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Effects of Enzymatic Deamidation by Protein-Glutaminase on Structure and Functional Properties of Wheat Gluten

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Protein-glutaminase (PG) purified from *Chryseobacterium proteolyticum* was used to investigate its deamidation effects on wheat gluten. Water-insoluble gluten was able to be deamidated to the extent of deamidation degree (DD) 72% in 200 mM sodium phosphate buffer (pH 7) at 40 °C for 30 h. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis exhibited an upper shift of gluten bands with only deamidation for 1.5−2.0 h (DD 35−45%) compared to the bands of nondeamidated gluten. Results of Fourier transform infrared analysis revealed alterations in secondary structure of gluten by PG deamidation. The assignment within amide I region showed decreases in both inter- (around 1695 cm^{-1}) and intramolecular β-sheets (around 1680 cm^{-1}) by deamidation suggesting the deterioration of the aggregation ability of gluten molecules. Solubility and emulsification properties of gluten at pH 7 were improved by deamidation, while both properties at pH 3 were deteriorated by deamidation. Enzyme-linked immunosorbent assay identified that allergenicity of deamidated gluten as compared to the nondeamidated cohorts was decreased remarkably as the deamidation time was prolonged.

KEYWORDS: Wheat gluten; protein-glutaminase; deamidation; secondary structure; solubility; emulsification; allergenicity

INTRODUCTION

Wheat, together with maize and rice, is known as one of the three important crops in the world (*1*). Processing of wheat for different foods has been practiced for centuries. The scientific study of wheat started for over 250 years ago (*2*), and a great deal of research attention has been focused on the study of its gluten proteins in the past 3 decades (*3*).

Wheat gluten proteins form a continuous viscoelastic network when flour is mixed with water to form dough (*4*). It is believed that its glutenin and gliadin fractions are responsible for the developed dough elasticity and extensibility, respectively (*5*). These proteins together represent almost 80% of the total protein in typical wheat flour (*6*).

Gluten proteins consist of various kinds of subunit polypeptides (*7*); these polypeptides commonly feature unusually high contents of glutamine, proline, and glycine (*8*, *9*). The most notable differences between these proteins are a higher content of glutamate plus glutamine in gliadin whereas glutenin is richer in proline and glycine (*10*).

While rice is frequently consumed by humans without any processing except cooking, wheat is almost always consumed after processing (*11*). Bread, pastries, pasta, and noodles are just the few examples of processed products. However, wheat gluten actually exhibits low solubility in aqueous solution (*12*). This causes limited applications of wheat gluten on various types of food, as solubility is the main characteristic of proteins selected for use in liquid foods and beverages (*12*, *13*). Furthermore, solubility is closely related to other functional properties of proteins such as foaming, emulsification, and gelling ability (*13*).

Deamidation can improve the solubility and other functional properties of food proteins by increasing the number of negative charges in the protein (*14*). It has been shown that even small levels of deamidation could result in a significant improvement of protein functional properties (*15*). Deamidation is believed to improve also the solubility of wheat gluten, since a high content of glutamine residues may cause the aggregation of the protein molecules via hydrogen bonding. Therefore, efforts have been made to change wheat protein solubility by acidic treatments (*16*) and enzymatic modifications (*17*, *18*) as well as cation-exchange resin catalysis (*19*).

Enzymatic protein modifications are becoming more desirable than chemical treatments due to their speed, mild reaction conditions, and high specificity (*20*). Transglutaminase, proteases, and peptidoglutaminases are the only enzymes reported literally for protein deamidation (*20*). These enzymes have disadvantages with respect to the application for protein deamidation. Side reactions are inevitable for transglutaminase

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and proteases, whereas the substrates of peptidoglutaminases are limited to short-size peptides.

Recently, a novel enzyme named protein-glutaminase (PG) was purified from *Chryseobacterium proteolyticum* (*21*), which is a monomer consisting 185 amino acids (*22*). It is thought to be a new type of enzyme with significant potential for the enzymatic modification of food proteins. The action of PG has been investigated for various proteins (*22*), especially α-lactalbumin (*23*) and α-zein (*24*).

Since gluten has a very high content of glutamine residues (*25*), we predicted this protein is a very suitable candidate for PG reaction. In this paper, we investigated the efficiency of PG to catalyze the deamidation of wheat gluten. The conformational changes of this protein after deamidation were observed. Alteration of some functional properties of enzymatically modified gluten was also characterized.

