Effects of Heat and Proteolysis on Deamidation of Food Proteins Using Peptidoglutaminase

Jamel S. Hamada

Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 1100 Robert E. Lee Boulevard, New Orleans, Louisiana 70179

Enzymatic methods were developed for the deamidation of food proteins. Modification of proteins by peptidoglutaminase was dependent on their size and conformation. After such treatments as heat or alkali solubilization, which might have partially broken hydrogen and disulfide bonds, peptidoglutaminase deamidation of proteins increased by proteolysis as a function of percent peptide bond hydrolysis (DH), up to 54-fold at 20% DH. With prior heat treatment at 100 °C for 15 min, followed by proteolysis and alkali solubilization of soy protein, casein, and gluten, over 48, 37, and 39% protein deamidation can be achieved, respectively.

INTRODUCTION

The structure of proteins is modified to improve the functional properties for effective use as food ingredients. Modification of proteins is also a possible route for increasing the world's food supply by making food products better suited for human and animal nutritional utilization (Feeney and Whitaker, 1985). Deamidation can improve the solubility and other functional properties of food proteins by increasing the number of negative charges in the protein. Conversion of glutamine (Gln) or asparagine (Asn) to glutamic acid (Glu) or aspartic acid (Asp) in food proteins is expected to have a major effect on proteins, particularly cereal proteins, where up to one-third of the total amino acids is Gln (Whitaker, 1977). Even small levels of deamidation (e.g., 2–6%) could result in a significant improvement of protein functional properties (Matsudomi et al., 1985; Hamada and Marshall, 1989). The use of enzymes provides a multitude of advantages over the use of chemicals in protein modification including speed, mild conditions, and, most importantly, high specificity. This will result in reduction of energy costs and increased processing efficiency. Accordingly, enzymatic methods are more attractive to the food processor and far more compliant to regulatory affairs than chemical treatments.

Transglutaminase, protease, and peptidoglutaminase are the only enzymes reported in the literature for protein deamidation. Peptidoglutaminase appears to be the most feasible for practical application. Food proteins must be chemically modified prior to transglutaminase deamidation (Motoki et al., 1986). This chemical pretreatment poses potential problems for the food processor, and its cost would be prohibitive. Shih (1990) indicated that protein deamidation by proteases, as reported by Kato et al. (1989), was most likely a chemical deamidation, rather than an enzymatic deamidation. Hamada et al. (1988) and Hamada (1991) investigated the potential use of peptidoglutaminase (PGase) for the modification of a variety of food proteins and concluded that the use of prior proteolysis substantially enhanced deamidation. In addition to proteolysis, other means to rupture the compact structure of intact proteins are also required to increase protein deamidation by PGase. Hamada and Marshall (1988) found that heating increased PGase activity toward soy protein and quantified the combined effects of heat and proteolysis of soy protein on its deamidation by PGase.

The procedures previously introduced for PGase deamidation of proteins were limited to basic research on soy protein. The extent to which any protein modification method can be used by industry will depend on the quantity and the quality of the research devoted to it (Feeney and Whitaker, 1985), as well as demonstrated practicality and economic viability. Creative advancement in protein modification research will lead to new markets and expand the food use of many proteins in such products as protein-fortified beverages, infant formulas, coffee whiteners, emulsifiers, and flavor enhancers. The objectives of this research were (1) to study the effects of altering the structure of soy protein, casein, and gluten, which have diverse structures, by solubilization with dilute alkali, heat treatment, and peptide bond hydrolysis on their PGase deamidation and (2) to develop enzymatic methods that may be suitable for industrial applications in the deamidation of food proteins.

MATERIALS AND METHODS

Materials. *Bacillus circulans* culture (ATCC 21590) was obtained from the American Type Culture Collection, Rockville, MD. Alcalase 2.4 L (2.4 Anson units/g) was obtained from Novo Laboratories Inc., Wilton, CT. Casein and wheat gluten were purchased from Sigma Chemical Co. (St. Louis, MO). Nutrisoy 7B flakes were obtained from Archer Daniels Midland Co. (Decatur, IL).

Preparation and Assay of PGase. *B. circulans* cells were grown and harvested and then PGase was extracted and assayed as previously described (Hamada et al., 1988).

Preparation of Soy Protein. The soy protein substrate was prepared by extracting Nutrisoy flakes with water as described by Hamada et al. (1988).

