba, Japan) at 22000 rpm for 3 min at room temperature and sonicated with an Ultrasonic Homogenizer US-150 (Nissei, Tokyo, Japan) at 100 μA for 2 min at room temperature. The particle size distribution of the resultant emulsions was measured using a Horiba LA500 laser diffraction particle size analyzer (Horiba Ltd., Kyoto, Japan) after the appearances of the emulsions had been compared. For determining emulsifying stability, the emulsions were kept overnight at room temperature in test tubes (6 × 50 mm) to observe visually the stability of liquid emulsions to creaming.

Evaluation of Antioxidative Properties of Deamidated α-Zein. Eicosapentanoic acid ethyl esters (EPE) were freed from peroxides by passing them through a Sep-Pak Vac 12 cm^3 (2 g) Florisil cartridge. Peroxide-free EPE were dissolved in chloroform (5% w/v) and stored at −4 °C.

All α-zeins were washed with ethyl acetate to remove antioxidants before preparation of the powder system. A mixture of EPE and stearic acid ethyl ester (SAE) (7:3, w/w) dissolved in hexane was added to α-zein to a ratio of 1:9 by weight. SAE was the internal standard to estimate the extraction efficiency of the EPE. Sample powders were subdivided into small portions and stored in a humidity-controlled plastic box at 40 °C for different intervals (0−7 days). Water acticity (A_w) was adjusted to 0.9 with 22% (v/v) sulfuric acid (*23*). Samples were taken from the box at stated intervals for gas chromatographic (GC) analysis (*24*) and peroxide value (POV) measurement. The POV was measured by using the ferric thiocyanate method as described by Iwami et al. (*23*). For data with good consistency, two replicates were taken for the data analysis. As for the analysis work with more varied data, three replicates were used. By using Microsoft Excel version 2000, all of the values were averaged and mean values were reported. Standard deviation for three replicate data was also shown.

RESULTS AND DISCUSSION

Deamidation of α-Zein by PG. The action of PG toward α-zeins was investigated with the aforementioned conditions. **Figure 1** shows the DD of α-zein after PG reaction. DD increased with reaction time, reaching 62% after 137 h. PG was added twice (0 and 44 h) with 13 units for 1 g of zein substrate, respectively. Without the second PG addition, the reaction would have eventually reached a plateau. **Figure 2** shows the appearance of the deamidated sample solutions of α-zein. For all of the reaction times (44, 89, and 137 h), solution and precipitate parts were noticeably separated. When reaction time was extended from 44 to 137 h, the turbidity of the solution increased slowly while the amount of the precipitate decreased.

This kind of reaction mode is possibly due to the strong hydrophobic interaction among α-zein molecules. In the early stage of PG reaction, most of the glutamine residues may be embedded in the aggregates of zein molecules induced by

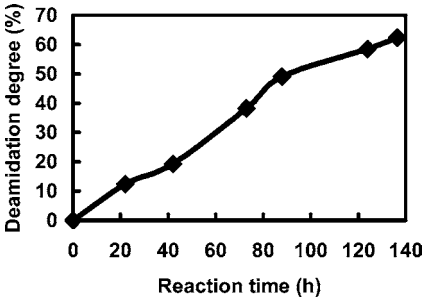

Figure 1. Time-dependent increase of DD of α-zein induced by PG reaction. α-Zein (10 mg) was dispersed in phosphate buffer (50 mM, pH 8.0) containing 11.7% ethanol. PG (0.13 unit/mL) was added twice at 0 and 44 h, and the incubation was carried out at 40 °C for 137 h.

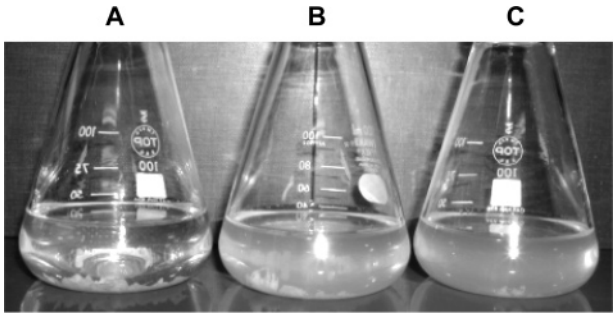

Figure 2. Appearance of α-zein dispersions incubated with PG. Reaction conditions were the same as those of **Figure 1**. Reaction times: (**A**) 44 h; (**B**) 89 h; (**C**) 137 h.

