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ARTICLE

Effects of Deamidation on Structure and Functional Properties of Barley Hordein

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Deamidated hordeins of various deamidation degree (DD) ranging from 0.7 to 40% were prepared using an alkaline method. The hordein peptide bond cleavage, secondary structure, surface charge and hydrophobicity as well as their solubility, emusifying and foaming properties were studied as a function of DD value. SDS−PAGE, size exclusion chromatography (SEC) and Fourier transform infrared (FTIR) results suggest that limited deamidation (≤4.7%) could dissociate hordein aggregates, leading to great increase of the protein solubility and surface hydrophobicty. A further increase of the DD value greater than 4.7% resulted in extensive protein hydrolysis and a marked change of protein secondary structure. The optimal functionalities were obtained at a narrow DD range (2.4−4.7%), where hordein samples demonstrated significantly improved solubility and the emulsifying and foaming properties at both acidic and neutral pHs. These results suggest that deamidated hordein would be an excellent candidate to be developed as an emulsifying and foaming ingredient.

KEYWORDS: Barley hordein; deamidation; molecular structure; solubility; foaming; emulsifying

INTRODUCTION

Deamidation is one of the most used chemical methods to improve food protein properties (*1*). For cereal proteins, deamidation is a particularly important modification since up to one-third of their total amino acid content is glutamine (Gln) (*2*, *3*). The conversion of the glutamine (Gln) to glutamic acid (Glu) is believed to improve cereal protein solubility as a high content of glutamine residues cause the aggregation of the protein molecules via hydrogen bonding (*4*). Deamidation can also partially unfold the protein and indirectly lead to protein hydrolysis (*5*). These changes at appropriate levels improve the functional properties of wheat, corn, rice and soy proteins, making them useful for the food and pharmaceutical industries (*4*−*11*). However, excessive deamidation could cause undesirable property changes that reduce protein utility (*5*). Thus, optimization of the DD is crucial to achieve better functionalities. Plus, since deamidation rates as low as 2−6% can enhance protein functionalities (*6*), protein structural and functional changes induced by limited deamidation may require special attention to enable a fine adjustment of protein structures.

As the fourth most widely cultivated cereal in the world after wheat, rice and corn (*12*), barley is gaining increasing popularity as a part of the human diet because of the recent health claim made about its β-glucan (*13*). This soluble dietary fiber component of barley is known to reduce both blood cholesterol (*14*) and the glycemic index (*15*). Additionally, barley represents a potential abundant and affordable source of plant proteins. The overall barley grain protein content is 8 to 13% (w/w) depending on the variety (*16*). Hordein, barley prolamin extracted with alcoholic solution, comprises approximately 35−55% of the total barley grain protein, and is the main storage protein of barley (*17*). Hordeins are divided into four groups based on their electrophoretic mobilities and amino acid compositions. The B (30−50 kDa, sulfur-rich) and C (55−80 kDa, sulfur-poor) hordeins take up 70−80% and 10−20% of the hordein fraction, respectively; and the D (80−90 kDa) and A (15 kDa) hordeins take up less than 5% of the total hordein fraction. C and some B hordeins appear as monomers, while most B and D hordeins are linked by interchain disulfide bridges (*18*). In addition to a high amount of glutamine, hordein is also rich in hydrophobic amino acids (around 40%), with the highest levels corresponding to proline, leucine, and valine (*19*). Such amino acid profile yields high surface hydrophobicity and strong protein aggregation. These features favor adsorption of protein at the hydrophobic interface and then form viscoelastic films to stabilize foams and emulsions (*19*). However, on the other hand, it leads to markedly reduced protein solubility, hindering hordein applications since protein water solubility is critical to impart other desired and necessary properties such as emulsifying and foaming functionalities (*20*). Owing to a high Gln content (*19*), hordein is a suitable candidate for evaluation of deamidation, targeting improved functionalities. However, research on hordein deamidation is limited to few publications on antioxidant property (*21*, *22*). The impacts of deamidation on hordein structure and functional properties are unclear.

The objective of this research was to investigate the structure, solubility, and foaming and emulsifying properties of barley hordein when it is subjected to deamidation. The protein structure transition at low deamidation level and its functionality change as a consequence of such structure transition were emphasized.

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MATERIALS AND METHODS

Materials. Regular barley grains (Falcon) were kindly provided by Dr. James Helm, Alberta Agricultural and Rural Development, Lacombe, Alberta, Canada. Protein content was 13.2% (w/w) as determined by combustion with a nitrogen analyzer (Leco Corporation, St. Joseph, MI) calibrated with analytical reagent grade EDTA. A factor of 6.25 was used to convert the nitrogen to protein. Canola oil used for the emulsification study was purchased from a local supermarket (Edmonton, AB, Canada). Unstained standard protein molecule marker for SDS−PAGE was purchased from Bio-RAD (Richmond, CA). Ammonia Assay Kit, *o*-phthaldialdehyde (OPA) reagent, 1-anilinonaphthalene-8-sulfonic acid (ANS) and standard molecular markers for HPLC analysis (BSA, 67 kDa; ovalbumin, 43 kDa; lactoglobulin, 35 kDa; cytochrome C, 13.6 kDa; aprotinin, 6.5 kDa and vitamin B_{12}, 1.4 kDa) were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). All other chemicals were of reagent grade.