In addition to the functional properties, the improvement or reduction of allergenicity of wheat gluten by PG was also targeted in this study. Wheat is categorized as one of the main eight food groups that cause food allergies (*26*), involving both adult and children (*27*). It has been identified that both gliadin and glutenin are important allergens responsible for food allergy to wheat when tested with patients' sera IgE (*28*, *29*). The sequence QQQPP involving glutamine residues is believed to be the major candidate of IgE-binding epitope in gluten (*30*), and the acid-catalyzed deamidation was found to be effective for the decrease of reactivity toward patients' sera (*31*). Acid-catalyzed deamidation, however, usually causes peptide scission, which obscure the intrinsic effect of deamidation on gluten allergenicity. Therefore, in this study, PG-catalyzed deamidation was used to understand clearly the relationship between the degree of deamidation and allergenicity decrease of gluten.

MATERIALS AND METHODS

Materials. PG derived from *C. proteolyticum* strain 9670 was purified according to the method of Yamaguchi et al. (*22*). The purified enzyme was judged to be homogeneous by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) (*22*). 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 an enzyme unit was described previously (*21*). The wheat gluten was supplied by Amylum UK Ltd. (Greenwich, London, U.K.). Other chemicals were purchased from Wako Chemical (Osaka, Japan), Nacalai Tesque, and Sigma Chemical Co. (St. Louis, MO) as analytical reagent grade.

Deamidation of Wheat Gluten by PG. Deamidation of wheat gluten was carried out in 200 mM sodium phosphate buffer (pH7.0) containing 10 mg/mL wheat gluten, and 0.13 unit/mL PG. The enzymatic reaction was conducted at 40 °C for various periods of time (0−30 h). A control sample for wheat gluten was treated under the same conditions without PG for 24 h. Amounts of ammonia released from deamidated glutamine residues were determined using an ammonia test kit (Wako Chemical) according to a method described in Yong et al. (*24*). Degree of deamidation (DD) was expressed as the ratio of the amount of released ammonia by PG reaction to 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 solutions were dialyzed toward 0.1 M acetic acid and lyophilized.

Determination of Band Patterns by SDS−PAGE. SDS−PAGE was performed on a 10% slab gel for wheat gluten (30 μg on each well) by using the procedure of Laemmli (*32*). The gels were stained with Coomassie brilliant blue R250 (Fluka Chemika, Buchs, Switzerland).

Measurement of Fourier Transform Infrared Spectroscopy (FT-IR). KBr pellets were prepared by admixing about 1 mg of wheat gluten samples (nonmodified gluten and gluten deamidated for 30 h), respectively, with approximate 100 mg of spectroscopy-grade KBr and pressing the mixture into a 3-mm disk diameter with a minipress (Jasco MP-1). Infrared spectra of each pellet were recorded as described by Yong et al. (*24*). Deconvolution of the infrared spectra was performed using Jasco Spectra Manager software (Windows 95/NT version) and according to the method of Kauppinen et al. (*33*). The band assignment to secondary structural components was carried out for amide region I (1600−1700 cm^{-1}).

Determination of Solubility. The lyophilized wheat gluten samples (1 mg) in microcentrifuge tubes (1.5 mL) were dissolved or dispersed in 1 mL buffer solutions of various pH values (100 mM acetate buffer for pH 3, 10 mM acetate buffer for pH 5, 10 mM phosphate buffer for pH 7). Each solution was kept overnight at 20 °C and vortexed. After being vortexed 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. (*34*).

Evaluation of the Emulsifying Properties. The lyophilized wheat gluten samples were dissolved or dispersed respectively in buffer solutions with different pH values (100 mM acetate buffer for pH 3, 10 mM acetate buffer for pH 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. Each of the mixtures (10 mL) was then homogenized and sonicated to produce emulsions according to method of Yong et al. (*23*). Small portions (100 μL) of the resultant emulsions were kept at room temperature in test tubes (6 × 50 mm) to observe visually the stability of liquid emulsions to creaming on the first day and eighth day. For the first day, the creaming of emulsions in test tubes was observed several hours after the preparation of emulsions. After visual observation, the emulsions were used for measuring particle size distribution using a Horiba LA500 laser diffraction particle size analyzer (Horiba Ltd., Kyoto, Japan). Before the measurement of particle size distribution, the emulsions were mixed homogeneously using glass pipets. The visual observation of creaming and the measurement of particle size distribution were also carried out in a similar way on the eighth day.

