Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 107 (2008) 1131–1137
Food Chemistry
www.elsevier.com/locate/foodchem

Influence of salt and pH on the solubility and structural characteristics of transglutaminase-treated wheat gluten hydrolysate ☆

Kingsley K. Agyare [a], Youling L. Xiong [a,*], Kwaku Addo [b]

[a] *Department of Animal and Food Sciences, 206 Garrigus Building, University of Kentucky, Lexington, KY 40546, USA*
[b] *Department of Nutrition and Food Science, University of Kentucky, Lexington, KY 40506, USA*

Received 24 March 2007; received in revised form 28 August 2007; accepted 17 September 2007

Abstract

Hydrolyzed wheat gluten (GH, 77–85% protein) was prepared by limited chymotrypsin digestion at 37 °C for 4 h (degree of hydrolysis = 6.4%) and 15 h (degree of hydrolysis = 10.3%). Microbial transglutaminase (MTGase) treatment (55 °C for 1 h, or 5 °C for 18 h) effect on the solubility and structural characteristics of GH was examined under selected food processing conditions (pH 4.0–7.0, 0–0.6 M NaCl). The MTGase treatment increased solubility of GH by 3–29-fold ($P < 0.05$) within pH 4.0–7.0. Addition of 0.6 M NaCl or changing the conditions of MTGase incubation did not significantly alter solubility characteristics of GH. The MTGase treatment decreased surface hydrophobicity, and increased carboxyl groups in GH, suggesting cross-linking and deamidation. Fluorescence and UV spectra attributed the improved GH solubility to MTGase-induced polar environment, and partial masking of some nonpolar aromatic amino acids possibly due to high-molecular-weight polypeptides formed.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Hydrolyzed wheat gluten; Structural properties; Solubility; Microbial transglutaminase

1. Introduction

Wheat gluten is an economically important co-product in the recovery of wheat starch in wet milling of wheat flour. It is available in large amounts at relatively low cost (Popineau, Blandine, Larré, & Bérot, 2002; Wang, Zhao, Yang, & Jiang, 2006). Wheat gluten consists of two storage proteins of the wheat endosperm, namely glutenin and gliadin. Both proteins are rich in the amino acids asparagine, glutamine and proline, but low in lysine and tryptophan (Ewart, 1967; Wu & Dimler, 1963). The gliadins are monomeric proteins stabilized by intra-molecular disulfide linkages while the glutenin subunits are linked together by inter- and intra-molecular disulfide bonds to form high-molecular weight polymers. The gluten network is formed via non-covalent interaction (mainly hydrophobic and hydrogen bonds) of glutenin polymers with the gliadins (Shewry, Tatham, Barro, Barcelo, & Lazzeri, 1995).

Wheat gluten is used mainly to improve the rheological and viscoelastic properties of bread-making flours when hydrated. It is also utilized as a functional protein additive in various non-bakery applications because of its desirable structure-enhancing properties (Ahmedna, Prinyawiwatkul, & Rao, 1999). However, gluten utilization in formulated food systems is limited by its low solubility in aqueous solutions (Mimouni, Raymond, Merle-Desnoyers, Azanza, & Ducastaing, 1994; Popineau et al., 2002). The low solubility is due to the high concentrations of nonpolar amino acid residues (such as proline and leucine) and the polar but nonionizable glutamine residue, and the low concentrations of charged side groups from ionizable amino acids (Krull & Wall, 1966).

Gluten application in food may be extended through chemical and enzymatic modifications. Limited hydrolysis results in improved solubility, foaming ability, and

☆ Approved for publication as journal article number 07-07-039 by the Director of Kentucky Agricultural Experiment Station.

* Corresponding author. Tel.: +1 859 257 3822; fax: +1 859 257 5318.
E-mail address: ylxiong@uky.edu (Y.L. Xiong).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.09.039

emulsifying capacity (Linarés, Larré, Lemeste, & Popineau, 2000; Mimouni et al., 1994; Panyam & Kilara, 1996; Popineau et al., 2002). Babiker, Fujisawa, Matsudomi, and Kato (1996) also reported that functionality of hydrolyzed gluten may be further enhanced by microbial transglutaminase treatment. In addition, they showed that the bitterness associated with hydrolyzed gluten was significantly reduced by microbial transglutaminase treatment. However, there is limited information on the effect of specific food processing parameters (e.g., ionic and pH conditions) on the solubility and structural characteristics of transglutaminase-modified hydrolyzed gluten. Such an investigation is important because physicochemical properties of the resulting gluten fragments may be influenced by environmental factors such as pH, temperature and ionic strength.