hydrophobic interaction. However, with the slow dissociation of such aggregates by PG reaction, the deamidation sites even on the buried hydrophobic regions are accessible; that is, the PG reaction in the initial step can activate the further modifying action by PG. The newly formed carboxyl group may enhance the unfolding of the protein via electrostatic repulsion, thereby increasing the possibility of modification of glutamine residues by the enzyme. Yamaguchi et al. (*15*) reported that the specific activity rate of zein by PG was very low (0.66 μmol·min^{-1}·mg^{-1}). However, the results of **Figures 1** and **2** hint at the possibility of the modification of α-zein by PG to large extents, if suitable reaction conditions are selected (usage of alcohol and addition pattern of enzyme), even though longer reaction times are required.

SDS-PAGE. The solution and precipitate parts of the zeins were separately applied to the SDS-PAGE to compare the band patterns of the deamidated samples and the non-deamidated substrates. In **Figure 3**, it is shown that the band patterns of the precipitate and solution parts of the deamidated α-zein were dissimilar. Despite reaction times, the band pattern of the precipitate part revealed exactly the same shifting mode with the non-deamidated α-zein. In contrast, the bands of the solution parts of deamidated α-zeins shifted to the upper part of the slab gel. These kinds of shifting phenomena were previously reported in the acid deamidation on wheat gliadins (*25*) and enzymatic modification by protein-deamidating enzyme on casein (*14*), suggesting the common feature that deamidated proteins are shifted toward the upper region of the slab gel. On the basis of this speculation, it is thought that no deamidation occurred for the precipitation part of the α-zeins after PG reaction, whereas soluble form α-zeins were deamidated. We confirmed the non-deamidation of precipitated α-zein (DD = 0.3%) and deamidation of soluble α-zein (DD = 64%) by determining the remaining glutamine contents of both α-zeins as released ammonia amounts after 3 N sulfuric acid treatment.

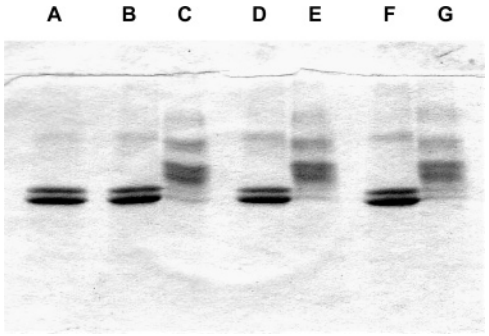

Figure 3. SDS-PAGE patterns of α-zein treated by PG: (**A**) α-zein incubated without PG for 20 h; (**B**) precipitate in α-zein dispersion incubated with PG for 44 h; (**C**) solution part of α-zein dispersion incubated with PG for 44 h; (**D**) precipitate in α-zein dispersion incubated with PG for 89 h; (**E**) solution part of α-zein dispersion incubated with PG for 89 h; (**F**) precipitate in α-zein dispersion incubated with PG for 137 h; (**G**) solution part of α-zein dispersion incubated with PG for 137 h.

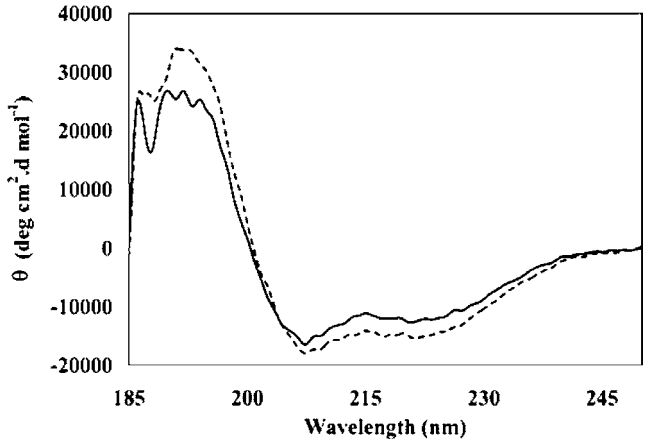

Figure 4. Far-UV CD spectra of α-zein. α-Zein was solubilized in 70% ethanol. The protein concentration was 0.05%. The measurement were carried out at 20 °C. The spectra of nonmodified α-zein and deamidated α-zein for 137 h (DD = 64%) are shown by dotted and solid lines, respectively.