Evaluation of Allergenicity of Deamidated Wheat Gluten. An enzyme-linked immunosorbent assay (ELISA) was performed from stock solutions of lyophilized wheat gluten samples. Each gluten sample was dissolved at 8 mg/mL in 100 mM Tris-hydrochloric acid (Tris-HCl) buffer (pH 7.4) containing 8 M urea and 1% (v/v) 2-mercaptoethanol (2-ME). The stock solutions were then diluted to different concentrations from 4 to 0.l25 mg/mL. Then each of these solutions (8 to 0.125 mg/mL) was dot-blotted to the wells of a Corning Inc. (Corning, NY) EIA/RIA Stripwell plate (1.25 μL/well) and dried up at 28 °C. After being coated, each well was blocked with 100 μL of 100 mM phosphate buffer (pH 7.4) containing 0.15 M sodium chloride (NaCl), 0.1% polyoxyethylene (20) sorbitan monolaurate (Tween 20) (PBST), and 1% human serum albumin (HSA) for 1 h at room temperature. After this step, five washes were performed with PBST. The wells were then incubated for 2 h at room temperature with sera of two patients who were allergic to wheat (patient A, RAST value was 41.5; patient B, RAST value was 355.0) in PBST−HSA and then washed 5 times with PBST. Horseradish peroxidase-labeled goat antihuman IgE (Dainippon Pharmaceutical, ST-AR96P) diluted 1:1000 was added for 2 h at room temperature. Wells were washed 10 times with PBST before the horseradish peroxidase reactivity was detected by using ELISA Pod Substrate ABTS kit (Nacalai Tesque, 14351-80). The absorbance was read at 405 nm with a microplate reader (model 680, Bio-Rad, Tokyo, Japan).

Data Analysis. For data with good consistency, two replicates were taken for 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. The standard deviation for three replicate data was also shown. Additional statistical analyses were carried out for the data of secondary structural contents (**Figure 4**) and droplet diameter of emulsions (**Figure 6**) (see the legends of these figures).

RESULTS AND DISCUSSION

Deamidation of Gluten by PG. Figure 1 shows a time-dependent increase of DD of gluten deamidated by PG. DD of

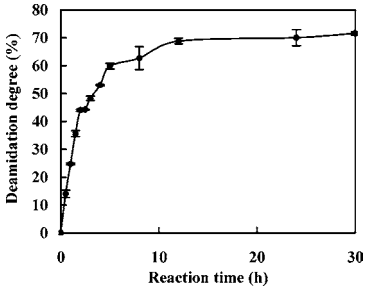

Figure 1. Time-dependent increase of DD of gluten induced by PG reaction. Gluten (10 mg) was dispersed in phosphate buffer (200 mM, pH 7.0). PG (0.13 U/mL) was added at 0 h, and the incubation was carried out at 40 °C for 30 h.

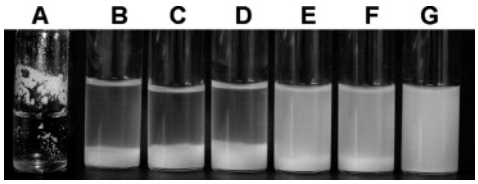

Figure 2. Appearance of gluten dispersions incubated with PG. The reaction conditions were the same as those of **Figure 1**. Reaction times: (A) 24 h (without PG); (B) 1.5 h (with PG); (C) 2 h (with PG); (D) 3 h (with PG); (E) 5 h (with PG); (F) 12 h (with PG); (G) 30 h (with PG).

gluten was increased rapidly for initial 5 h, but the reaction was slowed after that and eventually reached a plateau after 10 h. The final DD value after 30 h was 72%. The appearance of these gluten dispersions is illustrated in **Figure 2**. Glutens deamidated for 3 h or less showed the separation of precipitates. The gluten deamidated for 5 h started to show a mixture of turbid solution and precipitate. The extension of incubation time caused a decrease of precipitate, and finally a homogeneous turbid solution was obtained with deamidation for 30 h.

This result, as compared to the result of Yong et al. (*24*), clearly showed that deamidation of gluten was more dramatic and faster than that for α-zein. For α-zein, DD reached only 62%, and the precipitate without modification by PG still remain even after 137 h. In fact, these results are correspondent with the results of Yamaguchi et al. (*22*) whereby specific activity rate of gluten by PG (7.20 μmol•min^{-1}•mg^{-1}) was much higher than zein (0.66 μmol•min^{-1}•mg^{-1}).

Determination of Band Patterns by SDS−PAGE. Lyophilized glutens were applied for SDS−PAGE analysis. The result is shown in **Figure 3**. Bands patterns of deamidated samples (lanes C−J) were obviously different from those of their nonmodified cohort (lane B). It is apparent that all deamidated samples showed shifting bands toward the upper part of the gel. This kind of shifting phenomenon was already observed for various proteins that were deamidated by PG (*22*−*24*). It is unlikely that the upper-shifting of protein bands of PG-deamidated gluten is due to the molecular weight increment of deamidated subunits. As discussed in the previous paper (*24*), the upper shift of deamidated proteins in SDS−PAGE gel has two possible reasons. First, increased negative charge of deamidated molecules restricts the binding of SDS to the molecules, which may weaken the denaturation effects of SDS to form the compact rod complex for migrating effectively. Second, increased carboxyl groups by deamidation leads to the intensification of electrostatic repulsion within molecules, thereby producing molecules with an expanded shape, which is difficult to migrate through a gel matrix.

