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Effect of Alkaline Deamidation on the Structure, Surface Hydrophobicity, and Emulsifying Properties of the Z19 α-Zein

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Different deamidation conditions for the Z19 α-zein were studied in order to find the best conditions for the development of the emulsifying properties. Alkaline deamidation was chosen, and the effects on the peptide bond cleavage, secondary structure, emulsifying properties, and surface hydrophobicity were studied. The Z19 α-zein was deamidated by using 0.5 N NaOH containing 70% ethanol at 70 °C for 12 h. A deamidation degree (DD) of 60.6 ± 0.5%, and a degree of hydrolysis (DH) of 5 ± 0.5% were achieved. Analysis by far-UV circular dichroism showed that the denaturation was mainly promoted by the high temperature used during the incubation. The adequate balance between the DD and the DH results in an effective emulsifying property improvement for the Z19 α-zein. Thus, after the deamidation treatment, the surface hydrophobicity decreased from $9.5 \times 10^4 \pm 6.8 \times 10^3$ to $46 \times 10^4 \pm 2.1 \times 10^3$, and the emulsion stability increased from 18 ± 0.7% to 80 ± 4.7% since the oil globules stabilized by the modified protein were smaller (57.7 ± 5.73 nm) and more resistant to coalescence than those present in the native protein emulsions (1488 ± 3.92 nm).

KEYWORDS: α-Zein; protein emulsifying properties; protein deamidation; protein hydrolysis; surface hydrophobicity; circular dichroism; dynamic light scattering

INTRODUCTION

Zeins, maize (*Zea mayz*) prolamins, are synthesized during the endosperm development and stored in proteic bodies (*1*). α-Zeins are the most abundant (75−85% of the total) prolamins and are divided in two types: Z19 and Z22, with approximate weights of 22 and 25 kDa, respectively (*2*). The Z19 protein is rich in hydrophobic amino acids (more than 50%), especially aliphatic amino acids, with the highest levels corresponding to alanine, leucine, and proline (*3*, *4*). This structure yields high aliphatic indexes and high surface hydrophobicity. The low amounts of polar-charged amino acids explain their high insolubility in water and their tendency to aggregate. The amount of aromatic residues is low, and there are no tryptophan residues (*3*). Another of its characteristics is that almost all the *β*- and *γ*-carboxyl residues of aspartic and glutamic acids are amidated (asparagines and glutamines, respectively). α-Zeins possess a high α-helical structure content. The Z19 protein is 40% α-helical and 14.5% *β*-sheet (*4*). Various authors (*5*−*7*) propose that the Z19 protein is divided in two distinct regions: a hydrophobic hydrocarbon portion is thought to dominate one end of the zein molecule, while the other end is weakly polar resulting from an abundance of hydroxyl, ethoxyl, and keto groups. These features promote poor functional properties. Consequently, the α-zeins are not utilized as food ingredients in human food and are destined to animal consumption (*4*). However, due to all the features mentioned before, Z19 is suitable for modifications to improve its functional properties, particularly its emulsifying properties. Proteins are commonly used as emulsifiers of oil/water mixtures in food products, but the relationship between protein structure and emulsifying properties has not been clearly established (*8*). The amphipatic α-helix is a structural feature that has previously been proposed as favoring good emulsifying properties and contributing to the surface activity proteins (*9*−*11*). Furthermore, *in vitro* studies have shown that the presence of an interface can induce or increase the degree of α-helix formation (*12*−*14*). The structure−function relationship of food proteins can be studied by modifying the amino acid side chains (*15*).

The modification of the protein usually refers to physical, chemical, or enzymatic treatments that change its conformation and structure and, consequently, its physicochemical and functional properties (*16*). Deamidation, the hydrolysis of amino acid side chains of the protein, is one of such modifications, which is considered necessary for the development of desirable food-use functionality. Deamidation could be performed by chemical or enzymatic methods. The chemical reactions by mild acid hydrolysis and/or basic solutions have been used to modify many cereal proteins, such as wheat, corn, and oat proteins that have high levels of the amide-containing amino acids (glutamine

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and asparagine). These side chains are susceptible of hydrolysis on the amide bond, which leads to the release of ammonia and the transformation of acidic groups. The conversion of the amide groups into acid groups may partially unfold the protein, resulting in an amphiphilic molecule that can be used as a surface active agent or emulsifier for food processors (*17*).