The up-shifting of the protein bands was an indication of a slow migration due to the deamidation reaction. This slow migration reaction may be explained by two possible reasons. First, the deamidation of glutamine residues into glutamic acid increased the negative charge of the protein polypeptide. This leads to a weaker binding of SDS to the protein molecules in the slab gel because of strong electrostatic repulsion, which may weaken the denaturation effects of SDS to form the compact rod complex for migrating efficiently in the gel. Second, the increase of carboxyl groups by deamidation leads to the amplification of electrostatic repulsion within molecules, thereby producing molecules with an expanded shape, which is a disadvantage to the migration through gel matrix.

Secondary Structure Changes in CD Spectra and FT-IR of α-Zein by Deamidation. On the basis of the observation of the previous SDS-PAGE section, we decided to apply the solution part of the sample deamidated for 137 h to compare with non-deamidated control sample for the studies in structural changes and functional properties. The investigation of the effects of deamidation on the conformation of α-zein in 70% ethanol was conducted using far-UV CD spectra. The spectra of nonmodified and deamidated molecules are shown in **Figure 4**. In this figure, the spectra show two negative maxima around 207 and 221 nm. This kind of α-zein spectrum was very similar

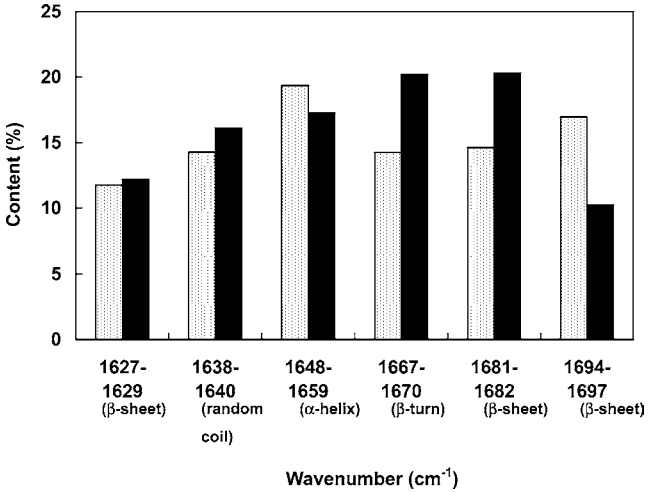

Figure 5. Secondary structural contents of α-zein determined by FT-IR spectroscopy: (dotted bars) nonmodified α-zein; (black bars) deamidated zein (reaction time, 137 h; DD = 64%). FT-IR measurements were conducted for the powdery form of α-zein. Each band was assigned to the component of the secondary structure according to the previous data. The positions and contents of the bands at the region from 1620 to 1700 cm^{-1} are shown.

to the one published earlier by Tatham et al. (*26*), and the two peaks were thought to reflect α-helical structure. A significant diminution of the two negative maxima was observed when deamidation was applied. Deconvolution of the spectra assigned non-deamidated and deamidated α-helix contents of 46 and 36%, respectively. The α-helix content of nonmodified α-zein was in agreement with the 49% α-helix of Tatham et al. (*26*). The analysis of CD spectra also pointed to an increase of β-sheet in α-zein from 24% (non-deamidated) to 32% (deamidated).

To study the secondary structural changes of α-zein in the powder form by deamidation, FT-IR analysis was applied. The amide I region (1600−1700 cm^{-1}) in spectra was analyzed according to the methods decribed under Materials and Methods, and the results of band assignment are shown in **Figure 5**. The content of the bands around 1690 cm^{-1}, which was assigned to be antiparallel intermolecular β-sheets, was diminished ∼7% by PG deamidation. PG reaction also slightly decreased the α-helix content by 2%. In contrast, the contents of β-turn (around 1670 cm^{-1}) and random coil (around 1640 cm^{-1}) exhibited 6 and 2% increments, respectively.

Three possible models of α-zeins have been proposed by Argos et al. (*27*), Garratt et al. (*28*), and Matsushima et al. (*29*), respectively. The first two papers (*27*, *28*) suggested relatively globular forms, although they differ in the packing fashion of α-helical tandem repeat units. However, from small-angle X-ray scattering of α-zein in 70% ethanol, the latter (*29*) proposed a linear stacking of antiparallel helices of tandem repeat in the direction perpendicular to the helical axis to form an elongated prism-like shape with an appoximate axial ratio of 6:1. This model of Matsushima et al. (*29*) is appropriate to explain the CD spectra results in the present paper in which the measurement was conducted in 70% ethanol. The high α-helix content of native α-zein was aligned linearly in tandem repeat units, which were joined firmly by the glutamine-rich turns. By PG reaction, the glutamines were deamidated into negatively charged glutamic residues. This may strengthen the electrostatic repulsion in turns, which changes the packing stacks of α-helix domain, hence destabilizing the α-helix formation.

