



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



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ABSTRACT

The effects of citric acid deamidation on the physiochemical 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, Akilloglu, 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,

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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.

Molecular weight of DWG hydrolysates collected from different digestion times was analysed using a TSK-Gel G2000SWXL column (7.8 mm i.d. × 300 mm length, Tokyo, Japan). The digests were filtered through 0.45 µm syringe filters and then injected into the column (20 µl) and run at 1 ml/min in a 50 mM sodium phosphate buffer (pH 7.2). The elution profiles were monitored at 214 nm. To establish the molecular mass of peptides, the following molecular weight (MW) markers (Sigma–Aldrich, Inc., St. Louis, MO) were run as follows: oralbumin (43,000 Da), cytochrome C (12,384 Da), aprotinin (6512 Da), vitamin B₁₂ (1855 Da), glutathione (307 Da), and gly (75 Da). The markers yielded a linear log MW versus elution time regression line ($r = 0.9924$).

2.6. Antioxidant activity by ORAC assay

The ORAC assay was determined according to Zheng et al. (2012). The reaction was done in a 75 mM sodium phosphate buffer (pH 7.4). First, 20 µl of the antioxidant and 120 µl of the 70 nM fluorescein solution (final concentration) were placed in a black 96-well microplate (Thermo Fisher Scientific, Waltham, MA). The mixture was preincubated for 15 min at 37 °C. Then, 60 µl of the 12 mM AAPH solution (final concentration) were added rapidly using a multichannel pipet (Thermo Fisher Scientific, Waltham, MA). The plate was shaken for 30 s before the first reading, and the fluorescence was recorded using a Varioskan Flash Spectral Scan Multimode Plate Reader (Thermo Fisher Scientific, Waltham, MA) every minute for 100 min. The excitation and emission wavelengths were 485 and 520 nm. A blank using phosphate buffer instead of the antioxidant solution and calibration solutions with 1–6 µM Trolox (final concentration) as the antioxidant were used for each assay. Each sample was done at least in triplicate. The area under the fluorescence decay curve (AUC) was calculated as follows:

$$\text{AUC} = 1 + \sum_{i=100}^{i=1} \frac{f_i}{f_0}$$

where f_0 is the initial fluorescence reading at 0 min, and f_i is the fluorescence reading at time i . The net AUC corresponding to a sample was calculated as follows:

$$\text{netAUC} = \text{AUC}_{\text{antioxidant}} - \text{AUC}_{\text{blank}}$$

The linear regression equation between the net AUC and the antioxidant concentration was calculated. The final ORAC values were expressed as µmol TE (Trolox equivalent)/g of antioxidant.

2.7. Amino acid analysis

2.7.1. Free amino acid analysis

The digests at different digestion stages were precipitated with 10% sulfosalicylic acid for 1 h at 4 °C and then centrifuged at 11,000g for 15 min. The sample was then diluted with HCl (pH 2.0), and the solution was passed through a microfiltration membrane (0.22 µm). The filtrate was subjected to a Membrapure A-300 amino acid analyser (Membrapure Co., Frankfurt, Germany) after precolumn derivatising with OPA.

2.7.2. Total amino acid analysis

The method of Zhu et al. (2008) was used with little modification. Samples of DWG hydrolysates (pancreatin 2 h, supernatant) were hydrolysed in sealed, evacuated glass tubes with 6 M HCl, at 110 °C for 24 h. The analysis was performed with a Membrapure A-300 amino acid analyser (Membrapure Co., Frankfurt, Germany) equipped with a C18 column (4.6 mm × 125 mm) for amino acid separation. Post-column reaction with ninhydrin yielded the amino acid derivatives. The concentrations of the specific amino acids

were determined from their respective absorption intensities, which were calibrated to the known concentrations of amino acid standards. Trp was determined by alkaline hydrolysis.

2.8. Statistical analysis

Three repetitions of each test were carried out to characterise the functional properties. Statistical calculation was investigated using the statistical package SPSS 11.5 (SPSS Inc., Chicago, IL) for one-way ANOVA. Differences were considered to be significant at $p < 0.05$, according to Duncan's Multiple Range Test.