The deamidation process may indirectly lead to protein hydrolysis by cleavage of the peptidic bond; its extent depends on the reaction conditions. Thus, a moderate hydrolysis will increase flexibility and exposure of different fragments in proteins containing a high proportion of secondary structures (*18*). It has been shown that these changes improve the functional properties of different proteins, making them useful products, especially for the food, pharmaceutical, and related industries (*19*, *20*). However, excessive peptide bond cleavage during hydrolysis could affect the polymeric structure of the protein, often resulting in undesirable properties such as bitter taste and reduced functionality (*18*).

The use of enzymes in protein modification is desirable due to their speed, mild reaction conditions, and high specificity. Transglutaminase, protease, peptidoglutaminase (PGase), glutaminase (*21*, *22*), and recently protein glutaminase (PG) (*23*) are the only enzymes reported in literature for protein deamidation. In both types of deamidation, the changes in structure and physicochemical properties as well as the effect on the functional properties are unclear. A partial protein unfolding with hydrophobic amino acids residue exposure could be verified as a consequence of chemical and physical modifications of proteins.

Hydrophobic interactions are extremely important in the protein folding as well as protein interactions. The changes in the hydrophobic surface of the protein can result in important changes in the functionality of the protein (*24*). A significant correlation has been obtained between the emulsifying activity and the protein hydrophobicity determined fluorometrically (*24*). These results suggest that the emulsification of oil by protein could be explained based on protein hydrophobicity. In addition to protein hydrophobicity, the protein flexibility is another important feature to consider for emulsifying capacity of a protein, since they could facilitate its interaction with the oil surface. An increment of surface hydrophobicity could decrease the interfacial tension between oil and water. Thus, the increment in emulsifying capacity could be a debt to a better hydrophobicity−hydrophilicity balance, which is more effective in the modified protein (*15*).

In this paper, we compared different methods for the deamidation of the Z19 α-zein. Alkaline deamidation was chosen in order to study the effect of deamidation on the structural characteristics (peptide bond cleavage, secondary structure content, and surface hydrophobicity changes) of the protein and relate them to the emulsifying properties.

MATERIALS AND METHODS

Biological Material. Waxy yellow dent from corn hybrid grains (*Zea mays* L.) were grounded in a disk mill until a particle size of 40 μm was obtained (Weber Bros. & White, Metal Works Inc., U.S.).

Z19 α-Zein Extraction and Purification. The extraction was performed according to Dickey et al. (*25*). with a slight modification. Lipids from maize flour were extracted with hexane. Carotenes and xanthophylls were removed using a chloroform−methanol mixture (2:1). Defatted, decolorized flour was mixed with 95% ethanol (5:1 solvent−flour (v/w)) through agitation for 12 h at 25 °C. This mixture was then centrifuged at 12000*g* for 30 min at 4 °C, and the supernatant was recovered. The protein was purified using the previously described protocol (*3*): a cationic exchange column was used (SP-Sepharose 2.6 × 11 cm Amersham Biotech, Uppsala, Sweden). The mobile phase was a citrate buffer, 0.02 M with 70% methanol, pH 3.5. The sample was solubilized in this phase to reach a final concentration of 0.5 mg/mL and injected into a high-pressure liquid chromatograph (AKTA prime, Amersham Pharmacia Biotech, Uppsala, Sweden). The elution buffer was 0.7 M NaCl, and a gradient from 0 to 1.2 mM NaCl was performed. The purified Z19 α-zein preparation was dialyzed against 70% ethanol, concentrated by ultrafiltration using YM10 membranes (Amicon, Millipore, U.S.), and dried under N_2 flux.

Deamidation of Z19 α-Zein. Three forms of deamidation were carried out:

(a) Enzymatic deamidation was performed in a 50 mM sodium citrate buffer, pH 4.9, containing 70% ethanol (such ethanol concentration was determined by preliminary experiments to ensure enzyme activity and solubility), 2 mg/mL purified Z19 protein, and 2 U/mL glutaminase (Catalogue G-5894, Sigma Aldrich, Steinheim, Germany). The deamidation was performed at 25 °C in 24 h intervals from 0 to 144 h. After the reaction, the protein solution was adjusted with NaOH to pH 7.

(b) For the acidic deamidation, Z19 protein (2 mg/mL) was solved in 70% ethanol 0.5 N HCl and incubated at 25 °C in 24 h intervals from 0 to 144 h. After the reaction, the reaction mixture was adjusted with 0.5 NaOH to pH 7.