Extraction of Barley Hordein. Barley hordein was extracted according to our previous work (*19*). Briefly, after pearling and milling processing, barley endosperm flour was dispersed in the 55% ethanol solution at a solvent-to-flour ratio of 6:1 (v/w) with stirring for 2 h at 60 °C. After extraction, the solid part was removed by centrifuge (Beckman Coulter Avanti J-E Centrifuge System) at 23 °C at 8500*g* for 15 min. The hordein fraction was isolated from the supernatant by cold precipitation at 4 °C overnight. The isolated hordein was lyophilized, and the dry powders were stored at 4 °C before further processing.

Preparation of Deamidated Hordein. Deamidated hordeins of different DD were prepared according to the method of Yong et al. (*23*) with a slight modification. Hordeins (5%, w/v) were suspended in 0.5 M NaOH solution. The reactions were conducted at 40 and 60 °C, respectively, under consistent stirring. Samples were withdrawn at different time intervals (10−120 min). After neutralization using 0.5 M HCl, the sample solutions were dialyzed against deionized water and then freeze-dried. The dried samples were stored at 4 °C until use.

Determination of the Degree of Deamidation, Hydrolysis and Surface Hydrophobicity. The deamidation degree (DD) was determined by measurement of the released ammonia after deamidation using an Ammonia Assay Kit according to the manufacturer's instruction. DD was calculated as the ratio of ammonia generated in the sample to that of the completely deamidated protein. Complete deamidation was achieved by refluxing hordein with 2 M HCl for 2 h.

The hydrolysis degree (HD) was measured by the *o*-phthaldialdehyde (OPA) method (*24*). The deamidated hordein sample (1.25 mg/mL) was dissolved in 12.5 mM borate buffer (pH 8.5) plus 2% (w/v) SDS. This solution (50 μL) was mixed with 1 mL of the reagents. The reagent was composed of 50 mL of 0.1 M borate buffer (pH9.3), 1.25 mL of 20% (w/v) SDS solution, 100 mg of *N*,*N*-dimethyl-2-mercaptoethylammonium chloride (DMMAC), and 40 mg of OPA dissolved in 1 mL of methanol. The mixture was allowed to stand for 2 min before measurement of the absorbance at 340 nm. The number of amino groups was determined with reference to the L-leucine standard curve (between 0.5 and 5 mM). The HD was calculated by the following equation: HD (%) = $[(\alpha - ni)/nT] \times 100$, where nT was the total number of amino groups in original hordein after total hydrolysis with 6 M HCl for 24 h and ni was the number of amino groups in hordein, while α was the number of free amino groups measured in the deamidated hordein.

The surface hydrophobicity of the deamidated hordeins in sodium phosphate buffer (pH 7) was determined using a fluorescence probe, 1-anilinonaphthalene-8-sulfonic acid (ANS), according to the method of Kato and Nakai (*25*). Fluorescence intensity (FI) was measured at wavelengths of 390 nm (excitation) and 470 nm (emission) using a fluorospectrometer (FP-6300, Jasco, Tokyo, Japan). The surface hydrophobicity degree (S_o) was calculated by linear regression analysis using the slope of the straight line obtained by plotting the FI as a function of the protein concentration.

Electrophoretic Mobilities. The electrophoretic mobilities of the deamidated hordein samples in pH 3, 5 (0.2 M acetate buffer) and 7 (0.2 M phosphate buffer) buffers were measured by laser Doppler velocimetry using a Zetasizer NanoS (model ZEN1600, Malvern Instruments Ltd., Malvern, U.K.). Electrophoretic mobility (i.e., velocity of a particle within an electric field) was related to the zeta potential (ζ) using the Henry equation (*26*)

$$U_E = \frac{2\varepsilon \times \zeta \times f(\kappa\alpha)}{3\eta}$$

where η is the dispersion viscosity, ε is the permittivity, and $f(\kappa\alpha)$ is a function related to the ratio of particle radius (α) and the Debye length (κ). The results are reported as the average of at least three measurements. The same buffers were used in the following studies.

Electrophoresis. SDS gel electrophoresis was performed to study hordein subunit molecular weight change after deamidation using a vertical mini-gel system (Mini-PROTEIN Tetra Cell, BIO-RAD, Hercules, CA). Isolated protein fractions were mixed with the loading buffer (0.125 M Tris-HCl, pH 6.8, 4% (w/v) SDS, 20% (v/v) glycerol, 0.5% 2-mercaptoethanol and 1% bromophenol blue (w/v)) and then heated at 100 °C for 5 min. After cooling, a 12 μL sample (3 mg/mL) was loaded on 5% stacking gel and 12% separating gel and subjected to electrophoresis at a constant voltage of 80 V. After electrophoresis, the gels were stained with 0.1% (w/v) Coomassie Brilliant Blue-R-250 in water−methanol−acetic acid (4:5:1, v:v:v) for 30 min and destained with water−methanol−acetic acid (4:5:1, v:v:v).

Size Exclusion Chromatography (SEC). SEC chromatography was performed using a HPLC system (Varian ProStar) combined with a size exclusion column (Superdex 200 10/300 GL, Amersham Biosciences, NJ). 50 mM phosphate buffer containing 150 mM sodium chloride was used as the mobile phase at a flow rate of 0.4 mL/min at 25 ± 0.5 °C. 50 μL of sample solution was injected into the HPLC system, and the protein was monitored at the UV wavelength of 280 nm. Standard molecular markers were used to calculate the weight-average molecular weight (M_w) of the deamidated hordeins.