3. Result and discussion

3.1. Characters of DWG

The deamidation degree (DD), ash content, zeta-potential and intrinsic fluorescence of wheat gluten, at different deamidation times, are shown in Fig. 1. The DD value increased from 8.64% for 3 h deamidation products to 40.61% for 16 h deamidation products. The absolute value of zeta-potential of the deamidated gluten at pH 7 showed an increase after deamidation. The introduction of the carboxyl group by deamidation explained the increase of the protein surface charge (Zhang, Luo, & Wang, 2011; Zhao, Tian, & Chen, 2010). After deamidation, the ash content increased as a result of neutralization, which was similar to a previous report on HCl deamidated soluble wheat gluten (Wong et al., 2009).

The polarity of the environment of the tryptophan and tyrosine residues determines the fluorescence spectrum and was an indication of the change of the proteins conformation (Sun, Zhao, Yang, Zhao, & Cui, 2011). As shown in Table 1, the wheat gluten exhibited a fluorescence emission maximum (λ_{max}) at around 345 nm, which is a characteristic fluorescence profile of tryptophan residues in a relatively hydrophobic environment (Sun et al., 2011). The λ_{max} shifted from 345 to 346 nm after deamidation, implying that tryptophan residues within the protein molecules were exposed to a more hydrophilic environment during citric acid deamidation. This was in accordance with a previous report by Wong et al. (2012) which demonstrated that the increase of λ_{max} indicated protein unfolding.

3.2. Solution properties and secondary structure of DWG

The pH-dependant solubility of citric acid deamidated protein is shown in Fig. 1 A. After deamidation, the solubility of DWG significantly increased at neutral pH, and the isoelectric point was shifted to an acid pH. The highest solubility of wheat gluten was 50.1% at pH 7 after deamidation for 16 h. This change of solubility was in accordance with previous reports (Mimouni et al., 1994; Yong et al., 2006). Deamidation by transforming glutamine to glutamic acid increased negative charges, thus shifted isoelectric to acid pH. Protein solubility can exert a great influence on the surface and hydrodynamic properties, which is important for its use as food ingredients (Wu et al., 1976).

Surface hydrophobicity (S_0) is an important characteristic of the protein, that is most likely to relate to its surface behaviours and consequently to the emulsifying properties (Kato et al., 1987; Agyarea, Addo, & Xiong, 2009). S_0 gradually increased with the increase of deamidation time (Fig. 1 B), suggesting the hydrophobic regions were exposed outside. Matsudomi et al. (1981) found a linear increase in S_0 with increase in the degree of deamidation. A previous report showed that a further increase of the DD value, resulted in a significant decrease of the surface hydrophobicity of hordein (Zhao et al., 2010). This phenomenon did not occur in this work, which