(c) Basic deamidation was carried out dissolving Z19 protein (2 mg/mL) in 70% ethanol 0.5 N NaOH. The protein solution was incubated at 25 °C in 24 h intervals from 0 to 144 h. After the reaction, the reaction mixture was adjusted with 0.5 HCl to pH 7. For the second part of the experiments, the deamidation conditions were 70% ethanol; 0.5, 1, 1.5, and 2 N NaOH; incubation temperature of 70 °C; and reaction times of 12, 18, 24, and 30 h.

The final protein solutions were dialyzed toward 70% ethanol, concentrated by ultrafiltration using YM10 membranes (Microcon, Millipore, U.S.), dried under N_2 flux, and lyophilized.

Determination of the Deamidation Degree (DD). After incubation, the amounts of ammonia released were determined for all the deamidated Z19 protein solutions by using an Ammonia Enzymatic BioAnalysis Test Kit according to the manufacturer's instruction (Boehringer Mannheim, Darmstadt, Germany). The DD was expressed as the ratio of the amount of released ammonia by deamidation reactions and the total released ammonia when the Z19 protein solution was treated with 3 N sulfuric acid.

Measurement of the Degree of Hydrolysis (DH). The DH is expressed as the percentage of the dissolved protein in the hydrolysate after precipitation in 0.2 N aqueous trichloroacetic acid, refers to the total dissolved protein (100%) obtained after complete hydrolysis with 3 N sulfuric acid.

Circular Dichroism (CD) Spectroscopy. The CD spectra were recorded on a Jasco J-715 spectropolarimeter equipped with a Peltier thermostatic cell holder, using 1 mm path length quartz cell in the far-UV, baseline and solvent corrected. The non-modified as well as the enzymatic, acid, and alkaline deamidated Z19 protein solutions containing a final concentration of 0.5 mg/mL in 70% ethanol were filtered with 0.22 μm membranes (Millipore, Ireland). The non-deamidated Z19 α-zein used as control was incubated at 25 °C, and CD spectra were monitored between 200 and 260 nm at 24 h intervals from 0 to 72 h. After the deamidation treatments, the CD spectra of the Z19 protein solutions were measured at 25 °C between 200 and

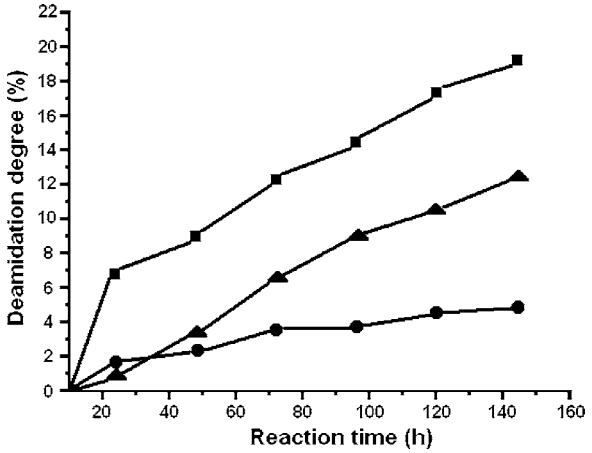

Figure 1. Deamidation degree as a function of time obtained by the (■) enzymatic, (▲) alkaline, and (●) acid deamidation treatments. In all cases, the Z19 protein solution concentration was 2 mg/mL containing 70% ethanol pH adjusted. The incubation was carried out at 25 °C for 144 h.

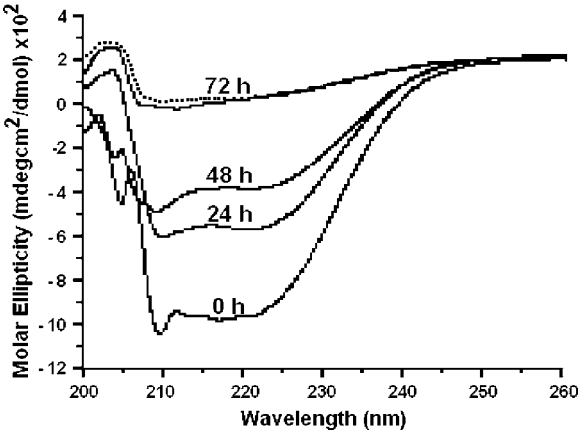

Figure 2. Far UV-CD spectra of the control non-deamidated (solid lines) and enzymatically deamidated (72 h/25 °C, DD = 19%) (dotted line) Z19 α-zein. The control non-modified Z19 zein sample was incubated for 72 h at 25 °C and measured at this temperature. In both cases, the lyophilized samples were solubilized in ethanol 70% with a final protein concentration of 0.5 mg/mL .