Transglutaminase (glutaminyl-peptide:amine γ-glutamyltransferase, EC 2.3.2.13) is an enzyme that catalyzes acyl transfer reactions between γ-carboxyamide of peptides or protein-bound glutamine residues (acyl donors) and primary amines (acyl acceptors). When lysine residues act as acyl acceptors, ε-(γ-glutamyl)lysine "isopeptide" bonds are formed in proteins, leading to intra- and intermolecular cross-linking. Such cross-linking reactions are responsible for functionality improvements of transglutaminase-treated proteins (Motoki & Seguro, 1998; Yokoyama, Nio, & Kikuchi, 2004). In the absence of suitable amine substrates, water acts as the acyl group acceptor, resulting in hydrolysis of the γ-carboxyamide group (deamidated) and leading to elevated protein solubility (Ando et al., 1989).

The present study investigated the efficacy of microbial transglutaminase in changing the solubility, surface properties, and structural characteristics of hydrolyzed gluten. The influence of selected food processing conditions (pH, salt concentration, and temperature) on the enzyme-induced protein changes was examined.

2. Materials and methods

2.1. Materials

Untreated commercial soft wheat flour, obtained from ADM Milling Company (Chattanooga, TN, USA), was used for making wheat gluten. Chymotrypsin (52 units/mg), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC hydrochloride), and *N,N,N*′,*N*′-tetramethylenediamine (TEMED) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Microbial transglutaminase (MTGase) used in all treatments was a crude enzyme preparation (Activa®-TI, 99% maltodextrin and 1% MTGase) donated by Ajinomoto Inc. (Teaneck, NJ, USA). All chemicals used were at least reagent grade.

2.2. Wheat gluten preparation

Gluten was prepared according to Method 38-10 of the AACC (2000) by washing wheat flour dough with water until the washings were free from water-soluble proteins and starch. The gluten ball was dialyzed against distilled water for 24 h at 4 °C and then lyophilized in a freeze-dryer (Lyph-Lock 4.5 Freeze Dry System, Labconco Corp., Kansas City, MO, USA). Freeze-dried gluten was pulverized with a mortar and pestle and then blended with a Waring blender (Dynamics Corp. of America, New Hartford, CT, USA) into a fine powder. The dry sample was stored in sealed containers at 4 °C until use. Protein content of the gluten powder (76.1%) was measured by the combustion method (AOAC, 2005) using a Leco Nitrogen Analyzer (Model CN-2000, Leco Corp., St. Joseph, MI, USA).

2.3. Preparation of chymotrypsin-digested gluten

In preliminary experiments, three proteases (chymotrypsin, papain and alcalase) were tested to hydrolyze wheat gluten. Because chymotrypsin was most effective in producing gluten fragments, it was selected for this study.

Gluten was partially hydrolyzed with chymotrypsin according to Babiker et al. (1996). Specifically, a freeze-dried sample of gluten (16 g) was suspended in 400 mL of 0.05 M Tris–HCl (pH 8.0) containing 0.05% sodium azide, and then 160 mg of chymotrypsin was added. The mixture was incubated at 37 °C for 4 or 15 h and then heated at 80 °C for 20 min to inactivate the enzyme. The chymotrypsin-digested gluten was centrifuged at 10,500*g* for 10 min to remove undigested residues. The degree of hydrolysis was determined by the trinitrobenzenesulfonic acid method of Adler-Nissen (1979). The supernatant was dialyzed (3500 molecular weight cut-off) against distilled water or 0.1 M phosphate buffer (pH 7.0) (Babiker et al., 1996). The former (supernatant dialyzed against distilled water) was freeze-dried (designated as gluten hydrolysate, GH), and the latter (supernatant dialyzed against phosphate buffer) was used for transglutaminase treatment.

2.4. Transglutaminase treatment

The supernatant of chymotrypsin-digested gluten (4 or 15 h hydrolysis) that had been dialyzed against 0.1 M phosphate buffer (pH 7.0), was adjusted to 10 mg/mL protein concentration and then reacted with MTGase ($E/S = 1/20$) at 5 °C for 18 h, or at 55 °C for 1 h. For all the MTGase-treated samples, 0.1% *N*-ethylmaleimide was added after the specific incubation times to inactive the enzyme. The MTGase-treated samples were dialyzed against distilled water, freeze-dried, pulverized with mortar and pestle, and stored in sealed containers at 4 °C until use (designated as MTGase-treated GH). Determination of protein in control (GH) and MTGase-treated GH was done by the Biuret method (Gornall, Bardawill, & David, 1949) using bovine serum albumin as standard.

2.5. Electrophoresis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of freeze-dried control and MTGase-treated GH samples was carried out using a 15% acrylamide resolving gel and a 3% acrylamide stacking gel in a Mini-PROTEAN 3 Cell electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA) (Laemmli, 1970). Aliquots of 20–30 μL of protein were loaded per well. A cocktail protein standard consisting of proteins of 6.5–200 kDa was also run. Molecular weights (MW) of unknown proteins were estimated from the regression line obtained by plotting the log (MW) versus relative mobility (R_f) of the protein bands.