FTIR Spectroscopy. Protein conformation was studied with Fourier transform infrared (FTIR) spectroscopy (Varian FTS-7000) in the wavenumber range from 400 to 4000 cm^{-1} during 128 scans, with 4 cm^{-1} resolution. Deamidated hordeins (5%, w/v) were dissolved in D_2O solution. To ensure complete H/D exchange, samples were prepared 2 days before and kept at 4 °C prior to infrared measurements. Samples were placed between two CaF_2 windows separated by 25 μm polyethylene terephthalate film spacer for FTIR measurement. To study the amide I region of the protein, Fourier self-deconvolutions were performed using the software provided with the spectrometer. Band narrowing was achieved with a full width at half-maximum of 20 cm^{-1} and with a resolution enhancement factor of 2.0 cm^{-1}.

pH Solubility Profile. Deamidated hordeins (125 mg) were dispersed in 25 mL, pH 3, 5, and 7 buffers. The dispersions were mixed for 1 h at 23 °C using a magnetic stirrer. After centrifugation at 1200*g* for 20 min at 4 °C, the protein concentration in the supernatant was determined by Bradford dye assay (*27*) with bovine serum albumin as the standard. The solubility was expressed as a percentage of the total protein content of the starting sample.

Foaming Properties. Foaming properties were determined according to the method of Ahmedna et al. (*28*) with a slight modification. Protein samples (0.5%, w/v) were dispersed in 50 mL, pH 3, 5, and 7 buffers. The solution was mixed for 2 min with a homogenizer (PowerGen 1000, Fisher Scientific, Fairlawn, NJ) at speed "three". Volumes were recorded before and after homogenization. The percentage volume increase was calculated according to the following equation: foaming capacity (FC) % = $(Vf_1 - Vf_0)/Vf_0 \times 100$, where Vf_0 and Vf_1 represent the volume of the protein solution and the formed foams after homogenization, respectively. Foam stability (FS) was determined as the volume of foams (Vf_2) that remained after standing for 0.5 h at 23 °C expressed as a percentage of the initial foam volume: FS % = $Vf_2/Vf_1 \times 100$.

Emulsion Properties. Emulsifying properties were determined according to Yasumatsu et al. (*29*) with a slight modification. Protein samples (0.5%, w/v) were dispersed in 50 mL, pH 3, 5, and 7 buffers, followed by the addition of 50 mL of canola oil. The mixture was homogenized at speed "six" for 2 min to form an emulsion. The emulsion was then centrifuged at 1500*g* for 5 min. The emulsion centrifugation stability (ECS) was calculated by measuring the volume of the emulsion (Ve_1) remaining after centrifugation and before (Ve_0), and recorded as ECS % = $Ve_1/Ve_0 \times 100$. The emulsion samples were then heated to 80 °C in a water bath for 30 min and cooled to 23 °C. Upon cooling, these tubes were centrifuged at 1500*g* for 5 min. The volume of the remaining emulsified fraction Ve_2 was recorded. The emulsion thermal stability (ETS) was calculated according the equation ETS % = $(Ve_2/Ve_0) \times 100$.

Statistical Analysis. All experiments were performed at least in triplicate. Error bars on graphs represent standard deviations. Statistical

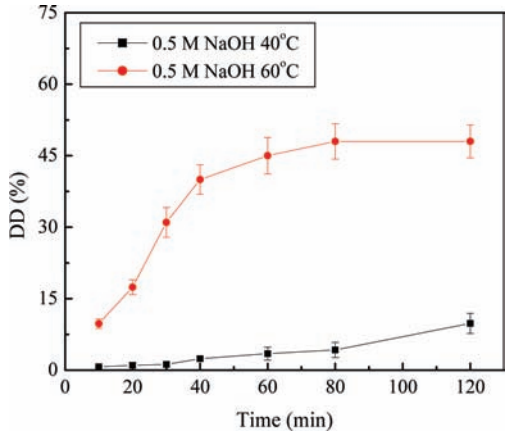

Figure 1. Time-dependent increase of DD value of hordein induced by alkaline reaction.

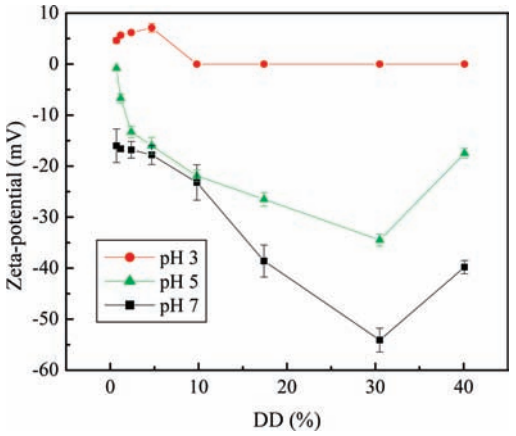

Figure 2. Electrophoretic mobilities of the deamidated hordeins at different pH as a function of DD value.

significances of the differences were determined by Student's t test. The level of significance used was $p < 0.05$.