260 nm. Three scanning acquisitions were gathered and averaged to obtain the final spectra in all cases. The results are expressed as mean of the residue ellipticity, [θ]mrw, using a value of 109.867 g/mol for the molecular weight of the mean residue. Secondary structure estimation from the far-UV CD spectra was calculated using CDNN (*26*).

Isoelectric Point Calculation. A prepared IEF acrylamide gel (Amersham Pharmacia, Uppsala, Sweden) was used, with a 3−9 pH gradient. The non-modified and deamidated Z19 protein lyophilized samples were solubilized in a 0.5% (w/v) 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate (CHAPS) solution, and 1 μL of each sample was used per lane. Bands were visualized using a silver staining kit (Amersham Biosciences, Uppsala, Sweden).

SDS−Polyacrylamide Gel Electrophoresis (SDS−PAGE). SDS−PAGE was used to examine the purity of the purified Z19 protein. All protein samples were resuspended in equal volumes of deionized water and buffer (0.125 M Tris-Cl, 4% SDS, 20% glycerol, 10% BME, 5 M urea, and 0.01% bromphenol blue, pH 6.8) and then submitted to heating at 100 °C for 10 min. SDS−PAGE was performed according to Laemmli (*27*). Electrophoresis was carried out on 15% (w/v) polyacrilamide gels for all the samples except for the zeins which were carried out on PhastGel 20% (w/v) polyacrylamide gels (Amersham Biosciences, Uppsala, Sweden). The gel was stained with silver (Amersham Biosciences, Uppsala, Sweden) for band visualization. The molecular weight standards (Bio-Rad, Hercules, CA) were as follows: phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa).

Surface Hydrophobicity Index (S_o). Surface hydrophobicity of protein solutions 0.1% (w/v) in 0.01 M 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 (*28*). Fluorescence intensity (FI) was measured at wavelengths of 340−380 nm (excitation) and 505−515 nm (emission) using a Versafluor Fluorometer System (Bio-Rad, Hercules, CA). The 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.

Evaluation of Emulsifying Properties. The emulsifying properties were measured as previously described (*29*−*31*). To prepare the emulsions, 0.2 mL of tricaprilin and 1.8 mL of protein solutions (0.1% w/v in 0.01M sodium phosphate buffer pH 7) were homogenized for 3 min at 22000 rpm with a high-speed homogenizer Ultraturrax (Tekmar, model TYPE STD 1810, Cincinnati, OH).

(*a*) *Emulsion Stability.* The stability of the emulsions was verified by filling microcentrifuge tubes with emulsion and centrifugating at 3000 rpm for 3 min to observe the cream phase formation and to quantify the amount of oil separated.

(*b*) *Particle Size Measurement.* The particle size distribution of the emulsions was measured using dynamic light scattering (DLS). Light scattering measurements were performed in a multi-angle light scattering instrument (Zetasizer Nano series Malvern Instruments, Ireland, U.K.). Emulsion sample aliquots of 25 μL were diluted in 225 μL of 0.01 M sodium phosphate buffer (pH 7) and filtered in 0.22 μm membranes (Millipore, Ireland). The measurements were carried out at 25 °C. Three replicate measurements for each sample were collected and averaged. The results were analyzed with the Zetasizer Nano series Software (2004).

RESULTS AND DISCUSSION

Enzymatic, Alkaline, and Acid Deamidation of Z19 α-Zein. The three different types of deamidation were compared, and their effects on the secondary structure of the Z19 α-zein were determined. **Figure 1** shows the DD obtained as a function of the enzymatic, alkaline, and basic deamidation reaction time. From our previous experiments and in agreement with data reported by other authors (*32*, *23*), the α-zein emulsifying properties improve when DD reaches values higher than 15−20%. Thus, the deamidation reactions were monitored until reach these values. The deamidation reactions catalyzed by the PG reached higher DD values, faster than those obtained by the chemical modifications. Among the chemical modifications, the alkaline diamidation was the most effective. In order to compare the secondary-structure changes obtained by the three different deamidation treatments, the far-UV CD spectra (200−260 nm) of the alkaline, acid, and enzymatic deamidated samples were recorded. To distinguish between the effects of the deamidation versus protein unfolding, a control non-deamidated Z19 α-zein sample was also monitored during its incubation in ethanol 70% at the same temperature and times as the deamidated samples (**Figure 2**). The Z19 α-zein native