Ma et al. (*35*) reported that mild acidic deamidation of gluten caused gradual decrease in the size of lower molecular weight

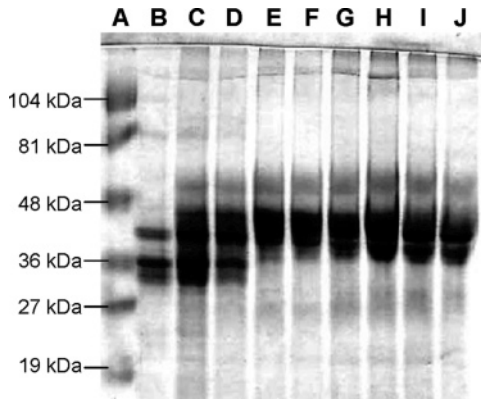

Figure 3. SDS–PAGE patterns of gluten treated by PG: (A) molecular weight markers; (B) gluten incubate without PG for 24 h (DD 0%); (C) gluten incubated with PG for 0.5 h (DD 11%); (D) gluten incubated with PG for 1 h (DD 22%); (E) gluten incubated with PG for 1.5 h (DD 34%); (F) gluten incubated with PG for 2 h (DD 45%); (G) gluten incubated with PG for 3 h (DD 52%); (H) gluten incubated with PG for 5 h (DD 60%); (I) gluten incubated with PG for 12 h (DD 72%); (J) gluten incubated with PG for 30 h (DD 72%).

subunits, suggesting the fragmentation of protein molecules as well as deamidation. In our case (**Figure 3**), the smaller peptides were not observed obviously in the deamidated glutens, indicating no protease activity in PG fraction. Therefore, PG is thought to be a good tool for the deamidation of gluten without touching macromolecular characteristics.

It is noticeable that the majority of the bands shifted up by only a short deamidation time, i.e., 1.5 h with DD 34%. This means that most of gluten molecules were subjected to PG reaction by 1.5 h despite the presence of precipitates as shown in **Figure 2**. The capability of PG to react substrate in the form of precipitate or aggregate is a great advantage because available food proteins are very often difficult to be solubilized or dispersed in aqueous media.

Another interesting point for the bands (lanes E−J) is that the shorter time deamidated bands (lanes E−G) initially became much more diffused (causing a smearing effect). Then, with longer deamidation time (lanes H−J), the bands began to appear as darker and more distinct bands. We speculate that the "diffused" set of protein bands represent many different partly deamidated gluten proteins, including the same gluten protein, with different degrees of deamidation. However, when deamidation is prolonged, there were fewer different "types" of gluten protein molecules, as these proteins became fully (or almost fully) deamidated, leading to fewer, more distinct protein bands on SDS−PAGE gels.

Secondary Structure Changes in FT-IR of Gluten by Deamidation. The study of the deamidation effect on the conformation of gluten in powder form was carried out by using FT-IR analysis. Gluten was analyzed in the powder form because there is no good solvent to solubilize appropriately nonmodified gluten for FT-IR measurement. By an analysis of the amide I region (1600−1700 cm^{-1}) in spectra of the gluten samples, the contents of secondary structural components were calculated (**Figure 4**). By PG deamidation, a decrement took place for both inter- (around 1695 cm^{-1}) and intramolecular β-sheets (around 1680 cm^{-1}). Total β-sheets content of wheat gluten dropped from 27% to 17% after reaction with PG. On the contrary, the contents of β-turn, α-helix, and random coil of deamidated gluten showed a clear rise of around 4%, 4%, and 2%, respectively.