According to the results of FT-IR on the secondary structure of α-zein, there were also obvious changes in the conformation

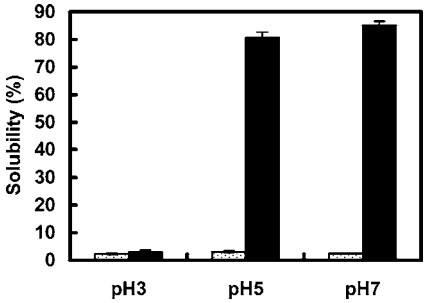

Figure 6. Solubility of α-zein in various pH conditions: (dotted bars) nonmodified α-zein; (black bars) deamidated zein (reaction time, 137 h; DD = 64%). α-Zein was dispersed in acetate buffer (10 mM, pH 3 and 5) and phosphate buffer (10 mM, pH 7), respectively, at 20 °C (protein concentration = 0.1 wt %).

of this protein by PG deamidation. Corresponding to the reduction in intermolecular β-sheets, it was apparent that by deamidation, the aggregation ability of the molecules in the powder was weakened. The increase in β-turn and random coil suggested the changing of deamidated α-zein into a more flexible or extended form.

Determination of α-Zein Solubility. The solubility of α-zein samples in different pH conditions is shown in **Figure 6**. The solubility test was carried out in acidic conditions (pH 3 and 5) and neutral condition (pH 7). From the figure, it is obvious that for α-zein in non-deamidated state, the solubility was very low in all cases of pH conditions. However, after deamidation by PG, even though there was not much change for the pH 3 condition, the solubilities at pH 5 and pH 7 were increased remarkably to 81 and 85%, respectively.

Panyam and Kilara (*30*) stated in a review that the most dramatic improvement of functionality as a result of enzymatic modification is the enhancement in solubility, depending on the type of enzyme used and the conditions of hydrolysis. Due to the high proportion of nonpolar amino acid residues and deficiency in basic and acidic amino acids, zein is soluble in water only with the presence of alcohol, high concentrations of urea, high concentrations of alkali (pH 11), or anionic detergents (*2*). Therefore, in this research, the low solubility for non-deamidated α-zein in either pH condition was as predicted.

The remarkable solubility at pH 5 and 7 for deamidated α-zein in this study provides a promising improvement for enabling a broader range of usage in food-processing industries. The result was in agreement with a study on zein whereby the zeins deamidated by chemical method improved their solubility from pH 6 to 11 compared to the native zein (*3*). The conversion of amide groups in zein to acid groups was responsible for the substantial increase in the ability to bind water, besides lowering the isoelectric point of zein (pH 6−7). As a result, zein became soluble effortlessly in weak acidic or neutral food systems. No change in solubility at pH 3 after deamidation may be due to the approaching of isoelectric point of α-zein to acidic region.

Evaluation of α-Zein Emulsifying Properties. After dispersion or solubilization the non-deamidated and deamidated α-zeins into different pH solutions, the samples were blended with corn oil to prepare emulsions for emulsifying tests. In **Figure 7A**, the bars show the mean droplet diameter (*31*) of the freshly blended emulsions. In the case of non-deamidated α-zeins, the mean droplet diameters of pH 5 and 7 were >100 μm. After the application of deamidation reaction, the emulsification at these two pH values was improved, especially in the case of pH 7, whereby the diameter was reduced to <4 μm. The diameter at pH 5 was reduced below 100 μm. For pH 3,