Table 1. Degree of Hydrolysis (DH) Obtained Using Different NaOH Concentrations for the Z19 α-Zein Deamidation[a]

	DH (%)[b]			
incubation time (h)	0.5 N	1 N	1.5 N	2 N
12	5 ± 0.5	18 ± 0.6	40 ± 3.1	81 ± 7.8
18	7 ± 0.3	45 ± 1.1	45 ± 2.7	85 ± 7.9
24	8 ± 0.4	57 ± 3.2	64 ± 4.9	86 ± 5.7
30	11 ± 0.5	60 ± 4.2	73 ± 5.1	91 ± 8.3

[a] The protein solutions were incubated at 70 °C in 70% ethanol. [b] Data are expressed as the average and standard deviation of three independent replicates.

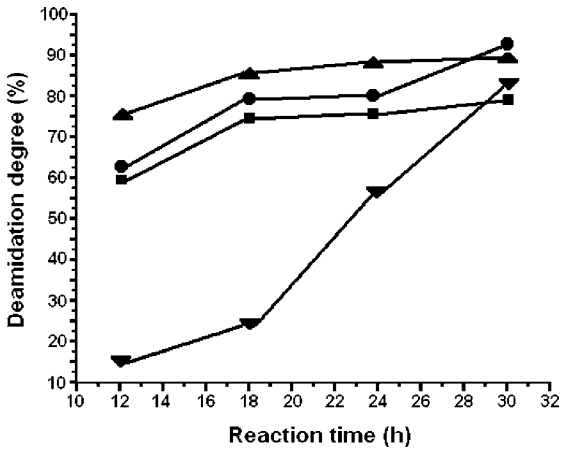

Figure 3. Deamidation degree as a function of incubation time obtained by the alkaline deamidation using different NaOH concentrations: (■) 0.5 N, (●) 1 N, (▲) 1.5 N, and (▼) 2 N. Z19 α-zein solutions (2 mg/mL) contained 70% ethanol. The incubations were carried out at 70 °C for 30 h.

spectra before the incubation at 25 °C shows two strong ellipticity values around 210 and 222 nm (characteristic of α-helix structures) with average molar ellipticity values similar to those reported previously for the Z19 α-zein (*3*, *6*). As expected, no signals were obtained for the chemically deamidated samples (data not shown), indicating a total protein unfolding and denaturation promoted by the high temperature and the strong acidic and alkaline incubation conditions. Signals at 210 and 221 nm were minimal for the enzymatically modified Z19 α-zein. The control non-deamidated Z19 α-zein sample was incubated under the same conditions, and it was totally unfolded. Incubation conditions were the only reason for changes in the secondary-structure content of the non-deamidated control. Thus, the Z19 α-zein proved highly thermolabile, as a result of its tendency to oligomerization, avoiding a reversible thermal renaturation (*33*). Yong et al. (*23*) reported the effects of enzymatic deamidation with PG on the conformation of an α-zein mixture in 70% ethanol at a higher temperature and a longer reaction time. They reported α-helix content of non-modified α-zeins of 46%, on the other hand, for the deamidated sample (64 DD, the incubation was carried out at 40 °C for 137 h), this value decreased to 36%. In contrast, our results show that, at lower temperature (25 °C) and shorter incubation time (72 h), the purified Z19 α-zein looses all the α-helix content. The thermal stability seems to be lower in the purified preparation than in the α-zein mixtures.

The enzymatic, alkaline, and acid deamidation resulted in a water (pH 7) solubility increment of 22 ± 1.7%, 34 ± 2.3%, and 9 ± 2.1%, respectively. The results are in agreement with the reported by previous studies on α-zeins for the enzymatic

Table 2. Results of the Emulsion Stability from Emulsions Obtained from the Z19 α-Zein Alkaline Deamidation Using Different NaOH Concentrations[a]

	emulsion stability (% of emulsified oil)[b]			
incubation time (h)	0.5 N	1 N	1.5 N	2 N
12	80 ± 4.7	40 ± 3.2	10 ± 0.8	0
18	69 ± 3.5	74 ± 4.8	0	0
24	65 ± 3.9	62 ± 4.3	0	0
30	59 ± 4.5	29 ± 1.3	0	0

[a] The protein solutions were incubated at 70 °C. All the protein dried samples were dispersed in 0.01 M sodium phosphate buffer pH 7 (0.1% w/v). 1.8 mL of protein solutions was homogenized with 0.2 mL of tricaprilin, and then they were homogenized and centrifuged. [b] Data are expressed as the average and standard deviation of three independent replicates.