It was identified that repeatitive domains in wheat gluten are rich in glutamine residues (*10*), and the list of these amino acid

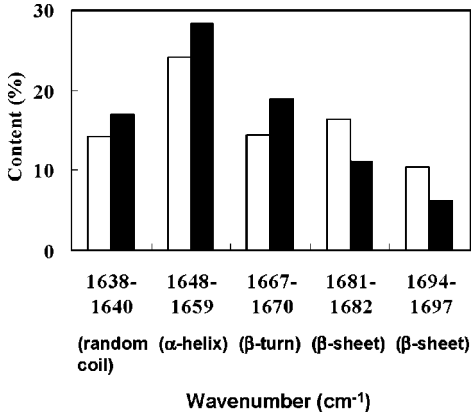

Figure 4. Secondary structural contents of gluten determined by FT-IR spectroscopy: (white bars) nonmodified gluten; (black bars) deamidated gluten (reaction time, 30 h; DD = 72%). FT-IR measurements were conducted for the powdery form of gluten. Each band was assigned to the component of the secondary structure according to the previous data. The positions and contents of the bands at the region from 1630 to 1700 cm^{-1} are shown. A χ^2 test was done to find a significant difference in each secondary structural content between nonmodified gluten and deamidated gluten. Although a significant difference was not found, the standard deviation was less than 1.3% for all contents, indicating the tendency of the alteration of secondary structure induced by deamidation.

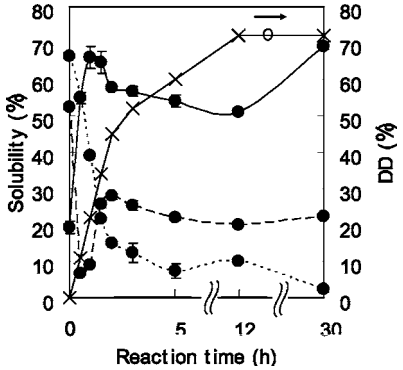

Figure 5. Time-dependent change of solubility of gluten induced by PG reaction. The deamidated glutens were dispersed in buffer solutions of various pH values at 20 °C (protein concentration: 0.1 wt %). Buffer solutions: (dotted line) acetate buffer (100 mM, pH 3); (dashed line) acetate buffer (10 mM, pH 5); (solid line) phosphate buffer (10 mM, pH 7). Time-dependent increase of DD of gluten induced by PG reaction is also shown (–×–).

sequences was reported by Shewry et al. (*4*). According to Belton (*36*), the repeat sequences would have a very high capacity to form intra- and intermolecular hydrogen bonds, which were associated with β-sheet formation. By developing a loop and train model, Belton (*36*) has also proposed possible β-sheet conformational changes through hydration.

Belton's loop and train model was considered to explain well the secondary structural changes observed in this research, although we known that Belton's model assumes a greater hydration state of gluten molecules compared to the powder state. Before reaction with PG, glutamine residues in powder form gluten would form β-sheets, which was expressed as a "train". When deamidation proceeded, these glutamine residues were converted into negatively charged glutamic residues. Eventually, it caused an augmentation of electrostatic repulsion among these β-sheets and forced them to be "unzipped". Therefore, β-sheet content as analyzed by FT-IR dropped 10% after PG deamidation. This led to the formation of unbounded mobile regions known as a "loop". The loop could involve the more flexible β-turn, α-helix, and random coil with a total increase of 10% after the gluten was deamidated by PG. The analysis by FT-IR totally indicates that deamidated gluten has a more flexible conformation than the nondeamidated cohort. Probably, such secondary structural changes lead to the extended form of deamidated gluten suggested by SDS−PAGE analysis.

Solubility of Wheat Gluten. The effects of PG deamidation on solubility of wheat glutens were investigated (*34*). Solubility in acidic conditions (pH 3 and 5) as well as neutral condition (pH 7) was plotted against the PG reaction time (**Figure 5**). DD of gluten (indicated by solid line with cross marks) that induced by PG reaction was also shown. For gluten in the nondeamidated state (0 h), solubility was good (66%) under strong acidic condition (pH 3) and fairly good (52%) under weak acidic condition (pH 5), but solubility at pH 7 was very low (19%). However, once the gluten was deamidated by PG, solubility at this neutral pH increased tremendously even when the DD was only around 20%. Meanwhile, decreasing solubility was observed at acidic conditions, especially for pH 3 whereby the solubility dropped to less than 10% when DD was more than 60%.

The most insightful improvement through this study was the remarkable solubility of gluten in neutral buffer even after a very short time deamidation (1 h, DD 22%) though undesirable performance of solubility happened under acidic conditions. This result was in agreement with a research done by Matsudomi et al. (*17*) attempting to deamidate gluten by an enzymatic method. In the study, enzymatically modified gluten (DD 25%) was obtained by treating gluten with chymotrypsin at pH 10 and 20 °C for 2 h. The deamidated gluten was solubilized greatly for pH more than 6 whereas minimum solubility was observed for pH 5.

Wu et al. (*16*) showed solubility of acid-treated gluten was greatly improved. However, the study concluded that the solubilization was not merely due to deamidation but also rupture of a few peptide linkages in gluten molecules. Conversely, our research demonstrated that the substitution of nonselective acidic treatment with specific PG deamidation is possible as a useful and more selective tool for improving the solubility of gluten.