Heat Treatment of Proteins or Protein Hydrolysates. Protein or protein hydrolysate was dispersed in water (10 g/200 mL) in Erlenmeyer flasks. The flasks were covered with screw caps, placed in a boiling water bath, and heated for 15 min at 100 °C, with 400 rpm stirring.

Protein Hydrolysis. Dispersions of both native and heat-treated proteins were hydrolyzed using Alcalase 2.4 L at 50 °C according to the pH-stat method of Adler-Nissen (1986c). The degree of hydrolysis (DH), or percent of peptide bonds cleaved, was determined according to the method of Adler-Nissen (1979). Figure 1 outlines the steps undertaken for the preparation of protein hydrolysates used as substrates for PGase deamidation.

PGase Deamidation of Proteins or Protein Hydrolysates. A soy protein sample was treated with PGase in 0.05 M sodium phosphate, pH 7.5, according to the method of Hamada and Marshall (1988). Except for this sample, proteins and protein hydrolysates were deamidated by PGase according to the method of Hamada (1991) as depicted in Figure 2. The amide content of proteins and protein hydrolysates, corrected for free ammonia,

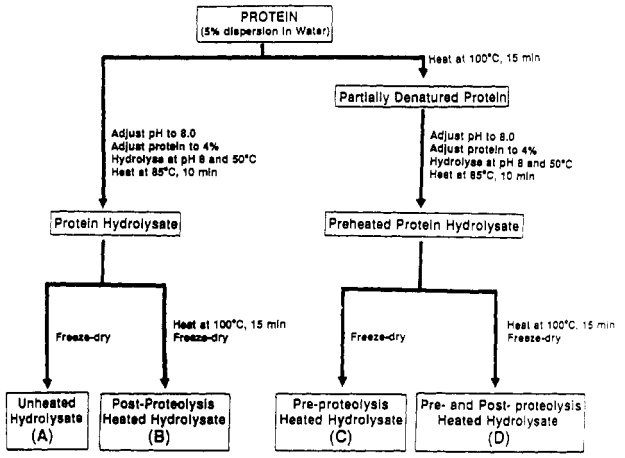

Figure 1. Flow diagram for the preparation of protein hydrolysates with various DH values.

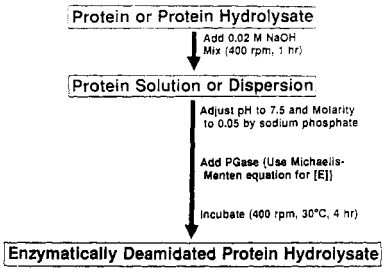

Figure 2. Steps for the preparation of PGase-modified food proteins or protein hydrolysates.

were measured according to the method of Wilcox (1967). The extent of deamidation was calculated as the ratio of ammonia released after each treatment, including PGase treatment, to the total amide content multiplied by 100. Control samples were analyzed for their contents of free ammonia at each step used for the preparation of hydrolysates (Figure 1) and before PGase treatment (Figure 2). The control data were taken into consideration in the calculation of percent deamidation, according to the method of Wilcox (1967).

Statistical Analysis of Variance. Two-factor analysis of variance (DH 0, 5, 10, 15, and 20 and treatments A–D, Figure 1) of the duplicate observations of percent deamidation was performed using the software package and the procedures of Statgraphics (Statistical Graphics Corp., Rockville, MD) to test for significant effects. Tukey's multiple-range test was used to compare the means of the different levels of each factor and to group the levels of a factor together if the different levels were not significantly different (i.e., homogeneous).

RESULTS AND DISCUSSION

Alkali Solubilization of Proteins Prior to Their PGase Deamidation. When soy protein was dispersed in 0.05 M sodium phosphate, pH 7–8, prior to its PGase deamidation, 1.2% deamidation was obtained. However, using a pretreatment, which included alkali solubilization of proteins at pH 11.3, followed by adjustment to pH 7.5 (Figure 2), the PGase deamidation was 6.4%. This 4.5-fold increase in deamidation was apparently due to the unfolding of the rigid and compact structure of the protein by cleavage of disulfide, hydrogen, and hydrophobic bonds (Ishino and Okamoto, 1975). Adding phosphate to lower the pH of the solution of alkali-treated proteins may cause some aggregation of proteins (Ishino and Okamoto, 1975), but some amino acid residues become more accessible through net intermolecular interactions (Nielson, 1985).