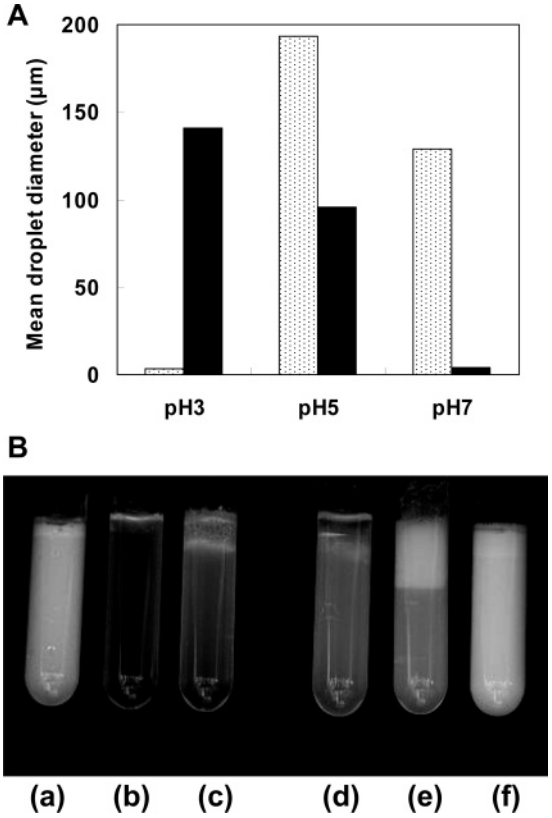

Figure 7. Emulsification properties of α-zein in various pH conditions: (**A**) mean droplet diameter of emulsions [(dotted bars) nonmodified α-zein; (black bars) deamidated zein (reaction time, 137 h; DD = 64%)]; (**B**) appearance of emulsions after overnight storage at 20 °C [(**a**) nonmodified α-zein at pH 3; (**b**) nonmodified α-zein at pH 5; (**c**) nonmodified α-zein at pH 7; (**d**) deamidated α-zein at pH 3; (**e**) deamidated α-zein at pH 5; (**f**) deamidated α-zein at pH 7]. α-Zein was dispersed in acetate buffer (10 mM, pH 3 and 5) and phosphate buffer (10 mM, pH 7), respectively, and the resultant dispersions were mixed with corn oil at 20 °C. The protein concentration and oil amount were 0.09 and 10 wt %, respectively. DD of deamidated α-zein was 64%.

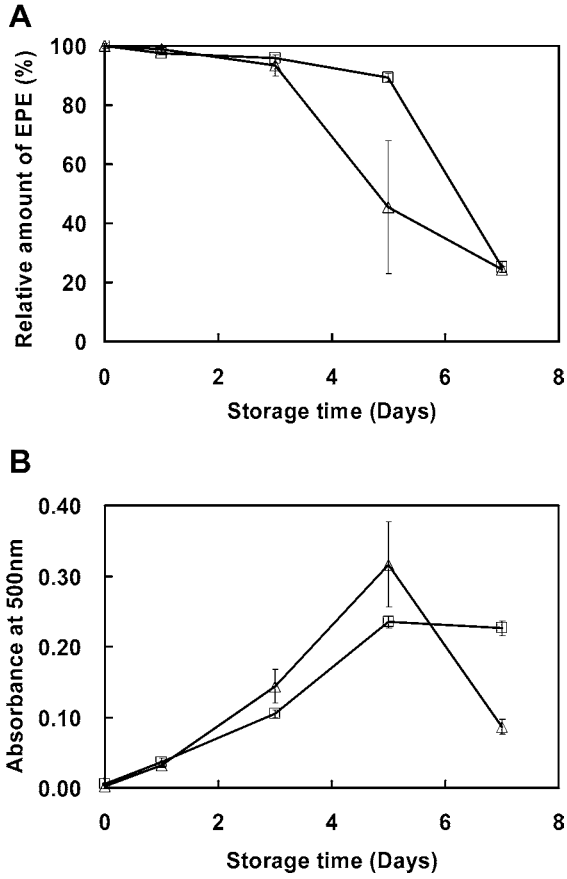

Figure 8. Antioxidative effects of α-zein in a powder model system: (**A**) relative amount of EPE content measured by GC; (**B**) POV measured by ferric thiocyanate method for three series of samples extracted at appropriate intervals; (□) nonmodified α-zein; (△) deamidated α-zein (reaction time, 137 h; DD = 64%). Powder samples consisting of α-zein and EPE–SAE mixtures at a 9:1 weight ratio were incubated at 40 °C (A_w = 0.9) under aerobic conditions.

the non-deamidated zein showed smaller particle sizes (<4 μm) as compared to its deamidated cohort. From these results, it might be concluded that the non-deamidated zein produced a better quality of emulsion at pH 3. However, a different conclusion might be drawn by the observation of the appearance of the emulsions after they had been kept at room temperature overnight (**Figure 7B**). This figure illustrates that corn oil was only able to "partially" be dispersed into the non-deamidated α-zein solutions in either pH condition, including pH 3. Excessive oil eventually oiled off on the top of the samples. Hence, the emulsification abilities of these non-deamidated solution systems were thought to be low. For the deamidated zeins, although the α-zein−corn oil system at pH 3 still demonstrated poor emulsifying properties, it was clear that at pH 7 excellent emulsion conditions were achieved; that is, neither oiling off nor creaming was observed. On the other hand, at pH 5, the creaming occurred clearly for the emulsion stabilized by the deamidated α-zein, although the emulsion stability was improved as compared to the case of using non-deamidated α-zein.