2.6. Measurement of solubility

Freeze-dried control and MTGase-treated GH samples (2 mg/mL) were used for the determination of solubility in distilled and deionized water (0 M NaCl) and 0.6 M NaCl solutions at various pHs, namely pH 4.0–5.5 (with 0.05 M acetate buffer) and pH 6.0–7.0 (with 0.05 M phosphate buffer). Samples were dissolved in the respective buffers at 20 °C for 5 min and vortexed for 10 s. Turbidity (optical density at 500 nm) was immediately measured. Triplicate GH samples were tested.

2.7. Determination of surface hydrophobicity

The surface hydrophobicity of control and MTGase-treated GH samples was determined by the method of Hayakawa and Nakai (1985). A specific amount of freeze-dried sample was dispersed in 0.01 M phosphate buffer (pH 7.0) to obtain 0.5 mg/mL protein. A series of dilutions of this dispersion were made with the same buffer to obtain a range of protein concentrations (0.08–0.5 mg/mL). The sample solutions were centrifuged at 10,000*g* for 15 min (4 °C) and 4 mL of the supernatants were reacted with 20 μL of 1-anilino-8-naphthalenesulfonate magnesium salt (ANS) (8 mM in 0.1 M phosphate buffer, pH 7.0) for 15 min. The relative fluorescent intensity (RFI) of each sample solution was measured using a photon-counting FluoroMax-3 spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ, USA) with excitation (390 nm) and emission (470 nm) slits set at 5 nm to obtain the fluorospectrum. The RFI values were plotted against protein concentrations and the slope (S_o = RFI/%protein) of the regression line was designated as surface hydrophobicity. Triplicate GH samples were measured.

2.8. Determination of carboxyl groups

Fluorescence of free carboxyl groups in control and MTGase-treated GH samples was measured according to Kobayashi and Chiba (1994) with some modifications. Specifically, test samples (2 mg/mL protein) dispersed in distilled and deionized water (0 M NaCl) or in 0.6 M NaCl solution were centrifuged at 10,000*g* for 15 min. An aliquot of the supernatant (0.25 mL) was mixed with 4% EDC hydrochloride (0.25 mL) freshly dissolved in 100 mM TEMED-HCl buffer (pH 4.75) and incubated at 30 °C for 30 min. After 10-fold dilution with water, a 0.1 mL aliquot was reacted with 0.1 mL *o*-phthalaldehyde (OPA) reagent (Kobayashi & Ichishima, 1990) and mixed thoroughly with a vortex. Thereafter, 8 mL of water was added and the relative fluorescence intensity, recorded as photon counts per second (pc/s), was measured using a FluoroMax-3 spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ, USA). The emission or excitation slits were set at 5 nm, and the excitation and emission wavelengths were 340 and 455 nm, respectively. Triplicate GH samples were measured.

2.9. Measurement of UV absorption

Ultraviolet (UV) absorption spectra of control and MTGase-treated GH samples were measured according to Tang, Yang, Chen, Wu, and Peng (2005). Test samples (2 mg/mL protein) were prepared with 0.05 M Tris–HCl buffer, pH 7.5 and the solutions were centrifuged at 10,000*g* for 15 min. The supernatants were subjected to UV scan in the range of 240–380 nm on a Shimadzu U-601 Spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA). Triplicate GH samples were measured.

2.10. Measurement of fluorescence intensity

The intrinsic fluorescence spectra of control and MTGase-treated GH samples were determined using a photon-counting FluoroMax-3® spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ, USA) with 5 nm emission and excitation slit according to Tang et al. (2005). The test samples (2 mg/mL protein) in 0.05 M Tris–HCl buffer (pH 7.5) were excited at 280 nm, and the emission intensities, recorded as photon counts per second (cps), were measured from 300 to 500 nm. Triplicate GH samples were measured.

2.11. Statistical analysis

The experimental design was a completely randomized, 2 × 2 factorial design with two MTGase incubation temperatures (5 and 55 °C) and two ionic strengths (0 and 0.6 M NaCl), with repeated measures. Data were analyzed using the general linear model procedures of the Statistix 7.0 software package (Analytical Software Inc., St. Paul, MN, USA). Analysis of variance (ANOVA) was performed to determine treatment effects. When significant treatment effects ($P < 0.05$) were found, the treatment means were separated by the least significant difference (LSD) test.