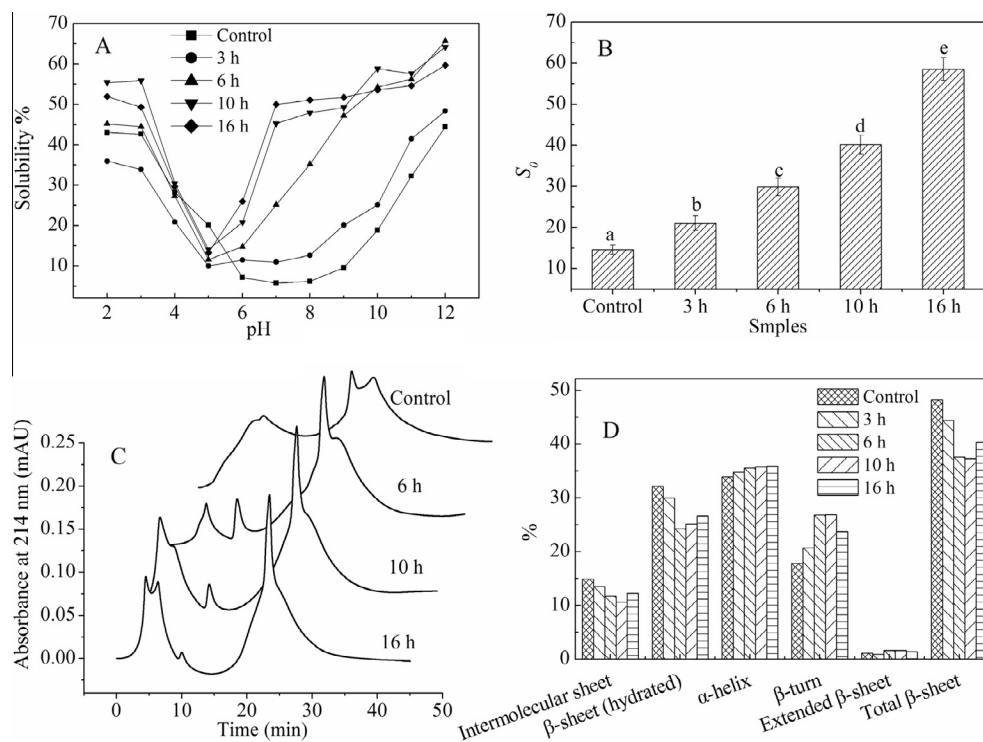


Fig. 1. Characters of DWG with a different deamidation time: (A) solubility; (B) surface hydrophobicity; (C) SE-HPLC elution profiles; (D) secondary structural compositions. Different letters (a–e) on the top of columns indicate significant differences among samples ($p < 0.05$).

Table 1
Characters of DWG after citric acid deamidation.

	DD (%)	Ash (%)	Zeta-potential (mV)	$\lambda_{\max}$ (nm)
Control	–	0.58 $\pm$ 0.05 ^a	–2.88 $\pm$ 0.15 ^a	345 $\pm$ 0.1 ^a
3 h	8.64 $\pm$ 1.23 ^a	5.35 $\pm$ 0.32 ^b	–3.78 $\pm$ 0.21 ^b	345 $\pm$ 0.3 ^b
6 h	21.4 $\pm$ 2.66 ^b	6.07 $\pm$ 0.91 ^b	–5.06 $\pm$ 0.22 ^c	345 $\pm$ 0.3 ^{b,c}
10 h	28.4 $\pm$ 2.15 ^b	5.66 $\pm$ 0.38 ^b	–6.84 $\pm$ 0.17 ^d	345 $\pm$ 0.1 ^{b,c}
16 h	40.6 $\pm$ 3.41 ^c	6.60 $\pm$ 0.44 ^b	–10.6 $\pm$ 0.25 ^e	346 $\pm$ 0.2 ^c

^{a–e} Mean values in the same column not followed by a common letter differ significantly ($p < 0.05$).

might be due to no cleavage of peptide bonds in this deamidation process.

SE-HPLC elution profile of deamidated wheat gluten (control, 6 h, 10 h and 16 h) is shown in Fig. 1 C. There were mainly two peak regions in the chromatogram. The first was around the first 10 min, and the second was around 20–30 min. According to the molecular weight of the standards and literature, the first region was glutenin with a high molecular weight and the latter was gliadin. Gliadin mainly includes α -, β -, γ -, ω -gliadins (25–75 kDa) and exhibits two peaks corresponding to ω -gliadin and α -, β -, γ -gliadin (Wong et al., 2009). As the deamidation time increased, the two peaks of gliadin gradually changed to one peak. Meanwhile, the glutenin region in the first 10 min of the elution also showed an evident difference. This may be the result of the cross-linking between α -, γ -gliadin and glutenin during hydrothermal treatment (Lagrain, Thewissen, Brijs, & Delcoul, 2008). There was no smaller molecule observed in DWG after deamidation. Besides, little free amino acid was detected by OPA methods (data was not shown here). Thus, we could assume that the ammonia released from gluten by heating at 70 °C with 0.2 M citric acid, was mainly the result of hydrolysis of a few accessible Gln γ -amide (and Asn σ -amide) groups to form carboxylic groups (deamidation), but not peptide bond cleavage (proteolysis) (Berti et al., 2007).