Excessively strong alkali treatment leads to destruction of cysteines and the formation of lysinoalanine (LAL), which may be toxic (Woodward and Short, 1973). The light alkaline treatment used here should not have such an undesirable impact on the final products. Friedman et al. (1984) investigated the factors governing the formation of LAL in soy proteins by alkali treatment. Exposing soy protein to severe alkaline conditions (high alkaline concentrations and long times of exposure) favored the formation of LAL. On the other hand, Friedman et al. (1984) found that addition of cysteine, copper salts, dimethyl sulfoxide, and glucose suppressed the formation of LAL.

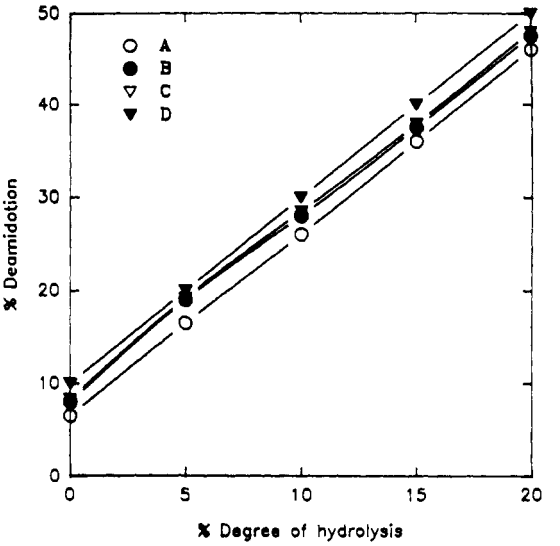

Figure 3. Effect of Alcalase proteolysis and heat treatment on the PGase deamidation of soy protein. Descriptions of symbols A–D are given in Figure 1.

Heat and Soy Protein Deamidation by PGase. When soy protein was heated at 100 °C, PGase deamidation increased only slightly, from 6.4 to 8.6% after 15 min to 9.5% deamidation after 30 min, but was not changed by continued heating, regardless of heating time. Hamada and Marshall (1988) obtained up to a 5.5-fold increase in deamidation of soy protein by the heat treatment at 100 °C for 90 min. This different response to heat might be due to changes in both protein conformation and molecular interactions accompanying the heat treatment and the subsequent NaOH solubilization of the protein prior to its deamidation by PGase. NaOH increased soy protein solubility and, subsequently, the capacity to be deamidated by PGase (4.5 times). This is thought to occur possibly through the breaking of some of the hydrogen and disulfide bonds (Ishino and Okamoto, 1975; Nielson, 1985). Therefore, the improvement in protein deamidation was likely due to the combined unfolding effect by heat and breaking of bonds including the S–S bonds by NaOH.

Heat and Proteolysis of Soy Protein and PGase Deamidation. Protein hydrolysis increased deamidation linearly from 6% in the intact protein to 44% in 20% DH hydrolysates (A in Figure 3). The method Hamada and Marshall (1988) used to deamidate soy protein by PGase increased soy protein deamidation linearly to 10% DH, but deamidation rapidly leveled off at higher DH values. In both cases, the significant improvement in soy protein deamidation was due to changes in both protein conformation and molecular size accompanying proteolysis. Breaking some hydrogen and S–S bonds by NaOH after proteolysis might have provided peptide structure with maximum accessibility of amide groups to PGase as evidenced from the straight line obtained for the relationship of DH with the rate of PGase deamidation (A in Figure 3).

Heat treatment of hydrolysates at 100 °C for 15 min after Alcalase hydrolysis increased PGase deamidation (B in Figure 3). In agreement with results for heating soy protein hydrolysates, Hamada and Marshall (1988) found

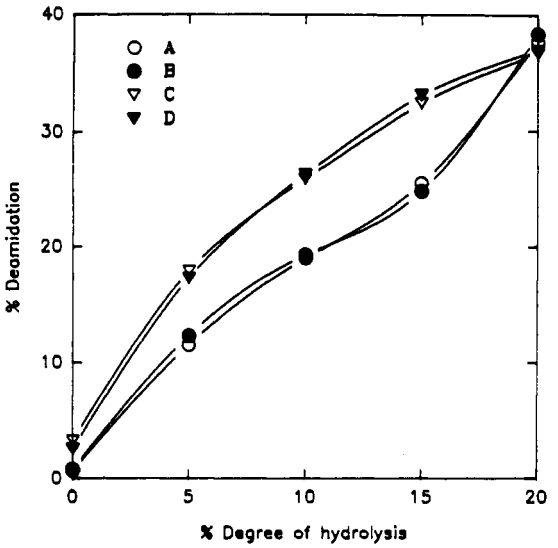

Figure 4. Effect of Alcalase proteolysis and heat treatment on the PGase deamidation of casein. Descriptions of symbols A–D are given in Figure 1.

