



Effect of citric acid deamidation on *in vitro* digestibility and antioxidant properties of wheat gluten



Chaoying Qiu^a, Weizheng Sun^{a,*}, Chun Cui^a, Mouming Zhao^{a,b,*}

^aCollege of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China

^bState Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China

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ABSTRACT

The effects of citric acid deamidation on the physicochemical properties of wheat gluten were investigated. *In vitro* digestion was carried out to determine changes of molecular weight distribution, amino acids composition and antioxidant efficacy of wheat gluten hydrolysates. Results indicated that citric acid deamidation significantly increased gluten solubility and surface hydrophobicity, at a neutral pH. Deamidation induced molecular weight distribution change of gluten with little proteolysis. Results from FTIR indicated that the α -helix and β -turn of deamidated gluten increased accompanied by a decrease of the β -sheet structure. After deamidation, *in vitro* pepsin digestibility of wheat gluten decreased, while *in vitro* pancreatin digestibility increased. The oxygen radical absorbance capacity (ORAC) activity of the *in vitro* digests decreased with increase of deamidation time. The high Lys and total essential AAs amounts in the final digests suggested that the nutritional values of wheat gluten after deamidation might be enhanced.

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1. Introduction

Wheat gluten as an abundant byproduct protein of wheat starch has attracted increasing attention owing to its unique characters and low cost (Berti et al., 2007; Wang, Zhao, Zhao, & Jiang, 2007). However, wheat gluten utilisation is limited by its low solubility for its large content of nonpolar amino acid residues and glutamine residues (Day, Augustin, Batey, & Wrigley, 2006; Liao et al., 2010). Deamidation can remove the amide groups primarily on glutamine residues of gluten protein to form acidic residues. It is an effective way to dissociate protein polymers and increase the electrostatic repulsion between protein molecules, which can greatly enhance the solubility of gluten (Mimouni, Raymond, Merledesnoyers, Azanza, & Ducastaing, 1994). Hydrochloric acid or enzymes have been used to deamidate wheat gluten (Mimouni et al., 1994; Wu, Nakai, & Powrie, 1976; Yong, Yamaguchi, & Matsumura, 2006). Compared with hydrochloric acid (HCl), carboxylic acid was reported to be a better choice with little proteolysis and possible generation of chloropropanol (Liao et al., 2010). Previous work, about acetic acid deamidation, demonstrated that deamidated wheat gluten had better properties and could effectively lower the potential risk for celiac people, by decreasing the immunoreac-

tivity of celiac IgA anti-gliadin antibodies (Berti et al., 2007; Wu, Nakai, & Powrie, 1976).

Deamidation allows gluten protein polymers to dissociate, thus increasing the surface hydrophobicity and flexibility of the gluten molecule (Matsudomi, Kaneko, Kato, & Kobayashi, 1981). The changes in protein structure were considered to be related with its hydrolysis susceptibility (Marmon & Undeland, 2013). The interaction between deamidation and proteolysis has been reported in a few studies. HCl deamidation was helpful for the enhancement of hydrolysis efficiency of gluten by Flavourzyme (Schlichtherle & Amado, 2002). Deamidation of wheat gliadin by cation-exchange resin had no influence on pepsin digestibility, but increased pancreatin digestibility (Kumagai et al., 2007). Recently, deamidation of wheat gluten by acetic acid was demonstrated to exert an influence on the release of free amino acids by pancreatin (Liao et al., 2010). So far, possible relations between changes in the protein structure during deamidation and digestibility of wheat gluten in the gastrointestinal model are still poorly documented.

Increasing attention has been paid to developing antioxidants from plant proteins (Zheng et al., 2012; Zhu, Chen, Tang, & Xiong, 2008). Previous studies have established that gluten hydrolysates and peptides have antioxidant properties (Suetsuna & Chen, 2002; Wang et al., 2007; Žilic, Akillioglu, Serpen, Barac, & Gökmen, 2012). The antioxidant activity of protein hydrolysates is correlated with the amino acid composition and sequence, as well as the configuration of peptides (Zhu et al., 2008). Kawase, Murakami,

* Corresponding authors: College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China. Tel./fax: +86 20 87113914.

E-mail addresses: fewzhsun@scut.edu.cn (W. Sun), femmzhao@scut.edu.cn (M. Zhao).

Matsumura, and Mori (2003) reported that the decrease of antioxidant activity of C-hordein was related with the change of the secondary structure by deamidation. However, the antioxidant performance of the hydrolysates of deamidated wheat gluten in an *in vitro* digestion model system is still unclear. The influence of deamidation on the release pattern of amino acids, as well as the antioxidant activity of the digests, are useful for understanding the modifications in wheat gluten properties by citric acid and facilitating its application development.

The objective of the present study was thus to investigate the effect of citric acid deamidation on the conformational and nutritional properties of wheat gluten. A two-stage *in vitro* digestion model system was used to simulate the process of human gastrointestinal digestion. The molecular weight distribution, antioxidant properties and amino acid composition of the digesta were evaluated.

2. Materials and methods

2.1. Materials and chemicals

Commercial wheat gluten with 71.5% (w/w, dry basis) crude protein was obtained from Lianhua Co. Ltd. (Zhoukou, China). Pepsin from porcine gastric mucosa (400 U/mg solid), pancreatin from porcine pancreas (8× USP), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), fluorescein disodium, 2,2'-azobis (2-methylpropionamide)-dihydrochloride (AAPH) were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals used in the present study were of analytical grade.