3. Results and discussion

3.1. Modification of gluten molecular mass

Hydrolysis of gluten with chymotrypsin for 4 h yielded a degree of hydrolysis (DH) of 6.4%, and that for 15 h

produced a DH of 10.3%. Two bands corresponding to molecular weight (MW) of 14 kDa and 43 kDa, and a smear of small peptides (<10 kDa), were observed in the 4 h and 15 h GH samples (Fig. 1). The presence of these bands was in agreement with the 10 and 40 kDa reported by Babiker et al. (1996) for chymotrypsin, papain, and pronase digests of gluten. The results demonstrated the presence of protease-resistant peptides in gluten. In addition, three bands corresponding to 16, 29, and 67 kDa were clearly present in the 4 h hydrolysate, but they were barely detectable in the 15 h hydrolysate.

With MTGase treatment, a new, high-MW band (>200 kDa), along with a stack of small peptides (lanes b, c, e and f), appeared; concomitantly, the bands at 29 and 67 kDa diminished (Fig. 1). These bands of higher-MW were attributed to transglutaminase cross-linking of the peptides in GH, as reported previously (Babiker et al., 1996). The wide distribution of glutamine residues along gluten polypeptide chains allows MTGase to produce various cross-linked oligomers with different molecular masses. The abundance of glutamine in gluten or gluten peptides also makes them excellent substrates for the amine-transfer enzyme. Wu and Dimler (1963) reported that glutamine and glutamic acid together accounted for 25.4% of total amino acid residues in gluten, most of which were shown to be in the amide form (glutamine) for both glutenin and gliadin (Ewart, 1967). Yet, the lack of total disappearance of some peptides in GH, notably the 14 kDa component, suggested that these peptides probably were still of a compact structure, and thus, not all the glutamine residue side chain groups were readily accessible by MTGase. Another plausible reason was that the 14 kDa polypeptide was relatively deficient in lysine, the receptor of transfer amine groups. However, this hypothesis is yet to be tested.

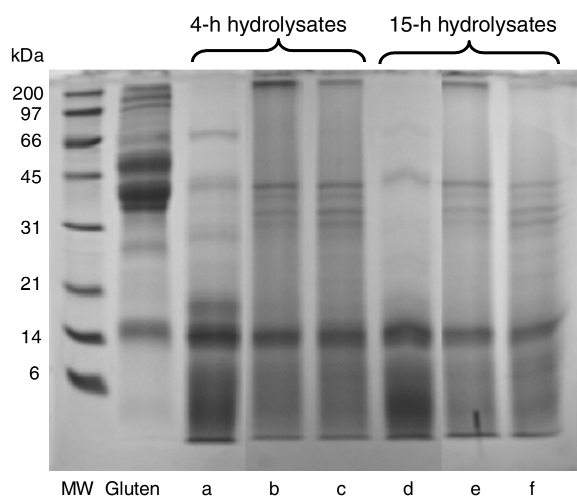

Fig. 1. SDS-PAGE of native gluten, hydrolyzed gluten, and MTGase-treated hydrolyzed gluten. Lanes: MW = molecular weight standards; Gluten = native gluten; a,d = gluten hydrolysates (GH); b,e = GH + MTGase, 1 h, 55 °C; c,f = GH + MTGase, 18 h, 5 °C.

The production of high-MW bands in MTGase-treated samples was notably non-extensive. This may be due to the fact that high-MW glutenin subunits (HMW-GS) almost disappeared completely after 4 h of hydrolysis. Mujoo and Ng (2003) reported that transglutaminase induced extensive cross-linking of high-MW wheat glutenin subunits (HMW-GS) when compared with other gluten proteins, probably because HMW-GS were richer in lysine residues than any other gluten protein.

3.2. Solubility

Fig. 2 shows the effect of MTGase treatment (5 °C, 18 h) on solubility of GH under varying pH and ionic strength (NaCl concentration) conditions. The MTGase treatment at the higher temperature (55 °C, 1 h) produced essentially an identical solubility profile to the low-temperature (5 °C, 18 h) solubility profile; hence, only the latter is presented. The relatively low solubility (high optical density) of GH over the 4.0–7.0 pH range was improved markedly following the MTGase treatment ($P < 0.05$). Addition of 0.6 M NaCl did not significantly affect the solubility characteristics. Increased solubility of GH by MTGase treatment has been attributed to the decrease in hydrophobicity and increase in electrostatic repulsion as a result of partial deamidation of glutamine and asparagine residues, i.e. production of ionizable carboxyl groups (Motoki, Seguro, Nio, & Takinami, 1986; Babiker et al., 1996). Indeed, concentration of carboxylic groups after MTGase treatment increased up to 10.1% (Fig. 3), confirming deamidation of glutamine and asparagine into their acidic residues. The MTGase treatment also shifted the pH for maximum insolubility of GH samples from 5.5 to a lower pH (pH 4.0). This can be explained by the significant increase in the carboxyl content.