(*23*) and chemical deamidation (*32*). The only deamidation conditions where the Z19 protein did not present precipitates were the alkaline ones. This preparation was easily dispersed in water at pH 7. For this reason, we chose the alkaline deamidation to continue with the surface and emulsifying properties.

Evaluation of Alkaline Deamidation. In order to find the best alkaline deamidation conditions to verify an improvement in the emulsifying properties, four different NaOH concentrations and four reaction times were tested. We decided to raise the temperature to 70 °C to reduce incubation time. This temperature was chosen based on previous studies, in which the high-temperature effects on the protein structure are the irreversible unfolding and protein aggregation (*33*). Disulfide-bonded polymers of zeins are also formed when maize samples are heated. The electrophoretic analysis has shown that during the heating of maize at 100 °C for 3 h, a disulfide-mediated polymerization occurs (*34*, *35*) without significant protein hydrolysis. In addition, heating promotes hydrophobic interactions and the aggregation of the denatured protein molecules, originated by the exposed hydrophobic core of the unfolded protein (*36*, *37*). The protein hydrolysis occurs under the conditions used for alkaline deamidation in this work. Due to protein hydrolysis, molecular properties of proteins change as follows: molecular weight decreased, charge increased, hydrophobic groups are exposed, and reactive amino acid side chains are disclosed (*38*, *39*). As a result, the functional properties are affected. It has been determined that, in general, protein solutions with a low degree of hydrolysis (1−10%) show improved functional properties (mainly foaming and emulsifying capacity) (*40*−*45*). Thus, the measurement of the DH was carried out for the samples (**Table 1**). The DH incremented in function of the incubation time and NaOH concentration. Protein DH, higher than 10%, were detected in almost all the deamidated protein samples, except in those incubated in NaOH 0.5 N for 12, 18, and 24 h.

The increment of DD of the Z19 α-zein obtained by the different NaOH concentrations in function of reaction time is presented in **Figure 3**. DD higher than 50% were reached after 12 h at NaOH concentrations of 0.5, 1, and 1.5 N; during the next 18 h the DD increment was slower. The use of 2 N NaOH caused protein insolubilization since the beginning of the incubation, which delayed the DD increase. After the reaction time reached 30 h, the four protein samples had DD higher than 80%.

Only five conditions promoted emulsion stabilities (ES) higher than 60%. (**Table 2**). The protein sample incubated in 0.5 N NaOH for 12 h showed the best ES, the minor DH and a DD of 60.6 ± 0.5%. The samples with DD near 60% but a

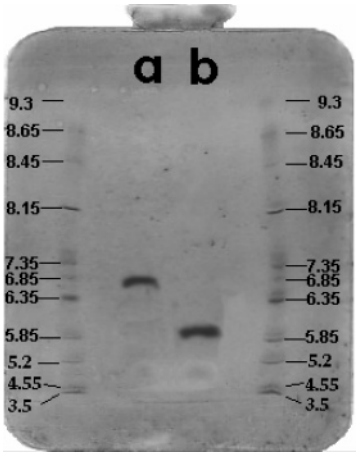

Figure 4. Isoelectric point determination for (**a**) native and (**b**) deamidated (incubation in 0.5 N NaOH/12 h/70 °C, deamidation degree 60.6%) Z19 α-zein. The IEF acrylamide gel pH gradient was 3–9.

Table 3. Surface Hydrophobicity Index (S_0) of the α-Zein Samples

	surface hydrophobicity index (S_0)[a]
α-zein mixtures[b]	500 (± 7) × 10^4
non-deamidated Z19 α-zein[c]	9.6 (± 0.7) × 10^4
deamidated Z19 α-zein[d]	46.2 (± 0.2) × 10^4

[a] Data are expressed as the average and standard deviation of three independent replicates. The S_0 was determined using 1-anilinonaphthalene-8-sulfonic acid (ANS) with protein solutions 0.1% (w/v) in 0.01 M sodium phosphate buffer (pH 7). [b] α-Zein mixtures were obtained before Z19 α-zein purification (Z19 and Z22 mixture). [c] Native Z19 protein. [d] Deamidated Z19 α-zein (incubation in 0.5 N NaOH/12 h/70 °C, deamidation degree 60.6%).