Evaluation of Wheat Gluten Emulsifying Properties. Nondeamidated and deamidated wheat glutens were initially dispersed or solubilized into different pH solutions. Then the samples were blended with corn oil to prepare emulsion for emulsifying tests. **Figures 6** and **7** show mean droplet diameter and appearance of these emulsions in various pH conditions on the first day and eighth day of storage at 20 °C, respectively. According to **Figure 6**, for nondeamidated gluten (0 h) on the first day storage, only emulsion at pH 3 showed a fine mean droplet diameter (in a few μm) whereas the values were more than 50 μm at pH 5 and more than 100 μm at pH 7. After 8 days of storage, while mean droplet diameter of nondeamidated emulsion at pH 3 and 7 remain unchanged as in the first day, mean droplet diameter of pH 5 increased to more than 90 μm. This shows nondeamidated gluten has good stability of emulsion particles in pH 3 but emulsified poorly in pH 7. The particles tend to coalesce into a bigger size at pH 5, showing a poor stability in emulsification.

After deamidation by PG, the emulsification properties of gluten demonstrated an abrupt change. First, in the case of neutral pH (**Figure 6A**), even very short time deamidation (0.5 and 1 h) caused a decrease of the mean droplet diameter of the emulsion into a few micrometers. Although the difference cannot

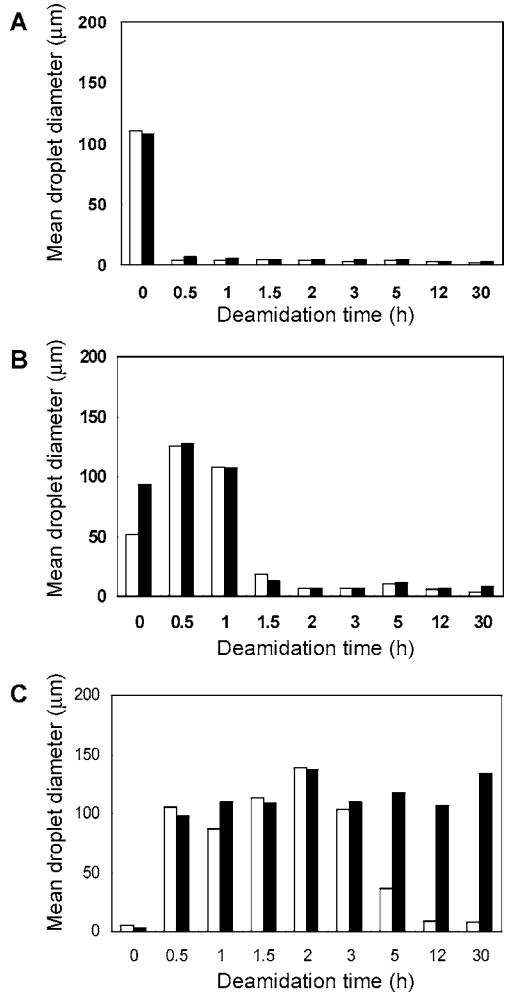

Figure 6. Mean droplet diameter of emulsions under various pH conditions on (white bars) the first day and (black bars) the eighth day of storage at 20 °C. Glutens, which were deamidated by PG from 0 to 30 h, were dispersed in (A) phosphate buffer (10 mM, pH 7), (B) acetate buffer (10 mM, pH 5), and (C) acetate buffer (100 mM, pH 3), 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. A significant difference in data among reaction times (at the same pH and the same storage day) was confirmed by an ANOVA test.

be indentified because of the large scale in **Figure 6A**, the longer PG reaction produced the gluten having more emulsifying activity. Samples deamidated for 12 and 30 h both yielded fine emulsion particles with a mean droplet diameter less than 3 μm. Even on the eighth day, only unnoticeable increments on mean droplet diameter were detected on these emulsions, exhibiting a high stability for these emulsion particles in neutral pH condition.

In **Figure 6B**, at pH 5, the emulsification property of gluten deamidated for 0.5 and 1 h was inferior to the nondeamidated cohort; i.e., more than 100 μm mean droplet diameter was observed. However, glutens with longer deamidation time (>2 h) produced fine emulsion particles. The mean droplet diameter was reduced to around 10 μm and remained stable even until the eighth day of storage.