3.3. Surface hydrophobicity

In corroboration of the solubility change, surface hydrophobicity of GH was altered by the MTGase treatment. Hydrophobicity of GH was found to be in the range of 3.87–5.45 in the absence of 0.6 M NaCl (Table 1), and after treatment with MTGase, the hydrophobicity decreased significantly ($P < 0.05$), to 1.42–1.58. Presumably, some hydrophobic residues in GH were partially occluded in cross-linked molecules as a result of peptide–peptide association. The large increase in negatively charged amino acids (glutamic and aspartic acids) due to deamidation would also lead to an overall decrease in GH hydrophobicity. In the presence of 0.6 M NaCl, hydrophobicity of GH decreased to 1.96–2.76 ($P < 0.05$), while hydrophobicity of the MTGase-treated GH samples decreased even further, to 1.14–1.49, when compared with samples containing no NaCl. The MTGase-induced deamidation appeared to complement the electrostatic interaction of NaCl with GH. The high concentrations of ions (Na^+ and Cl^-) probably decreased the exposure of hydrophobic residues via an electrostatic shield or an electric double layer that may

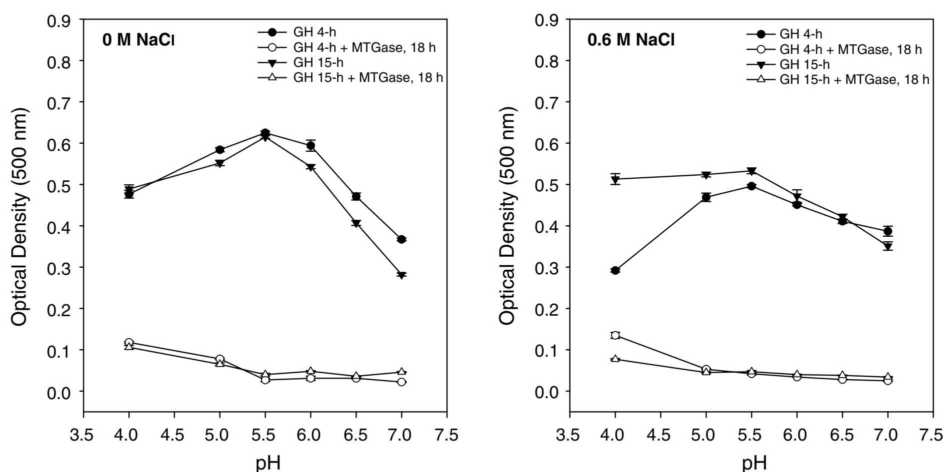

Fig. 2. Solubility of chymotrypsin-digested (4- or 15-h) gluten with or without microbial transglutaminase treatment in 0 or 0.6 M NaCl at 5 °C for 18 h. The solubility was measured at different pH levels.

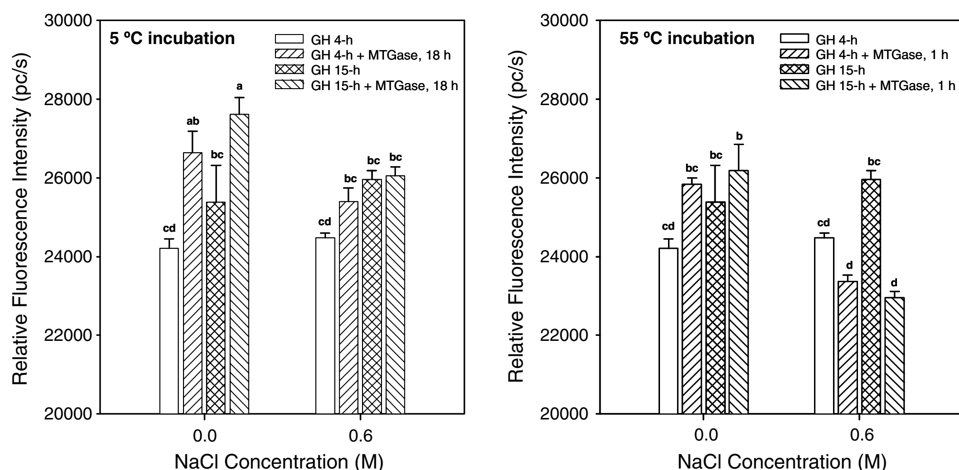

Fig. 3. Carboxyl groups of chymotrypsin-digested (4- or 15-h) gluten with or without microbial transglutaminase treatment in 0 or 0.6 M NaCl at 5 °C for 18 h or 55 °C for 1 h. ($n = 6$).[a–d] Means of vertical bars without a common letter differ significantly ($P < 0.05$).