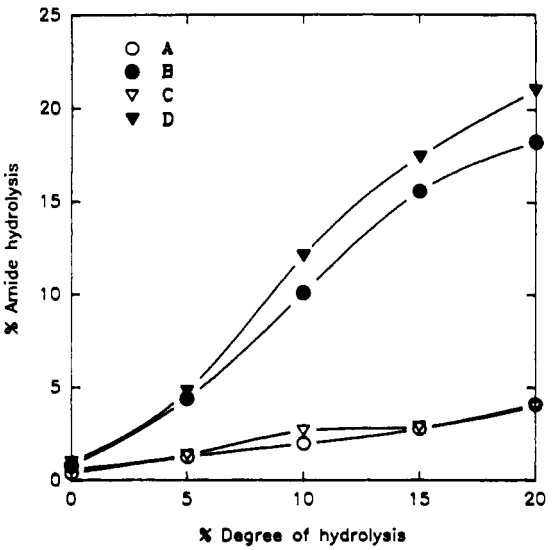

Figure 5. Effect of heat treatment of gluten and gluten hydrolysates on non-PGase deamidation. Descriptions of symbols A–D are given in Figure 1.

that heating soy protein hydrolysates also increased the rate of deamidation as a function of DH. Also, heat treatment of soy protein at 100 °C for 15 min prior to its hydrolysis (C in Figure 3) increased the deamidation of hydrolysates compared to the protein of plain proteolysis. There was not a significant difference observed between the B and C treatments of Figure 3. Increased deamidation could be a result of more uniform split of the peptide bonds in the course of protein hydrolysis, which may increase accessibility of amide to PGase attack. Adler-Nissen (1986a,d) showed that protein denaturation prior to hydrolysis increased the rate of the proteolysis reaction and resulted in hydrolysates with a fairly continuous distribution of intermediate-size peptides, reducing the possibility of obtaining small peptides or intact proteins.

Multiple-range analysis categorized the four pretreatments into three homogeneous groups (A, B and C, and D) with significant difference between groups. Heating increased deamidation of hydrolysates produced from proteins that were preheated prior to their hydrolysis (D in Figure 3). As a result of the two heat treatments, proteolysis and alkali solubilization, the limited PGase deamidation of soy protein increased up to 50% for 20% DH hydrolysates. Although proteolysis combined with heat treatment prior to protein hydrolysis was effective in enhancing protein deamidation by PGase, the extent of deamidation observed by Hamada and Marshall (1988) was nearly half that found using the NaOH solubilization method. The different responses of the proteins deamidated according to the two methods may be due to breaking of some S–S and other bonds by NaOH. This resulted in maximum amide accessibility, and the PGase deamidation was, thus, dependent on the molecule size, i.e., the DH value.

Heat and Proteolysis of Casein and PGase Deamidation. Protein hydrolysis increased deamidation of casein from 0.8 to 25% in protein hydrolysates with DH value of 15% and increased markedly to 37% for hydrolysates with DH value of 20% (A in Figure 4). The dramatic change in deamidation in the DH range 15–20% may indicate that substantial amounts of glutamine residues are hidden inside the molecule, probably by noncovalent bonds and/or disulfide bonding from κ-casein. The κ-casein, constituting about 15% of the total caseins, contains disulfide bonds, which cross-link the 19-kDa monomer to 60–600-kDa proteins (Wong, 1989). The results presented differ from observations of Gill et al. (1985), who found no PGase deamidation of whey and casein proteins after protein hydrolysis to DH = 2%. However, Gill et al. (1985) reported that limited portions of 0.2 and 0.36 μmol of glutamine, of the 17.5 and 9.7 μmol present, were converted to glutamic acid by PGase at DH values of 3.5 and 4.0% for casein and whey hydrolysates, respectively.