Contradictory, emulsions of deamidated gluten in pH 3 demonstrated a very different trend for mean droplet diameter as compared to pH 5 and 7 (**Figure 6C**). Large particle sizes (around or more than 100 μm) were obtained for samples deamidated for 0.5−3 h. On the first day of storage, though the diameter of the emulsion stabilized by glutens with longer

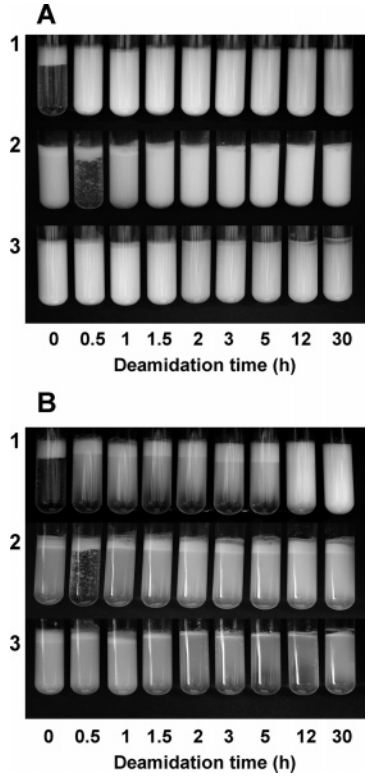

Figure 7. Appearance of emulsions on (A) the first day and (B) the eighth day of storage at 20 °C. Glutens, which were deamidated by PG from 0 to 30 h, were dispersed in (1) phosphate buffer (10 mM, pH7), (2) acetate buffer (10 mM, pH 5), and (3) acetate buffer (100 mM, pH 3), respectively, and the resultant dispersions were mixed with corn oil at 20 °C. The protein concentration and oil amount were 0.09 and 10 wt %, respectively.

deamidation time was reduced to less than 40 μm (for 5 h) and 10 μm (for 12 and 30 h), by the eighth day, the diameter of these emulsion particles was increased to more than 100 μm.

Observation on the appearance of the emulsions in **Figure 7** further illuminated the effects of PG reaction on emulsification properties of gluten. The appearance of emulsions on the first day is shown in **Figure 7A**. Nondeamidated gluten at pH 7 and 0.5 h deamidated gluten at pH 5 demonstrated very poor emulsions, i.e., the clear separation of cream and aqueous phases. In the case of other glutens, fine emulsions were generated. However, on the eighth day of storage (**Figure 7B**), nondeamidated glutens showed creaming was at pH 5 and 7. For their deamidated cohorts, they illustrated most unstable emulsions at pH 3. Particularly for samples 12 and 30 h, both creaming and oiling off were occurred. For emulsions at pH 5, the creaming process also happened for all the deamidated samples. However, the small diameter of these emulsions particle sizes as stated earlier (**Figure 6B**, especially deamidation times 1.5−30 h) indicates that these particles did not coalesce to form large particles. The most successful and stable emulsions were observed at the pH 7 conditions. By the deamidation of gluten for more than 12 h, especially after 30 h, excellent emulsion conditions were achieved in term of particle sizes and emulsion stability.

An obvious correlation can be made for soblubility and emulsification at pH 7. Improvement in solubility by deamidation actually enhanced the emulsification properties of these wheat glutens. The same trend was reported for emulsification of α-zein deamidated by PG (*24*). Introduction of more new negative charges by PG created an amphiphilic nature to the gluten, since gluten is originally rich in hydrophobic amino acid residues. This amphiphilic nature is able to make the protein

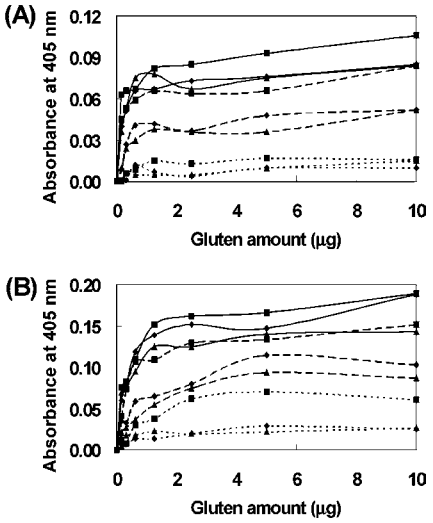

Figure 8. ELISA performed on serum of (A) patient A (RAST value 41.5, diluted 5 times) and (B) patient B (RAST value 355.0, diluted 20 times) with food allergy to wheat to analyze IgE reactivity against gluten treated by PG. Gluten was incubated (solid line, square mark) without PG for 24 h, (solid line, diamond mark) with PG for 0.5 h, (solid line, triangle mark) with PG for 1 h, (dashed line, square mark) with PG for 1.5 h, (dashed line, diamond mark) with PG for 2 h, (dashed line, triangle mark) with PG for 3 h, (dotted line, square mark) with PG for 5 h, (dotted line, diamond mark) with PG for 12 h, and (dotted line, triangle mark) with PG for 30 h. For this experiment, only 1 trial was done because the patients' sera were insufficient for repetitive experiments.