Table 1
Surface hydrophobicity (S_o) of chymotrypsin-digested gluten with or without microbial transglutaminase treatment[A]

Sample[A]	S_o (×1000 Photons)	
	0 M NaCl	0.6 M NaCl
4-h Gluten hydrolysates		
Control (no MTGase)	3.87 ± 0.27^b	1.96 ± 0.10^{de}
+MTGase, 18 h, 5° C	1.42 ± 0.46^e	1.14 ± 0.16^e
+MTGase, 1 h, 55° C	1.57 ± 0.46^e	1.31 ± 0.02^e
15-h Gluten hydrolysates		
Control (no MTGase)	5.45 ± 0.32^a	2.76 ± 0.18^c
+MTGase, 18 h, 5 °C	1.50 ± 0.34^e	1.44 ± 0.10^e
+MTGase, 1 h, 55 °C	1.58 ± 0.31^e	1.49 ± 0.15^e

[a–e] Means without a common letter differ significantly ($P < 0.05$).
[A] The hydrolysis was done for 4 or 15 h, and the subsequent enzyme treatment in 0 or 0.6 M NaCl was performed at 5 °C for 18 h or 55 °C for 1 h. Values were expressed as mean ± standard deviation of three replications with duplicate measurements in each ($n = 6$). (For detail, see Materials and Methods).

form surrounding GH. This premise was consistent with the report by Linarés, Larré, and Popineau (2001) that increase in pH or salt concentration decreased the solubility of more hydrophobic peptides derived from gluten.

3.4. UV spectra

Fig. 4 displays the ultraviolet (UV) spectra of GH and its MTGase-treated samples (2 mg/mL protein) at 5 °C for 18 h, with 0 or 0.6 M NaCl. All samples had similar characteristic peaks of absorbance in the UV region near 270–280 nm. The UV absorption was attributed to aromatic amino acid residues, i.e. phenylalanine, tyrosine, and tryptophan. The intensity of the peak at ~276 nm was reduced considerably after MTGase treatment, and there was no visible difference between samples treated for 1 h (55 °C) or 18 h (5 °C). Hence, data from the 1 h

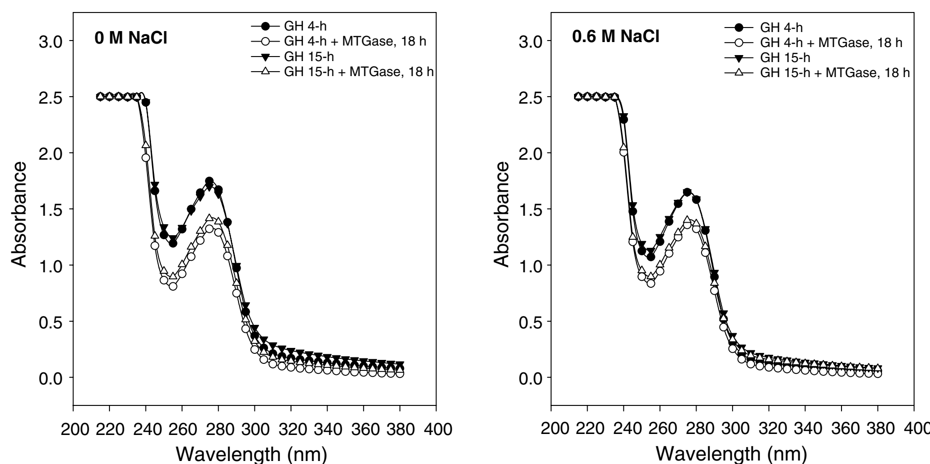

Fig. 4. UV spectra of chymotrypsin-digested (4- or 15-h) gluten with or without microbial transglutaminase treatment in 0 or 0.6 M NaCl at 5 °C for 18 h.

(55 °C) MTGase treatment are not presented. According to the general interpretation of the absorption spectra of proteins (Freifelder, 1982), the decrease in absorbance indicated that the chromophores (aromatic amino acid side chains) were shifted to a more polar environment. The decrease in absorbance may also be attributed to the formation of high-MW polymers/polypeptides induced by MTGase which resulted in partial masking of some nonpolar aromatic amino acid residues (Kim & Kinsella, 1986; Tang et al., 2005). These results were consistent with the findings from the SDS-PAGE, surface hydrophobicity, and carboxyl group analyses.

3.5. Fluorescence spectra

Intrinsic fluorescence spectra (wavelength λ_{em}, 280 nm) of GH and its MTGase-treated samples are presented in Fig. 5. No spectral difference was found between the 55 °C (1 h) and the 5 °C (18 h) controls or MTGase treatments. Hence, only the 5 °C incubation data are presented. The amino acid residues in proteins and peptides that could produce fluorescence include phenylalanine, tyrosine and tryptophan. These three amino acid residues having different side chain aromatic groups exhibit different fluorescence spectra, with the λ_{max} being 282, 304, and 355 nm, respectively (in neutral aqueous solution at ~20 °C) (Eftink, 1991). The fluorescence spectra mostly reflect the contribution of tryptophan residue, which has a high quantum yield, compared with phenylalanine and tyrosine residues (Freifelder, 1982).