RESULTS AND DISCUSSION

Alkaline Deamidation of Hordein. The protein content of isolated hordein was 93% (w/w) on a dry basis. Preliminary data showed that an alkaline method was more efficient to prepare deamidated hordein than an acidic method. This is in agreement with a previous report for zein (*4*). Thus an alkaline method was selected in this research. In order to systematically study the effects of deamidation on hordein structure and functional properties, samples of a relatively broad range of DD were prepared. Those within a limited DD range (DD ≤ 10%) were emphasized. Thus, reactions were conducted in 0.5 M NaOH at two selected temperatures (40 and 60 °C) based on preliminary data. **Figure 1** shows the DD obtained as a function of the reaction time. At 40 °C, the DD value reached 9.8% after 2 h. Prolonged time did not result in further increase of the DD value. Increasing temperature to 60 °C significantly enhanced the reaction rate ($p < 0.05$), and DD reached more than 45% within 2 h. Samples that possessed DD values of 0.7, 1.2, 2.4, 4.7, 9.8% obtained at 40 °C and DD values of 17, 31, 40% obtained at 60 °C were selected for the following study.

Characterization of Deamidated Hordein. The zeta-potential of the hordein samples in different pH buffers is expressed as a function of their DD value (**Figure 2**). Limited surface charge (+0.8 mV) was observed for hordein with a DD of 0.7% at pH 5. This value, however, increased greatly to −33 mV at DD 31%, and then decreased to −17 mV at DD of 40%. The zeta-potential of the deamidated hordein changed in the same way at pH 7, but the surface charge was generally higher than those at pH 5. On the other hand, the protein molecule surfaces were slightly positively charged (+5 mV) at pH 3 when the DD was 0.7%. With increasing of the DD value to 9.8%, hordein surface charge decreased to near zero. Wu et al. (1979) reported that the isoelectric point (IEP) of barley proteins is close to pH 6 (*30*). After deamidation, the IEP of hordein shifted to pH 3 due to an increase in the amount of carboxyl group on protein chains. This is in agreement with research obtained from other proteins where their IEP normally shift to acidic pH after deamidation (*5*). The introduction of the carboxyl group also explains the increase of the protein surface charge with DD until 31% at pH 5 and 7. The decrease of the surface charge at a DD of 40% in both pH 5 and 7 buffers was unexpected. This may be attributed to the formation of very small peptides enriched with deamidated Glu residues at high DD because the occurrence rate of peptide bond cleavage at Glu residues is higher than that for Gln (*31*). These small peptides with high charge density ($-COO^-$) permeated the dialysis tube during dialysis, even though low molecular weight cutoff (3.5 kDa) dialysis tubing was used in this research. Thus, the remaining samples exhibited decreased surface charge.

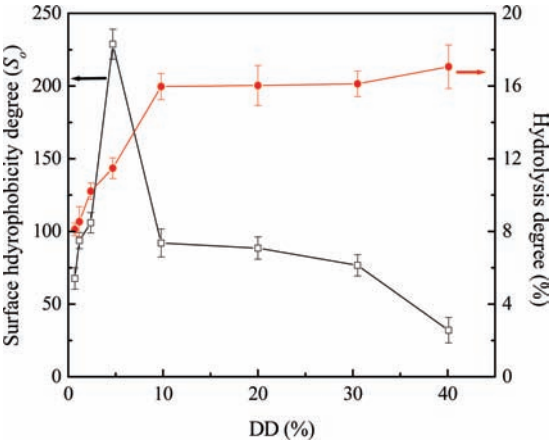

Figure 3. Degree of hydrolysis and surface hydrophobicity of the deamidated hordeins as a function of DD value.

The hydrolysis degree (HD) values and surface hydrophobicity (S_o) of the deamidated hordein samples are shown in **Figure 3**. The HD increased linearly in proportion to the degree of deamidation until a DD of 9.8%, and then the HD value leveled off. This suggests that hordein peptide bond cleavage occurred quickly within the DD range of 0.7−9.8% and the hydrolysis rate slowed down after DD of 9.8%. S_o of the deamidated hordein increased markedly ($p < 0.05$) with the DD increased to 4.7%, suggesting that the hydrophobic regions were exposed outside. A further increase of the DD value resulted in significant decrease ($p < 0.05$) of the hordein surface hydrophobicity.

The SDS−PAGE patterns of the deamidated hordeins are shown in **Figure 4**. Three subunits of hordein were identified with bands at 55−80, 30−50 and <15 kDa corresponding to C, B and A hordeins, respectively (*32*). A weak band at 80−90 kDa corresponding to D hordein was also observed in this work when ethanol was used as the sole extraction agent (*33*). Most bands remained visible in SDS−PAGE pattern until a DD value of 4.7%, but the band intensity of C and B hordeins decreased gradually. After DD of 4.7%, all bands disappeared. The result indicates that partial hydrolysis occurred at DD ≤ 4.7%, whereas extensive hydrolysis took place afterward.

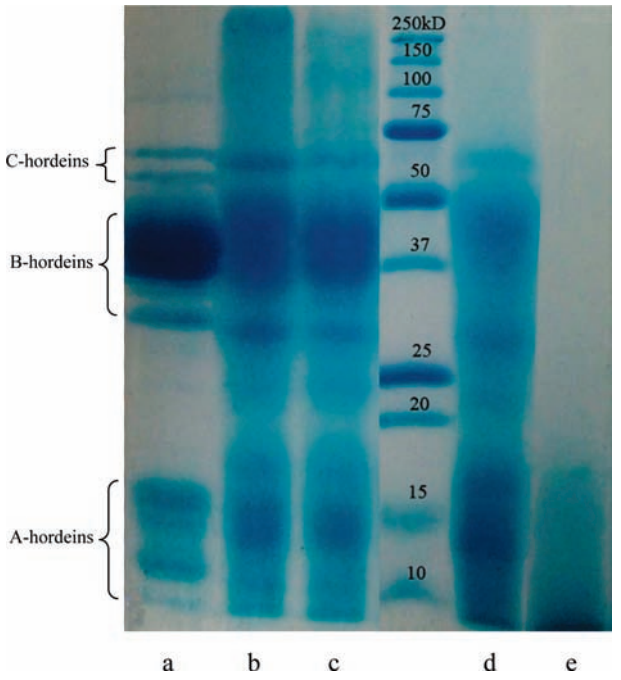

Figure 4. SDS−polyacrylamide gel electrophoresis of the deamidated hordeins: **a**, unmodified hordein; **b**, DD 0.7%; **c**, DD 1.2%; **d**, DD 4.7%; **e**, DD 9.8%.