Analysis of variance indicated that heat treatment of hydrolysates had no effect on their susceptibility to deamidation by PGase (B in Figure 4). Contrary to results for heating casein hydrolysates, heating soy protein hydrolysate at 100 °C for 15 min after Alcalase hydrolysis increased deamidation as a function of DH at all degrees of hydrolysis. The different response of casein and soy proteins may be due to difference in their structures. The colloidal structure of casein micelles is very stable, persisting at 140 °C for 20 min (Wong, 1989). Although caseins are not true random coils, they have a low percent helix due to high proline contents. As a result, casein is not denatured by heat (van den Hoven, 1987). These results of heat treatment are in agreement with the work of Gill et al. (1985) in which PGase deamidation of casein or casein hydrolysates was not affected when heat or other protein-denaturing conditions were used.

Heat treatment of casein prior to its hydrolysis significantly increased the deamidation of hydrolysates with DH less than 20%, compared to plain proteolysis or post-proteolysis heat treatment (C in Figure 4). This could be due to a more uniform peptide bond hydrolysis and access to some disulfide bonds from κ-casein, which were cleaved by the alkali treatment. Heating after protein hydrolysis, however, had no effect on the deamidation of hydrolysates, preheated prior to hydrolysis (D in Figure 4). Therefore, the best pre-PGase treatment of casein is heat followed by proteolysis when DH is below 20%.

Deamidation during Pre-PGase Treatments of Gluten. There was no considerable nonenzymatic deamidation during the preparation of soy protein or casein hydrolysates prior to the application of PGase as described in Figures 1 and 2. The greatest levels of ammonia, which was brought about by the pre-PGase treatments of these two proteins, were less than 3 and 2%, respectively. But unlike the effect of heat treatment on the nonenzymatic hydrolysis of the amide of soy and casein hydrolysates, heating gluten hydrolysates generated considerable free ammonia (Figure 5). Free ammonia concentration was also higher for preheated hydrolysates than hydrolysates

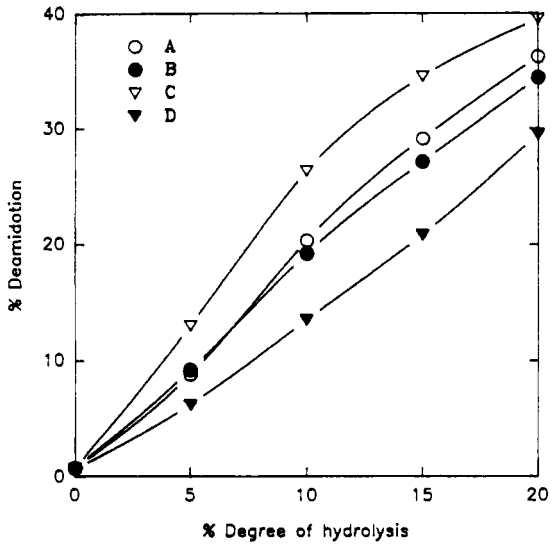

Figure 6. Effect of Alcalase proteolysis and heat treatment on the PGase deamidation of gluten. Descriptions of symbols A–D are given in Figure 1.

prepared from native gluten. Deamidation ranged from 1.0 to 20.4% and was dependent on the DH values. In addition to amide hydrolysis, there might have been another source for the nonenzymatic deamidation during the heat treatment. Succinimide rearrangement reaction could lead to the deamidation of both Asn and Gln during the heat treatment (Lura and Schirch, 1988). While Asn was specified in the paper on nonenzymatic deamidation of protein by internal elimination mechanism (Lura and Schirch, 1988; Patel and Borchardt, 1990), Gln can be also deamidated by the same mechanism.

Kato et al. (1989) reported that up to 20% of the amide bonds in proteins were hydrolyzed using the proteases papain, Pronase E, or chymotrypsin at pH 10 and 20 °C. Deamidation resulted in an increase in gluten solubility at all pH values in the range 2–12 and improved emulsifying and foaming properties of the proteins as well. Shih (1990) found that proteases showed no deamidating activity toward soy protein and reported the amide hydrolysis was nonenzymatic and catalyzed by anions such as phosphate and bicarbonate. It is obvious from Figure 5 that this nonenzymatic deamidation was due to the direct effect of heat on protein hydrolysates and not due to proteolysis. Possibly, the heat treatment of the hydrolysates at pH 8 catalyzed the hydrolysis of the amide of accessible Gln and Asn residues. The extent of deamidation was dependent either on the presence of ions produced during protein hydrolysis or on the extent to which the originally hidden Gln residues could be accessed as evidenced by the proportional relationship of this type of deamidation with the degree of hydrolysis (DH).