adjust itself to a polar−nonpolar interface (*37*). This may boost a stable interaction of gluten molecules with corn oil at the interface. In fact, an earlier study by Matsudomi et al. (*38*) on acid-treated gluten concluded that deamidation increased surface hydrophobicity of gluten at pH 7.4. Emulsifying properties of the gluten were correlated linearly with the surface hydrophobicity. Later, Matsudomi et al. (*17*) applied chymotrypsin to deamidate gluten at an alkali pH. They concluded that the increase of emulsifying property in the study was owed to induction of an amphiphilic nature by chymotripsin-catalyzed deamidation.

As mentioned earlier, lowering of pI by PG reaction led to precipitation of gluten at acidic pH. Therefore, for pH 3, probably due to this low solubility, corn oil could only be dispersed in a very limited amount and sustained weakly in the buffer, which eventually separated from the aqueous phase. For the case of pH 5, it is noticeable that correlation of solubility−emusification did not apply well. Hamada (*39*) actually explained why solubility is not the only precondition for emulsion stability. It is thought that more critical roles were played by the electrostatic repulsive force and steric hindrance occurring among adsorbed protein layers at oil droplet surfaces.

It is assumed that deamidated glutens have modest negative charges at pH 5, which cannot disperse gluten molecules well in aqueous media but adjust the molecules at the oil droplet surface and prevent the close contact of oil droplets during emulsification via electric repulsive force. However, such electrostatic repulsive force at pH 5 is not strong enough to hold the emulsion components apart from each other for a long time as compared to the case at pH 7. This situation may lead to the creaming or aggregation of droplets stabilized by deamidated gluten. However, the thick adsorbed layer of deamidated gluten at the oil droplet surface could protect and allow the oil droplets to coalesce a via steric hindrance mechanism.

Allergenicity of Deamidated Wheat Gluten. The final section in this study describes how deamidation affects the allergenicity of gluten. The study was carried out by using 2 patients' sera with different degrees of food allergy to wheat. The result of ELISA is shown in **Figure 8**. **Figure 8A**,**B** illustrated the ELISA results of patient's serum with medium and strong food allergies to wheat, respectively. Both of them demonstrated that nondeamidated gluten showed the highest absorbance value at 405 nm, connoting IgE reactivities of these patients' sera are strongest with native gluten. However, by PG deamidation, glutens lost their allegernicity toward patients' sera in proportion to the deamidation degree. Yet, it revealed that the sera with different degrees of RAST values exhibited variable responses to deamidated glutens. It is interesting to notice a higher deamidation degree (DD 72%) is needed for gluten to loss its allergenicity toward strong RAST value patient's serum than the medium RAST value serum (DD 60%), indicating that the epitopes for these two sera might be different.

Globally, wheat is one of the most common food allergens among children (*40*). In this ELISA study, patients' sera with specific IgE to wheat allergens were applied. This is because food allergy is commonly an IgE-mediated hypersensitivity reaction where symptoms appear rapidly following exposure to related macromolecules (generally proteins) (*41*). Our result shows how a modification on single type of amino acid (glutamine) in wheat gluten could alter its original allergenicity. In fact, a study on ovomucoid has proved that substituting a single amino acid within each epitope produced an interesting loss of allergy reactivity (*42*). A few papers have identified glutamine as an important amino acid in IgE-binding epitopes that is responsible for wheat gluten allergenicity (*27*, *30*, *43*). Since the glutens were treated with urea and 2-ME before the ELISA test, it is unlikely the loss of allergenicity of glutens by PG deamidation was due to the alteration of conformation on secondary and tertiary structures. Instead, it is most probably that the lowering of allergenicity was caused by the modification of glutamine into glutamic acid in the epitope regions.

In summary, PG deamidation was proved to be an effective approach to modify wheat gluten, which is difficult to be solubilized in aqueous solution. The wheat gluten maintained its long-chain subunits even after a large amount of glutamine was converted into glutamic acid. Deamidated glutens possess an amphiphilic nature. Under neutral condition, these glutens, particularly the long-hour deamidated samples, showed excellent solubility and emulsification properties. Furthermore, these deamidated glutens proved to have a great reduction in allergenicity as compared to their native cohort. These new features of deamidated gluten suggest that PG could be a potential tool for enhancing the usability of wheat gluten in the food industry as well as producing hypoallergic product for patients of wheat allergy. However, as wheat gluten is a very complicated protein consisting of gliadin and glutenin subunits, we will focus on these two proteins separately in our following studies.

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