SEC chromatograms of the deamidated hordeins in phosphate buffer are depicted in **Figure 5**. Deamidated hordein with the DD value of 0.7% contains two main broad peaks (peak 1 and peak 2) corresponding to subunits with M_w of < 15 kDa and 20−67 kDa, respectively. The former can be assigned to A hordeins, whereas the latter could be a mixture of B and C hordeins. A small sharp peak (peak 3) was also observed at 114 kDa, which could be assigned to some aggregated large peptides (*34*). Interestingly, increasing the DD value to 2.4−4.7% significantly altered the chromatogram patterns. Peak 2 was markedly sharpened, and peak 3 amplitude was dramatically enhanced. The sharpened peak 2 corresponds to the remaining of more hydrolysis-resistant subunits. The increased peak 3 intensity can be attributed to an increased solubility of the large polypeptides (*34*). A further increase of the DD value ≥9.8% resulted in the dissociation of the aggregated large peptides as peak 3 almost disappeared. Additionally, an obvious shift of peak 2 to lower molecular weight range was observed. This indicates that the resistant subunits in hordein started to be hydrolyzed after DD values of 4.7%. Their degradation may account for the extensive hydrolysis of the hordein samples.

Fourier transform infrared spectroscopy (FTIR) is a powerful tool to determine the secondary structure of a protein by analyzing the amide I band. The hordein sample with DD of 1.2% shows several bands in the amide I region (**Figure 6a**), which were assigned to protein secondary structures according to previous reports (*8*, *35*−*38*): α-helices (1653 cm^{-1}), *β*-sheets (1620, 1635, and 1683 cm^{-1}), *β*-turn (1669 and 1675 cm^{-1}), and random coils (1645 cm^{-1}). The band at 1660 cm^{-1} could be mainly assigned to the carbonyl stretching of the glutamine side chain (*38*). The bands at 1683 and 1920 cm^{-1} are believed to be associated with the aggregation process (*35*). When the DD value was increased to 4.7%, the intensity of the bands at both 1683 and 1620 cm^{-1} decreased, suggesting disassociation of protein aggregates, probably due to increased repulsions between protein molecular chains as a result of deamidation. With further increase of the DD value, these two bands disappeared. Additionally, marked shifts

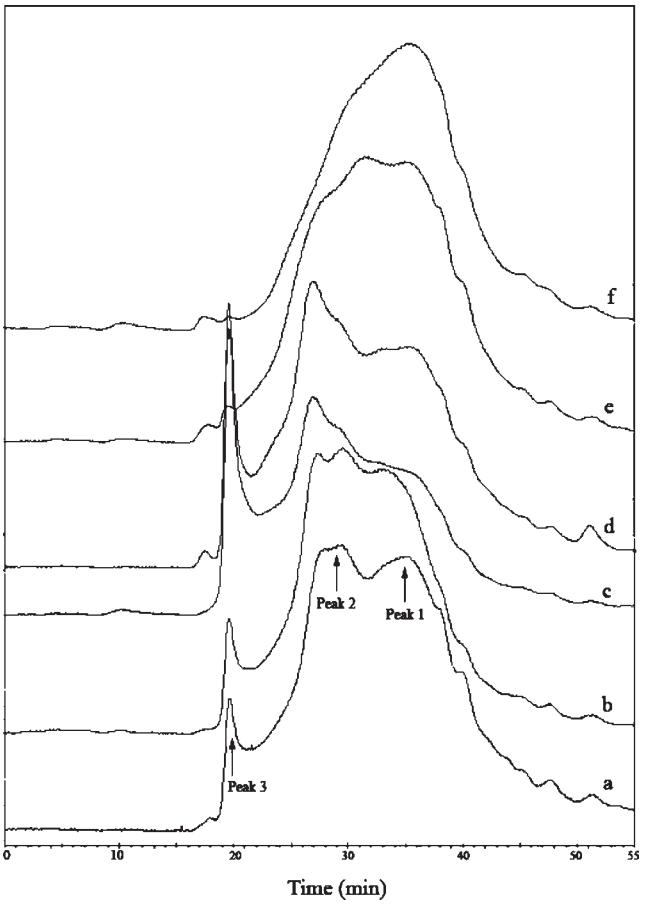

Figure 5. SEC-HPLC chromatograms of the deamidated hordeins: **a**, unmodified hordein; **b**, DD 0.7%; **c**, DD 1.2%; **d**, DD 4.7%; **e**, DD 9.8%; **f**, DD 17%.