Heat and Proteolysis of Gluten and Deamidation by PGase. Gluten hydrolysis increased PGase deamidation (A in Figure 6) from less than 0.8% for intact gluten to about 35% for 20% DH gluten hydrolysates. Heat treatment of gluten hydrolysates had little or no effect on PGase deamidation (B in Figure 6). The deamidation of heated protein hydrolysates was similar to that of unheated hydrolysates at DH 0–10% and was slightly lower at DH 10–20%. The nonenzymatic deamidation of hydrolysates (B in Figure 5) accounted for approximately one-third of the total deamidation brought about by both the heat treatment (B in Figure 5) and the PGase action (B in Figure 6). This substantial amount of amides hydrolyzed nonenzymatically may have been the reason for the decreased subsequent deamidation by PGase.

Heat treatment prior to hydrolysis, however, considerably increased the deamidation of hydrolysates at all DH values (C in Figure 6), compared to the unheated or the postproteolysis heat-treated hydrolysates. Protein deamidation by PGase increased 54-fold as a result of the combined effects of proteolysis (to 20% DH) and heat treatment prior to protein hydrolysis. Also, reheating the preheated hydrolysates after protein hydrolysis had a deleterious effect on the rate of deamidation (D in Figure 6), compared to the effect of preproteolysis heating alone. Decline in PGase deamidation may be partially due to the reduction of the number of the amides accessible to PGase. From D in Figure 5 and D in Figure 6, nearly half of the total deamidation of hydrolysates was the result of the heat treatment alone. Accordingly, substantial numbers of amides were already hydrolyzed before the PGase treatment and thus were not available for PGase attack. At 20% DH, the total deamidations of gluten hydrolysates with the four different pretreatments (sets A–D) were 38, 49, 42, and 49%, of which 35, 33, 39, and 29% resulted from PGase attack. Furthermore, the difference between the means of the four treatments (A–D) was statistically significant as estimated by multiple-range analysis at 95% confidence level.

As in the case of soy protein and casein, proteolysis of gluten increased PGase deamidation. However, heat treatment of gluten hydrolysates resulted in remarkable nonenzymatic hydrolysis of amides and thus substantially decreased their subsequent deamidation by PGase. Gluten structure is different from that of casein or soy protein, being distinguished by its high S–S bonds and high hydrophobic bonding that lead to high molecular weight and highly aggregated structure in which Gln residues are hidden inside the molecule (Wall, 1979). Hydrogen bonding is more extensive in gluten than in most proteins and is caused by the high content of glutamine. Accordingly, gluten is a tightly coiled protein containing substantial numbers of S–S bonds that give the protein its rather fibrous, rigid structue. Therefore, proteolysis or breaking of either or both of the S–S and hydrogen bonds ruptures the protein and increases amide exposure to further PGase deamidation.

Anticipated Changes in Protein Functionality. The negative charges created in the deamidated polypeptides lead to increased solubility and improved emulsification and foaming properties. Solubility of soy protein, deamidated to 6–16% by PGase, increased at pH 4–7 and under alkaline conditions. Deamidation also increased emulsifying activity, emulsion stability, and foaming power (Hamada and Marshall, 1989). The improvement in solubility and emulsification under the mildly acidic conditions imparted by deamidation should make the deamidated soy protein better suited for a number of food applications, such as carbonated and noncarbonated beverages, pourable and nonpourable dressings, and coffee whiteners. Therefore, protein deamidation can be a particularly useful modification method for soy and other Gln-rich proteins, in which even small levels of deamidation result in a significant improvement of functional properties of the proteins.

A substantial improvement in emulsification, foaming, and other functional properties of the casein proteins is expected upon deamidation. Because of its extensive disulfide and hydrogen bonding, gluten is not easily solubilized. After the hydrolysis of the amide group, however, the hydrolysate becomes more soluble through the abolition of some amide groups and hence the dissociation of gluten aggregates, caused by hydrogen bonding and hydrophobic interactions, and the creation of acidic peptide segments. The uneven distribution of glutamate-bound peptides, combined with a high content of glutamic

acid itself, should result in an acidic and highly soluble segment of gluten subunits. These polypeptides, therefore, should play a major role in enhancing solubility and other functional properties under mildly acidic conditions when applied to foods.