higher DH showed poor emulsion stability. It is important to point out that a high DD does not necessarily promote an improvement in the emulsion stability. These results are evidence of the importance of the balance between the DD and the DH in an effective emulsifying property. These results show that, when deamidation of the Z19 α-zein is accompanied by a limited protein hydrolysis, the emulsifying properties are improved. As it was mentioned before, α-zeins tend to aggregate when they are heated. We suggest that the hydrolysis could reduce the formation of large aggregates, allowing the formation of a proteic web that covers the oil drop. DH results indicate that peptides generated by an extensive Z19 protein hydrolysis tend to destabilize the emulsion, possibly as a consequence of inadequate hydrophobicity−hydrophilicity balances. Conde et al. (*46*) suggested that this misbalance causes the decrease of the monolayer thickness as the degree of hydrolysis increases. These phenomena explained the poor functional properties for the formation and stabilization of an emulsion by protein hydrolysates from sunflower flour isolates at high degrees of hydrolysis.

There was an increment in the negative charge density of the deamidated Z19 α-zein (DD = 60.6%,; DH = 5%, and ES = 80%) (**Figure 4**). Before the deamidation reaction, the isoelectric point diminished from 6.8 to 5.9. This caused the increment in the protein solubility at pH 7. In order to compare the electrophoretic pattern of the α-zeins, the SDS−PAGE of the mixture, composed by the Z19 and the Z22 α-zeins, the non-deamidated, and deamidated Z19 α-zeins is shown in **Figure 5**. The deamidation reaction did not affect the Z19 α-zein electrophoretic pattern.

Evaluation of Surface Hydrophobic and Emulsifying Properties. Due to the propensity of nonpolar amino acid

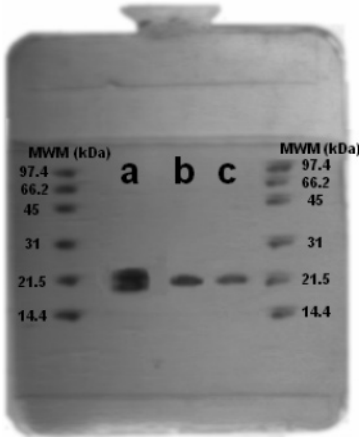

Figure 5. SDS-PAGE analysis of (**a**) α-zeins mixture obtained before Z19 α-zein purification (Z19 and Z22 mixture), (**b**) native Z19 α-zein, and (**c**) deamidated Z19 protein (incubation in 0.5 N NaOH/12 h/70 °C, deamidation degree 60.6%). The amounts of protein loaded were 500, 200, and 100 ng, respectively. The gel was silver stained.

Table 4. Results of the Average Particle Sizes Obtained from α-Zein Emulsions[a]

	mean droplet diameter (nm)
α-zein mixtures[b]	2380 (± 8.4)
non-deamidated Z19 α-zein[c]	1488 (± 3.9)
deamidated Z19 α-zein[d]	57.7 (± 5.7)

[a] Data are expressed as the average and standard deviation of three independent replicates. Protein solutions were 0.1% (w/v) in 0.01 M sodium phosphate buffer (pH 7). [b] α-Zein mixtures were obtained before Z19 protein purification (Z19 and Z22 mixture). [c] Native Z19 α-zein. [d] Deamidated Z19 α-zein (incubation in 0.5 N NaOH/12 h/70 °C, deamidation degree 60.6%).

residues to position themselves in the interior of protein molecules in solutions, thus avoiding contact with the aqueous surroundings, only a portion of them participate in the emulsification of oil into aqueous phase. Hydrophobicity thus measured would be "surface" or "effective hydrophobicity", which does not directly correlate with the "total hydrophobicity" (*47*). The α-zein mixture presented a high surface hydrophobicity index (S_0) (**Table 3**). The S_0 of the non-deamidated Z19 α-zein was smaller than that obtained for the α-zein mixture. Proteins minimize their energy by folding and associating into structures of low energy. In corn seed, the two α-zeins are bonded by electrostatic and hydrophobic, not covalent, interactions. This suggests that the more stable arrangement for these proteins are the large and highly hydrophobic arrangements. When the native Z19 α-zein was purified, rearrangements in the protein structure took place, and many of the nonpolar amino acid residues were hidden. As a consequence of the deamidation treatment and unfolding of the protein, some of these amino acid residues were exposed to the aqueous surroundings, and the S_0 of the deamidated Z19 α-zein was higher than in the native protein. The deamidation reaction promoted the Z19 α-zein S_0 increment due to hydrophobic zones exposure which before the deamidation were hidden inside the protein. As a consequence of protein unfolding and electrostatic and hydrophobic forces rearrangement, the hydrophobic regions reoriented to the surface, and the fluorescence signal increased. Surface hydrophobicity is an important factor in determining the emulsifying properties. As previously reported by other workers (*47*, *48*), the hydrophobicity exposed by the protein would allow a better molecular anchorage to be established in the oil−water interface, giving more stable emulsions. They reported that the