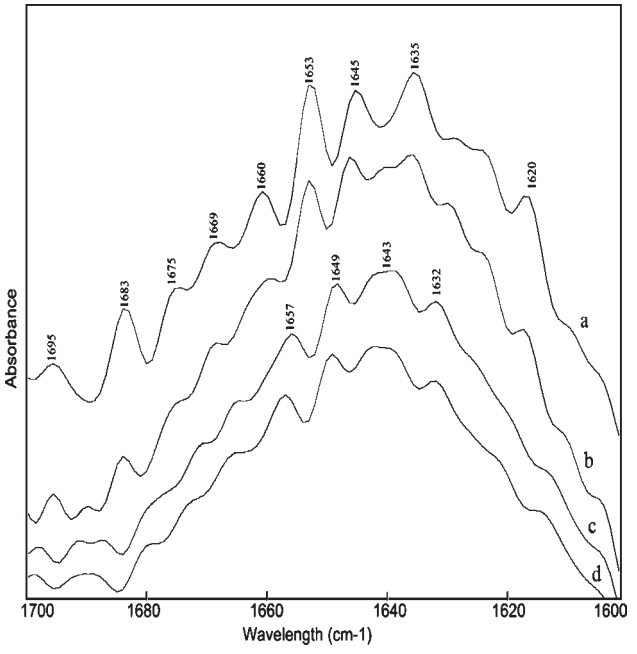

Figure 6. FTIR spectra of the deamidated hordeins: **a**, DD 1.2%; **b**, DD 4.7%; **c**, DD 9.8%; **d**, DD 17%.

in the band positions in the wavelength range of 1620−1660 cm^{-1} were observed. The absorption corresponding to glutamine side chain shifted to 1657 cm^{-1}, reflecting change of intra- or

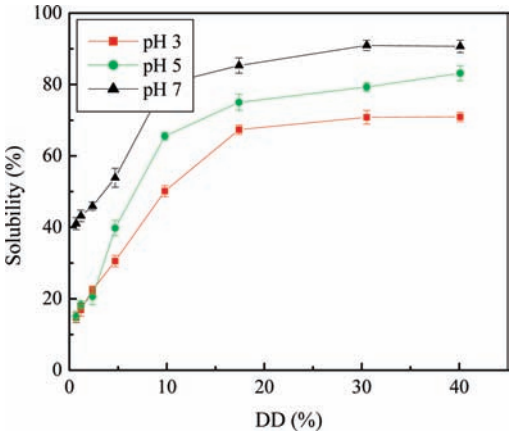

Figure 7. Solubility of the deamidated hordeins at different pH as a function of DD value.

intermolecular hydrogen bonds between glutamine side chains (*38*). Additionally, the α-helix band shifted to lower wavelength (1649 cm^{-1}) and the random coil band (~1643 cm^{-1}) broadened. This suggests that marked protein conformation changes occurred after a DD value of 4.7%, likely associated with protein partial unfolding as a result of both strong negative charge on molecular chains and extensive protein hydrolysis.

A model structure has been proposed for gliadin by Friedli (*39*). In this model, molecules of gliadin aggregate through hydrogen bonding to form a unit with a diameter of 32 nm with their hydrophobic groups exposed outside. These units then aggregate with each other through surface hydrophobic patches to form large aggregates, making them insoluble in water. It is proposed that hordein may have a similar aggregation structure as gliadin owing to their similarity in amino acid composition and biological function in cereal grains. It is obvious that the IEP of the hordein shifted from a pH of around 6 to 3 due to the introduction of additional carboxyl groups on the protein side chains. Limited deamidation (DD value ≤4.7%) can dissociate protein aggregates to a certain extent as revealed by the FTIR result. As a consequence, more hydrophobic patches on protein unit surfaces were exposed outside, thus increasing hordein surface hydrophobicity. The dissociation of these large aggregates may have led to formation of water-soluble peptide aggregates as observed in the deamidated hordein SEC chromatograms. On the other hand, higher deamidation (DD > 4.7%) has led to protein unfolding and extensive hydrolysis. As a result, more polar groups on protein side chains were exposed outside; thus a decreased hordein surface hydrophobicity was observed.

Solubility. Our previous research showed low solubility (<20%) of unmodified hordein at pH 3−7 (*19*). After deamidation, hordein solubility significantly increased ($p < 0.05$) at all the pHs tested (**Figure 7**). The most insightful improvement was the remarkably increased protein solubility at pH 5 from 15 to 83% with DD value increasing to 40%. The solubility was slightly lower at pH 3 due to shift of the hordein IEP to acidic pH. The improvement in solubility within a DD range of 0.7−4.7% could be due to the dissociation of hordein aggregates and partial protein hydrolysis. Further increased solubility after a DD value of 4.7% could be attributed to protein partial unfolding and extensive hydrolysis. These structure changes led to the exposure of more charged and polar groups to the surrounding water, thus promoting protein−water interaction and an increased solubility (*34*). Due to the high proportion of nonpolar amino acid residues and high surface hydrophobicity, hordein is soluble in water only with the presence of alcohol, high concentrations of urea, high concentrations of alkali (pH 11), or anionic detergents (*40*). The remarkably improved solubility after deamidation at both acidic and neutral pHs will enable a broader range of hordein usage in food and nonfood areas potentially.