For a long period of time, the problem of bitterness of protein hydrolysates has been of major concern to many food processors. One way to avoid the bitterness of hydrolysates is to restrict the DH values to less than 5.0% (Adler-Nissen, 1986b). However, one should not arbitrarily limit protein hydrolysis to produce hydrolysates with DH less than 5% to avoid bitter peptide bond formation. The taste of protein hydrolysates is a more complicated phenomena than just whether the bitter peptides are present or absent. There are other tastes contributed by other amino acid residues such as sweet, sour, brothy, or beefy sensations. Furthermore, bitter peptide formation can be dealt with as a separate issue if and when the problem arises. Several methods have been developed for the debittering of the bitter protein hydrolysates. These methods included the use of immobilized protease (Deter et al., 1975), the selective separation of bitterness components, such as adsorption and ultrafiltration, the plastein reaction, the application of exopeptidases (e.g., degrading of proline-rich, bitter peptide), and the masking by a number of components such as glutamic acid, malic acid, and other organic acids (Adler-Nissen, 1986b). Glutamate as part of a protein is not a flavor enhancer (Institute of Food Technologists, 1987), but glutamate bound into a peptide structure may have the flavor-enhancing properties of the free form. Noguchi et al. (1975) incorporated glutamic acid in the hydrolysates through the plastein reaction. The hydrolysate had no bitterness because of the masking effect of glutamic acid residues in the acidic oligopeptide fraction of the plastein hydrolysate. Thus, it is possible to use deamidated protein hydrolysates to obtain oligo- or polypeptide fractions having flavor enhancement capabilities.

Conclusions. New, specific, and reproducible enzymatic methods for the deamidation of food proteins were developed. A wide range of PGase deamidation of proteins can be achieved by preproteolysis alone or a combination of both proteolysis and other means to disrupt hydrogen or S–S bonds. Heat treatment followed by proteolysis and alkali solubilization of soy protein, casein, and gluten significantly increased the degree to which proteins can be deamidated by PGase. The procedures developed should benefit the commercial product developer by allowing the production of modified proteins with remarkably improved functional properties.

LITERATURE CITED

Adler-Nissen, J. Determination of the Degree of Hydrolysis of Food Proteins by Trinitrobenzenesulfonic Acid. *J. Agric. Food Chem.* **1979**, *27*, 1256–1262.

Adler-Nissen, J. A. Review of Food Protein Hydrolysis-General Issues. In *Enzymatic Hydrolysis of Food Proteins*; Elsevier Applied Science Publishers: New York, 1986a; pp 30–35.

Adler-Nissen, J. A Review of Food Protein Hydrolysis-specific Areas. In *Enzymatic Hydrolysis of Food Proteins*; Elsevier Applied Science Publishers, New York, 1986b; pp 57–74.

Adler-Nissen, J. Methods in Food Protein Hydrolysis. In *Enzymatic Hydrolysis of Food Proteins*; Elsevier Applied Science Publishers: New York, 1986c; pp 116–124.

Adler-Nissen, J. Kinetic Aspects of Food Protein Hydrolysis. In *Enzymatic Hydrolysis of Food Proteins*; Elsevier Applied Science Publishers: New York, 1986d; pp 170–186.

Deter, C. C.; Mason, R. D.; Weetall, H. H. Protein Hydrolysis with Immobilized Enzymes. *Biotechnol. Bioeng.* **1975**, *17*, 451–455.

Feeney, R. E.; Whitaker, J. R. Chemical and Enzymatic Modification of Plant Proteins. In *New Protein Foods*; Altschul, A. M., Wilcke, H. L., Eds.; Academic Press: New York, 1985; Vol. 5, pp 181–219.

Friedman, M.; Levin, C.; Noma, A. Factors Governing Lysinoalanine Formation in Soy Proteins. *J. Food Sci.* **1984**, *49*, 1282–1288.

Gill, B. P.; O'Shaughnessey, A. J.; Henderson, P.; Headon, D. R. An Assessment of Potential of Peptidoglutaminase I and II in Modifying the Charge Characteristics of Casein and Whey Proteins. *Ir. J. Food Sci. Technol.* **1985**, *9*, 33–41.

Hamada, J. S. Peptidoglutaminase Deamidation of Proteins and Peptides for Improved Food Use. *J. Am. Oil Chem. Soc.* **1991**, *68*, 459–462.