The MTGase treatment altered the fluorescence intensity of GH. With MTGase incubation, the λ_{max} shifted slightly from 355.5 to 354.5 (blue shift). In general, the λ_{max} is closely related to the position of tryptophan residues in a protein molecule (Kim & Kinsella, 1986). The shift in λ_{max} to a shorter wavelength reflected the "masking" of some of the tryptophan residues in GH to a less polar environment, possibly because of peptide–peptide association (Freifelder, 1982; Tang et al., 2005). Furthermore, the increased emission intensity of MTGase-treated samples seemed to also indicate that some of the chromophores were translocated into a less polar environment. However, this hypothesis apparently contradicted the result, as the presence of 0.6 M NaCl (a more polar environment than water only) did not cause appreciable change in either λ_{max} or the fluo-

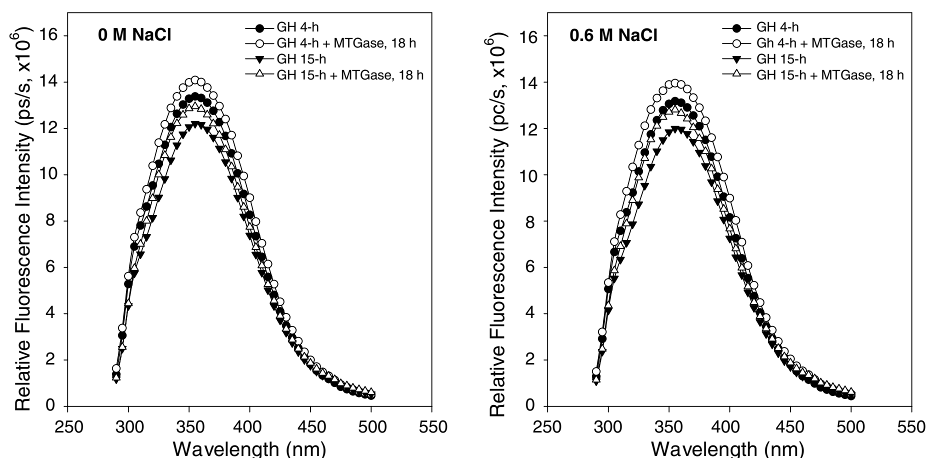

Fig. 5. Fluorescence spectra of chymotrypsin-digested (4- or 15-h) gluten with or without microbial transglutaminase treatment in 0 or 0.6 M NaCl at 5 °C for 18 h.

rescence intensity (Fig. 5). Thus, within the ionic strength range 0–0.6 M NaCl, the occlusion of contributing aromatic chromophores was insensitive to the specific salt concentrations.

4. Conclusions

This study showed that microbial transglutaminase treatment of chymotrypsin-digested gluten increased the protein solubility over a broad range of food processing conditions (pH 4.0–7.0, NaCl concentrations 0 M or 0.6 M, incubation temperatures 5 °C long time or 55 °C short time). This solubility enhancement was attributed to deamidation or formation of ionizable carboxyl groups and the reduction in surface hydrophobicity. The limited peptide–peptide cross-linking by transglutaminase led to the formation of oligomers or polymers with somewhat compact structures. However, neither the solubility change nor the structural alteration of gluten peptides was affected by ionic strengths corresponding to 0–0.6 M NaCl, suggesting that use of microbial transglutaminase to modify hydrolyzed wheat gluten can be achieved in both low and high salt concentration conditions commonly employed in food processing.

Acknowledgements

The authors thank Ajinomoto Inc. (Teaneck, NJ, USA) for donating the microbial transglutaminase, and ADM Milling Company (Chattanooga, TN, USA) for donating untreated soft wheat flour for this project.

References

AACC, (2000). *Approved Methods of the American Association of Cereal Chemists (AACC)*, tenth edition. St. Paul, MN: American Association of Cereal Chemists.

Adler-Nissen, J. (1979). Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *Journal of Agricultural and Food Chemistry, 27*, 1256–1262.

Ahmedna, M., Prinyawiwatkul, W., & Rao, R. M. (1999). Solubilized wheat protein isolate: Functional properties and potential food applications. *Journal of Agricultural and Food Chemistry, 47*, 1340–1345.

Ando, H., Adachi, M., Umeda, K., Matsuura, A., Nonaka, M., Uchio, R., et al. (1989). Purification and characteristics of a novel transglutaminase derived from microorganism. *Agricultural and Biological Chemistry, 53*, 2613–2617.

AOAC (2005). Official Methods of Analysis of Association of Official Analytical Chemists (AOAC) International, 18th ed.. Gaithersburg, MD: Association of Official Analytical Chemists International.