Foaming and Emulsifying Properties. Our previous data (*19*) showed good foaming capacity and emulsifying stability of unmodified hordein at both acidic and neutral conditions (FC 150−160%, ECS 57−61%, ETS 51%), whereas these values were relatively low at pH 5 (FC 90%, ECS 31%, ETS 18%). Unmodified hordein demonstrated FC values of 32−52% at acidic and neutral pHs. However, due to its poor solubility, unmodified hordein requires dehydration at pH 11 followed by adjusting pH back to acidic and neutral conditions to enable foaming and emulsifying functionalities. This procedure is not practical in commercial food systems. Deamidation significantly improved hordein solubility even within a limited DD range, thus allowing functionality testing by dispersing samples at different pH buffers directly in this study. As shown in **Figure 8**, with increasing of the DD value, the foaming capacity initially increased until a DD value of 2.4−4.7%, then decreased at all the pHs tested. A much more rapid decrease in FC value was observed at pH 3 and pH 5 than pH 7. The optimal FC values were obtained at a narrow DD range (2.4−4.7%) where a significant improvement of foaming capacity was observed at pH 5 (145%) and pH 7 (190−200%) compared to unmodified hordein. The optimal FC obtained at pH 3 is on the same level as that of the unmodified sample. The deamidated hordein samples also possessed an increased foaming stability (20% to 50−60%) at both pH 5 and 7 when the DD value was raised from 0.7 to 4.7%, and then leveled off after a DD value of 4.7%, whereas low FS values were observed at a DD range of 0.7−4.7% at pH 3, and this value increased rapidly after a DD value of 4.7%.

The initial increase of the FC value within the DD of 0.7−4.7% is probably due to an increase of the protein solubility, enabling easy protein diffusion to the air/water surface. Moreover, the exposed hydrophobic patches on hordein surface facilitated their binding and aggregation at hydrophobic air surfaces, forming viscoelastic films to stabilize foams. Although deamidated hordeins exhibited better solubility at a DD value >4.7%, their surface hydrophobicity decreased, which can be one major reason accounting for the decreased FC values at DD range of 9.8−40%. A significantly greater foaming capacity ($p < 0.05$) observed at pH 7 compared to pH 3 and 5 within the optimal DD range may be related to a greater surface charge on protein molecular chains at neutral pH. This created a strong repulsion between adjacent bubbles, preventing quick foam coalescence during homogenization process. This greater surface charge could also explain the slower decrease in the rate of the FC values at pH 7 after a DD value of 4.7%. The overall stability of a foam is related to the resistance of the lamella to drain and of the bubbles to collapse. Normally high molecular weight proteins form stronger films around air bubbles to stabilize the foams. Thus, the aggregated large peptides at the DD range of 2.4−4.7% may have contributed to the increased hordein foaming stability at both pH 5 and 7. However, the FS values did not decrease after a DD value of 4.7% when the large peptide aggregates were dissociated. It is noticed that the FC values decreased significantly ($p < 0.05$) after a DD value of 4.7% at both pH 5 and 7. Thus, it is deduced that fewer protein molecular chains have suitable molecular structures for foam forming compared to samples with a DD of 2.4−4.7%. However, once foams were prepared, the protein chains with a suitable structure could form continuous and rather rigid films around bubbles. Previous literature reported that the optimal foaming stability was observed near the protein IEP. Proteins can adsorb better to the air/water interface at minimum electrostatic

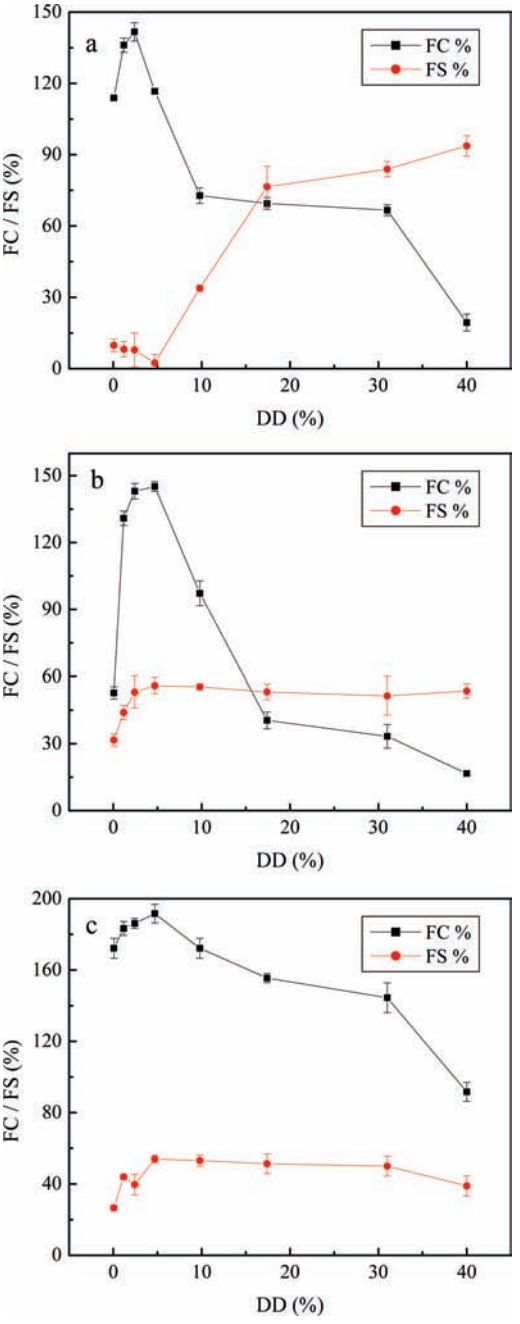

Figure 8. Foaming capacity and stability of the deamidated hordeins at (**a**) pH 3, (**b**) pH 5 and (**c**) pH 7 as a function of DD value.