Hamada, J. S.; Marshall, W. E. Enhancement of Peptidoglutaminase Deamidation of Soy Proteins by Heat Treatment and/or Proteolysis. *J. Food Sci.* **1988**, *53*, 1132–1134, 1149.

Hamada, J. S.; Marshall, W. E. Preparation and Functional Properties of Enzymatically Deamidated Soy Proteins. *J. Food Sci.* **1989**, *54*, 598–601, 635.

Hamada, J. S.; Shih, F. F.; Frank, A. W.; Marshall, W. E. Deamidation of Soy Peptides and Proteins by *Bacillus circulans* Peptidoglutaminase. *J. Food Sci.* **1988**, *53*, 671–672.

Institute of Food Technologists. Expert Panel on Food Safety and Nutrition. Monosodium glutamate (MSG): A Scientific Status Summary. *Food Technol.* **1987**, *41* (5), 143–148, 152–153.

Ishino, K.; Okamoto, S. Molecular Interaction in Alkali Denatured Soybean Proteins. *Cereal Chem.* **1975**, *52*, 9–21.

Kato, A.; Lee, Y.; Kobayashi, K. Deamidation and Functional Properties of Food Proteins by the Treatment with Immobilized Chymotrypsin at Alkaline pH. *J. Food Sci.* **1989**, *54*, 1345–1347, 1372.

Lura, R.; Schirch, V. Role of Peptide Conformation in the Rate and Mechanism of Deamidation of Asparaginyl Residues. *Biochemistry* **1988**, *27*, 7671–7677.

Matsudomi, N.; Sasaki, T.; Kato, A.; Kobayashi, K. Conformational Changes and Functional Properties of Acid-Modified Soy Protein. *Agric. Biol. Chem.* **1985**, *49*, 1251–1256.

Motoki, M.; Seguro, K.; Nio, N.; Takinami, K. Glutamine-Specific deamidation of α_{s1}-Casein by Transglutaminase. *Agric. Biol. Chem.* **1986**, *50*, 3025–3030.

Nielsen, N. C. Structure of Soy Proteins. In *New Protein Foods*; Altschul, A. M., Wilcke, H. L., Eds.; Academic Press: New York, 1985; Vol. 5, pp 27–64.

Noguchi, M.; Yamashita, M.; Arai, S.; Fujimaki, M. On the Bitter-masking Activity of a Glutamic Acid-rich Oligopeptide Fraction. *J. Food Sci.* **1975**, *40*, 367–369.

Patel, K.; Borchardt, R. T. Chemical Pathways of Peptide Degradation. III. Effect of Primary Sequence on the Pathways Deamidation of Asparaginyl Residues in Hexapeptides. *Pharm. Res.* **1990**, *7*, 787–793.

Shih, F. F. Deamidation during Treatment of Soy Protein with Protease. *J. Food Sci.* **1990**, *55*, 127–129, 132.

Tatham, A. S.; Shewry, P. R. The conformation of wheat gluten proteins. The secondary structures and thermal stabilities of α-, β-, γ and ω-Gliadins. *J. Cereal Sci.* **1985**, *3*, 103–113.

van den Hoven, M. Functionality of Dairy Ingredients in Meat Products. *J. Food Technol.* **1987**, *41* (10), 72–76.

Wall, J. S. Properties of Proteins Contributing to Functionality of Cereal Foods. *Cereal Foods World* **1979**, *24* (7), 288–292, 313.

Whitaker, J. R. Enzymatic Modification of Proteins Applicable to Foods. In *Food Protein Improvement Through Chemical and Enzymatic Modification*; Feeney, R. E., Whitaker, J. R., Eds.; American Chemical Society: Washington, DC, 1977; p 95.

Wilcox, P. E. Determination of Amide Residues by Chemical Methods. *Methods Enzymol.* **1967**, *11*, 63–76.

Wong, D. W. S. Proteins. In *Mechanism and Theory in Food Chemistry*; Van Nostrand Reinhold: New York, 1989.

Woodward, J. C.; Short, D. D. Toxicity of Alkali-treated Soybean in Rats. *J. Nutr.* **1973**, *103*, 569–574.

Received for review October 15, 1991. Revised manuscript received January 2, 1992. Accepted February 28, 1992.

Registry No. Peptidoglutaminase, 37228-70-9.