2.2. Preparation of deamidated wheat gluten (DWG)

Wheat gluten (8%, w/v) was mixed with citric acid (0.2 M) to form suspensions. The suspensions were hydrated for 3, 6, 10 and 16 h, respectively, in a shaking water bath at 70 °C. After the treatment, the suspension was neutralised with sodium hydroxide, dialysed against distilled water (MWCO 12–14 kDa) and the contents were freeze-dried. The degree of deamidation was determined according to Kato, Tanaka, Lee, Matsudomi, and Kobayashi (1987). Total amount of ammonia in the wheat gluten was determined by dissolving 0.4 g gluten in 5 ml of 3 M HCl, sealed in a 10 ml glass ampoule and heated at 121 °C for 3 h to reach complete deamidation. Ash content was measured according to standard AACC methods (1995).

2.3. Determination of protein characters

2.3.1. Solubility

Protein dispersions (10 mg/ml, dissolved in deionised water) were adjusted to a specific value within the range of pH 2–10 by 0.5 M HCl or NaOH. The dispersions were agitated with a magnetic stirrer for 1 h at room temperature, and then centrifuged at 12,000g for 20 min (HITACHI CR22G, Japan). Protein contents of the supernatant were determined according to Lowry, Rosebrough, Farr, and Randall (1951). Protein solubility was calculated as the nitrogen solubility index (NSI) = (protein content of supernatant/amount of proteins added) × 100%.

2.3.2. Zeta-potential

The zeta-potential of the DWG was determined using a Nanosizer ZS instrument (Malvern instruments, Worcestershire, UK). Solutions (1 mg/ml, w/v) at pH 7.0 were filled into the zeta-potential folder capillary cell (DTS1060) for test.

2.3.3. Intrinsic fluorescence spectroscopy and protein surface hydrophobicity (S_0)

Intrinsic emission fluorescence spectra of gluten and S_0 were determined using a F4500 fluorescence-spectrophotometer (Hitachi Co., Japan). Protein dispersions (0.15 mg/ml) were prepared in 10 mM phosphate buffer (pH 7.0). Protein solutions were excited at 290 nm, and emission spectra were recorded from 300 to 400 nm at a constant slit of 5 nm. S_0 was determined using ANS⁻. A series of dilutions of each sample were made with 10 mM phosphate buffer (pH 7.0) to obtain a range of protein concentrations at 0.05, 0.1, 0.2, 0.5, and 1.0 mg/ml. Then, 4 ml of the protein dispersion was mixed with 20 µl of stock solutions of 8 mM ANS⁻. Fluorescence intensity (FI) was measured at wavelengths of 390 nm (excitation) and 470 nm (emission). The initial slope of the FI versus protein concentration plot was calculated by linear regression analysis and used as an index of S_0 .

2.3.4. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of DWG were recorded using a Nicolet 8210E FTIR spectrometer (Nicolet, WI) equipped with a deuterated triglycine sulphate detector. The sample powder included 1 mg sample per 200 mg of KBr. FTIR spectra were obtained of wave number from 400 to 4000 cm⁻¹ during 128 scans, with 2 cm⁻¹ resolution (Paragon 1000, Perkin-Elmer, USA). Interpretation of the changes in the overlapping amide I band (1600–1700 cm⁻¹) components was made possible by deconvolution using Peak-Fit v 4.12 software (SPSS Inc.).

2.4. *In vitro* digestion

In vitro digestion process was carried out according to Zhu et al., 2008 with a little modification. Wheat gluten and DWG solutions (3% w/v, in Milli-Q water) were adjusted to pH 2.0 with 0.5 M HCl, and pepsin was added (4% w/w, protein basis). After incubation at 37 °C for 1 h, the solution was adjusted to pH 5.3 with 0.9 M NaHCO₃. Pancreatin (4% w/w, protein basis) was then added, and the pH was adjusted to 7.5 with 0.5 M NaOH. The solution was incubated at 37 °C for 2 h. Protein recovery was evaluated by terminating the digestion through heating the solution in boiled water for 10 min. The digesta were neutralised and centrifuged at 11,000g for 15 min to get the supernatant to evaluate protein recovery by the micro-Kjeldahl method. Another way of terminating digestion was through addition of 15% (final concentration) trichloroacetic acid (TCA) at various digestion times (pepsin 10 min, pepsin 1 h and pancreatin 2 h). After centrifugation for 10 min at 4000g, the TCA-soluble nitrogen in the supernatant was determined by the micro-Kjeldahl method to evaluate the *in vitro* digestibility.

2.5. Size exclusion high performance liquid chromatography (SE-HPLC) elution profiles of DWG and DWG hydrolysates

Molecular weight distribution of DWG was analysed using SE-HPLC according to Wong, Day, McNaughton, and Augustin (2009) with modifications. Samples were analysed using a Waters HPLC system (Water 600, Milford, MA). Samples were prepared in 50 mM sodium phosphate buffer (pH 7.2) containing 0.5 wt% SDS, then 20 µl was injected into a TSK-Gel G4000SW column (7.5 mm i.d. × 600 mm length, Tokyo, Japan) and run at 1 ml/min in a 50 mM sodium phosphate buffer (pH 7.2) containing 0.2 M NaCl. The elution profiles were monitored at 214 nm. Blue dextran 2000 (2000 kDa), thyroglobulin (669 kDa), aldolase (158 kDa), conalbumin (75 kDa) and ovalbumin (43 kDa) (Sigma Co., St. Louis, MO, USA) were used as the standard proteins for calibration. The estimation of molecular weight was based on the elution profiles of protein standards.

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