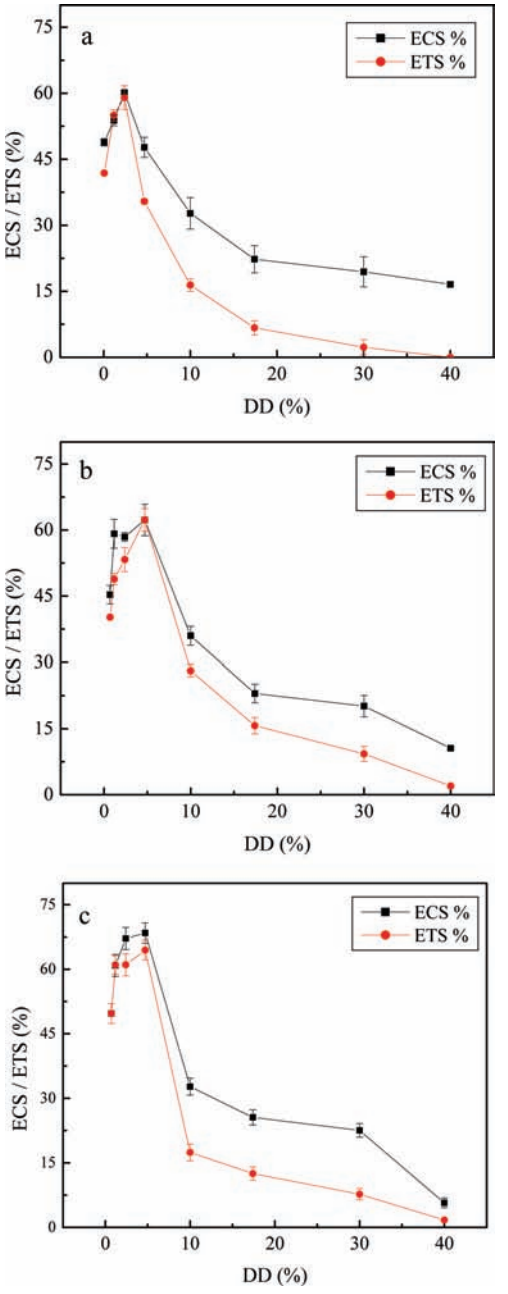

Figure 9. Emulsifying centrifuge and thermal stability of the deamidated hordeins at (**a**) pH 3, (**b**) pH 5 and (**c**) pH 7 as a function of DD value.

repulsion to form a rigid film against coalescence (*41*). However, a very low FS value (≤9.8%) was observed for deamidated hordeins at a DD range of 0.7−4.7% when the pH was 3 (near the IEP). It is assumed that the deamidated hordeins, including the large peptide aggregates, also formed a thick and rigid film at the air/water interface at pH 3. This film may have a strong tendency to aggregate when the surface charge is low. This resulted in extensive aggregation of protein films between adjacent gas bubbles, leading to film rupture, and foam instability. When the DD value was increased to >4.7%, large peptides were dissociated and the hordein peptide bonds were cleaved. The hydrolyzed peptides stabilizing the air bubbles may exhibit less tendency to aggregate. This explains a significant increase ($p < 0.05$) of the FS value observed with a DD value increasing from 9.8 to 40% at pH 3.

As shown in **Figure 9**, an increase of the ECS value was also observed until a DD value of 2.4−4.7% at all the pHs tested. When the DD value was increased beyond 4.7%, the ECS value decreased rapidly. The change of the ETS values as a function of DD value followed the same trend. The initial increase of the ECS value can be attributed to increase of both protein solubility and exposed hydrophobic side chains, since protein solubility and hydrophobicity have a strong correlation with emulsifying properties (*42*, *43*). The aggregated large peptides observed in the SEC chromatograms may also play an important role to stabilize the emulsions. Large peptides can generally form a rigid film at the oil/water interface to prevent the close contact of oil droplets, and decrease flocculation and coalescence (*44*, *45*). A further increase of the DD value (>4.7%) resulted in decreased protein surface hydrophobicity as a result of protein unfolding to expose the polar side chains, dissociation of the large peptides, and extensive protein hydrolysis. All of these factors would prevent the formation of a continuous protein film at the oil−liquid interface, leading to reduced emulsion stability. The deamidated hordein

demonstrated an excellent capacity to stabilize the emulsion at a DD value of 2.4−4.7% as around 70% of formed emulsions remained even after heating and centrifuge processing. This favorable property is likely due to hordein's unique molecular structure such as a strong surface hydrophobicity and a tendency to form aggregates. The excellent thermal stability may be due to further gelation of the deamidated hordein around the oil droplets during thermal treatment to form a reinforced film.

In conclusion, deamidation was an effective approach to modify barley hordein. The hordein structural changes as a result of deamidation greatly influenced their functionalities. Limited deamidation (≤4.7%) could dissociate hordein aggregates, leading to a great increase of protein solubility and protein surface hydrophobicity. These changes occurred without dramatically altering the protein secondary structure, and therefore improved protein foaming and emulsifying properties. With a further increase of the DD value to greater than 4.7%, hordein underwent extensive hydrolysis and a marked conformational change, leading to decreased functionalities. The optimal functionalities were obtained at a narrow DD range (2.4−4.7%) where hordein samples demonstrated significantly improved solubility and emulsifying and foaming properties at both acidic and neutral pHs. These results suggest that the deamidated hordein would be an excellent candidate to be developed as an emulsifying and foaming ingredient. To ensure wide applications in food and cosmetic industries, research toward a better understanding and control of solubility of hordein and its aggregates is necessary.

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