Babiker, E. E., Fujisawa, N., Matsudomi, N., & Kato, A. (1996). Improvement in the functional properties of gluten by protease digestion or acid hydrolysis followed by microbial transglutaminase treatment. *Journal of Agricultural and Food Chemistry, 44*, 3746–3750.

Eftink, M. R. (1991). Fluorescence techniques for studying protein structure. *Methods of Biochemical Analysis, 35*, 127–205.

Ewart, J. A. D. (1967). Amino acid analyses of glutenins and gliadins. *Journal of the Science of Food and Agriculture, 18*, 111–116.

Freifelder, D. (1982). *Physical Biochemistry: Applications to biochemistry and molecular biology*. New York: W.H Freeman and Company.

Gornall, A. G., Bardawill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry, 177*, 751–766.

Hayakawa, S., & Nakai, S. (1985). Relationships of hydrophobicity and net charge to the solubility of milk and soy proteins. *Journal of Food Science, 50*, 486–491.

Kim, S. H., & Kinsella, J. E. (1986). Effects of reduction with dithiothreitol on some molecular properties of soy glycinin. *Journal of Agricultural and Food Chemistry, 34*, 623–627.

Kobayashi, M., & Chiba, Y. (1994). Water-soluble carbodiimide for the fluorescent measurement of the carboxyl group produced by enzyme reactions. *Analytical Biochemistry, 219*, 189–194.

Kobayashi, M., & Ichishima, E. (1990). Use of water-soluble 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide for the fluorescent determination of uronic acids and carboxylic acids. *Analytical Biochemistry, 189*, 122–125.

Krull, L. H., & Wall, J. S. (1966). Synthetic polypeptides containing side-chain amide groups. *Biochemistry, 5*, 1521–1527.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature, 227*, 680–685.

Linarés, E., Larré, C., Lemeste, M., & Popineau, Y. (2000). Emulsifying and foaming properties of gluten hydrolysate with an increasing degree of hydrolysis: Role of soluble and insoluble fractions. *Cereal Chemistry, 77*, 414–420.

Linarés, E., Larré, C., & Popineau, Y. (2001). Freeze- or spray-dried gluten hydrolysates. 1. Biochemical and emulsifying properties as a function of drying process. *Journal of Food Engineering, 48*, 127–135.

Mimouni, B., Raymond, J., Merle-Desnoyers, A. M., Azanza, J. L., & Ducastaing, A. (1994). Combined acid deamidation and enzymic hydrolysis for improvement of the functional properties of wheat gluten. *Journal of Cereal Science, 21*, 153–165.

Motoki, M., Seguro, K., Nio, N., & Takinami, K. (1986). Glutamine-specific deamidation of α_{S1}-casein by transglutaminase. *Agricultural and Biological Chemistry, 50*, 3025–3030.

Motoki, M., & Seguro, K. (1998). Transglutaminase and its use for food processing. *Trends in Food Science and Technology, 9*, 204–210.

Mujoo, R., & Ng, P. K. W. (2003). Identification of wheat protein components involved in polymer formation on incubation with transglutaminase. *Cereal Chemistry, 80*, 703–706.

Panyam, D., & Kilara, A. (1996). Enhancing the functionality of food proteins by enzymic modifications. *Trends in Food Science and Technology, 7*, 120–125.

Popineau, Y., Blandine, H., Larré, C., & Bérot, S. (2002). Foaming and emulsifying properties of fractions of gluten peptides obtained by limited enzymatic hydrolysis and ultrafiltration. *Journal of Cereal Science, 35*, 327–335.

Shewry, P. R., Tatham, A. S., Barro, F., Barcelo, P., & Lazzeri, P. A. (1995). Biotechnology of breadmaking – unraveling and manipulating the multi-protein gluten complex. *Bio-Technology, 13*, 1185–1190.

Tang, C. H., Yang, X. Q., Chen, Z., Wu, H., & Peng, Z. Y. (2005). Physicochemical and structural properties of sodium caseinate biopolymers induced by microbial transglutaminase. *Journal of Food Biochemistry, 29*, 402–421.

Wang, J., Zhao, M., Yang, X., & Jiang, Y. (2006). Improvement on functional properties of wheat gluten by enzymatic hydrolysis and ultrafiltration. *Journal of Cereal Science, 44*, 93–100.

Wu, Y. V., & Dimler, R. J. (1963). Hydrogen-ion equilibria of wheat gluten. *Archives of Biochemistry and Biophysics, 102*, 230–237.

Yokoyama, K., Nio, N., & Kikuchi, Y. (2004). Properties and applications of microbial transglutaminase. *Applied Microbiology and Biotechnology, 64*, 447–454.