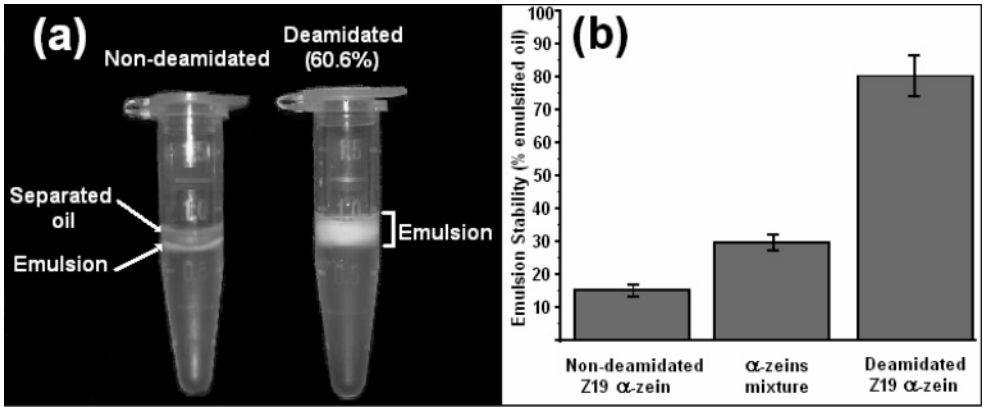

Figure 6. (**a**) Z19 α-zein emulsions obtained from the non-deamidated and deamidated (incubation in 0.5 N NaOH/12 h/70 °C, deamidation degree 60.6%) proteins. Dried samples were dispersed in 0.01 M sodium phosphate buffer pH 7 (0.1% w/v). 1.8 mL of protein solutions was homogenized with 0.2 mL of tricaprilin, and then they were homogenized and centrifuged. The cream as well as the separated oil amount indicates the stability of liquid emulsions to creaming. (**b**) Emulsion stability results obtained from the non-deamidated Z19 protein, the α-zeins mixture obtained before Z19 α-zein purification (Z19 and Z22 mixture), and the deamidated Z19 α-zein.

surface hydrophobicity of the soy protein increased at an early stage of the mild acid treatment and then gradually increased until a deamidation degree of approximately 10% was reached. However, it slightly decreased after that. In the case of the deamidated Z19 α-zein, when compared with that of the non-deamidated, the higher emulsifying capacities might be due to its lower hydrophobicity value. The deamidation treatment significantly improved the emulsion stability of the Z19 α-zein from 18 ± 0.7% to 80 ± 4.7% of emulsified oil (**Figure 6**) since smaller oil globules, more resistant to coalescence, are formed in the deamidated protein (**Table 4**).

The main factors of the improvement of emulsifying properties of the Z19 α-zein are the charge increment and the protein unfolding, which promotes the formation of protein arrangements with better hydrophilic−lipophilic balances to interact in the water−oil interface, stabilizing the oil drop. However, a discrete protein hydrolysis may play an important roll because it could diminish the large aggregates. The generated small peptides, with higher solubility, may facilitate the diffusion at oil−water interfaces and enhance the interaction between the protein and the oil.

ABBREVIATIONS USED

CD, circular dichroism; BME, β-mercapthoethanol; DLS, dynamic light scattering; IEF, isoelectric focusing; DD, deamidation degree; DH, degree of hydrolysis; ANS, 1-anilinonaphthalene-8-sulfonic acid; FI, fluorescence intensity; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate; SDS, sodium dodecyl sulfate; DTT, dithiothreitol; PGase, peptidoglutaminase; PG, protein glutaminasa; ES, emulsion stability; S_o, surface hydrophobicity index.

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