One reason for the outstanding emulsification properties of deamidated α-zein at pH 7 can be explained from the viewpoint of solubility demonstrated in **Figure 6**. Because α-zein is rich in hydrophobic amino acid residues, introducing a negative charge by new glutamic acids provided α-zein molecules with an amphiphilic nature as well as elevated solubility. The induction of these amphiphilic properties by deamidation was able to enhance the interaction between proteins and lipids. Indeed, by virtue of this amphiphilic nature, some food proteins are capable of orienting at the polar−nonpolar interface and are good emulsifiers (*32*). However, in the case of pH 5, although the solubility was greatly enhanced by deamidation, the emulsion stability was somehow inferior. In fact, Hamada (*12*) stated that solubility is not a prerequisite for increased emulsion stability. It is thought that the electrostatic repulsive force and steric hindrance occurring among adsorbed protein layers at oil droplet surfaces are more crucial. For deamidated α-zein covering oil droplet surfaces, the introduced charge may not be enough to produce such an electrostatic repulsive force and/or the conformation of the molecules may be too compact to cause the steric hindrance at pH 5.

Antioxidant Properties of Deamidated α-Zein. This section describes the antioxidant properties of α-zein in powder form before and after deamidation by PG. The study was carried out using GC analysis to examine EPE content variations with time (**Figure 8A**) and using the POV test to evaluate the peroxides in EPE (**Figure 8B**). In **Figure 8A**, the relative amount of nonoxidized EPE was plotted against time of storage. According to **Figure 8A**, the decrease in the amount of nonoxidized EPE begins in both control and deamidated α-zein after 3 days of

storage, reaching a significant decrease after 7 days of storage, with such a decrease being more dramatic and considerably faster for the deamidated α-zein. Corresponding to the GC result, as shown in **Figure 8B**, the POV of the deamidated α-zein system exhibited a faster and higher increase as compared to the non-deamidated sample. In short, the antioxidant ability of the α-zein powder system to suppress EPE oxidation became inferior by the deamidation.

Some research has been conducted with regard to the antioxidant activity of prolamins including wheat gliadin (*23*, *24*), barley hordein (*24*, *33*), and maize zein (*7*, *24*, *34*−*37*). In the present paper, poor antioxidant properties for deamidated zein as compared to native zein were detected. In fact, Chiue et al. (*36*) also found that zein lost its antioxidant effect in proportion to the degree of deamidation, with a correlation coefficient of $r = -0.946$. The antioxidant effect was later reported to be closely correlated with the fatty acid-binding capacity ($r = 0.98$), but not the surface hydrophobicity (*37*). It was suggested that abundant glutamine contents of cereal prolamins play a critical role in antioxidation (*33*). Therefore, the cleavage of these amide bonds would affect the antioxidant activity to a considerable extent by modifying the conformation of α-zein (*36*).

In native zein, glutamine residues in the hinge region may stabilize the compact stacking of α-helix domains, keeping its capacity to bury unsaturated lipid in the inter- and intramolecular hydrophobic spaces (*37*). The substrate oil was embedded in the shield formed by zein to avoid outer oxygen attack (*34*). In fact, the structure of deamidated α-zein proved to be more extended or flexible by CD and FT-IR analyses, suggesting the decrease of aggregation ability of zein molecules in the powder. Such lower aggregation ability of the deamidated form may be responsible for the inferior antioxidant activity of α-zein after deamidation.

In conclusion, PG was shown to be an effective enzyme in deamidating zein, which is difficult to solubilize in aqueous solutions. The glutamine residues were successfully converted into glutamic acids without the destruction of the long-chain form. This enzymatic deamidation caused a great leap in α-zein solubility and emulsification, predominantly in the neutral condition. Thereby, extensive usage of this modified zein in food materials can be expected. However, a favorable feature of non-deamidated zein, the antioxidant property, was degenerated by this deamidation reaction, due to the partial unfolding of α-zein. In a nutshell, this successful deamidation of maize α-zein by PG promises a new approach for the enzymatic modification on other prolamin subunits.

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