Secondary structure results using FTIR in Fig. 1 D show that control gliadin contained 33.9% α -helix, 17.8% β -turn and 48.3% β -sheet. Decrease of the β -sheet while increase of the α -helix and β -turn was observed after deamidation. As Glu and Asp are the main constituents of β -turn and glutamine residues mainly form β -sheet in gluten (Yong et al., 2006), the increase of the β -turn and decrease of the β -sheet indicated that more Glu and Asp were released. Thus the charge density and electrostatic repulsion of protein increased after deamidation. Changes in the secondary structure of protein by citric acid deamidation were similar to a previous report by Yong et al. (2006), who also showed a significant decrease of β -sheets and increase of α -helix and β -turn of gluten after protein glutamine deamidation. In contrast, Wong et al. (2012) reported that wheat gliadin deamidated by HCl exhibited an increase of β -sheet while a decrease of the α -helix. Cabra, Arreguin, Vazquez-Duhalt, and Farres (2007) also reported that after alkaline deamidation, the α -helix of Z19 α -zein decreased. These discrepancies might be mainly due to the different effect of HCl or alkali and citric acid on protein molecule.

3.3. Molecular weight distribution of *in vitro* digests of DWG

The molecular weight distribution of hydrolysates from control and DWG is summarised in Table 2. The amount of hydrolysates with molecular weight beyond 10 kDa decreased evidently after deamidation. Meanwhile, molecular weight less than 5 kDa in the hydrolysates after deamidation increased only at pancreatin digestion stage. This indicated that after deamidation, gluten was more resistant to pepsin hydrolysis while more susceptible to pancreatin hydrolysis. Results from FTIR, SE-HPLC, zeta-potential and intrinsic fluorescence (Table 1 and Fig. 1) indicated that changes in the chemical properties of wheat gluten treated by citric acid were significant. These changes might exert an influence on the *in vitro* digestion process. This phenomenon was similar to a previous report which demonstrated the increase of pancreatic digestibility *in vitro* of deamidated wheat gliadin (Kumagai et al., 2007).

Table 2Molecular weight distribution of *in vitro* digests of DWG.

	>10 kDa	5–10 kDa	3–5 kDa	1–3 kDa	<1 kDa
Control-pepsin 1 h	36.3	24.1	19.4	16.2	4.17
3 h-Pepsin 1 h	39.2	24.1	18.3	14.6	3.84
6 h-Pepsin 1 h	39.9	24.2	18.3	14.2	3.45
10 h-Pepsin 1 h	39.2	24.3	18.6	14.4	3.60
16 h-Pepsin 1 h	37.0	24.4	19.5	15.5	3.65
Control-pancreatin 2 h	13.1	20.9	28.4	28.2	9.43
3 h-Pancreatin 2 h	12.4	21.6	28.5	28.2	9.23
6 h-Pancreatin 2 h	10.5	20.6	29.5	29.4	10.0
10 h-Pancreatin 2 h	8.26	19.6	31.2	31.1	9.73
16 h-Pancreatin 2 h	7.50	19.7	31.4	31.6	9.87

Because of the unique peptide bond specificity of digestive proteases, the pepsin and pancreatin digestion profiles mainly depend on the characteristics of the substrates. Deamidation limitedly increased the protein solubility at an acid pH and the proteins tend to aggregate in the acid solution (Kumagai et al., 2007), so the susceptibility of all samples to pepsin digestion was slightly decreased. At a neutral pH, the deamidation process increased the protein solubility and loosened the protein structure, thus the digestibility of DWG was increased (Chan & Ma, 1999; Kumagai et al., 2007). Moreover, the digestion velocity of proteins by α -chymotrypsin and trypsin greatly correlated with the protein flexibility (Kato, Komatsu, Fujimoto, & Kobayashi, 1985). S_0 was in relation to the flexibility of protein molecules, and the increase in S_0 was important for the proteolytic recognition of proteins (Davies, 2001). As S_0 (Fig. 1B) significantly increased after deamidation, it was reasonable that the DWG digests had more low molecular weight fractions.

3.4. Characters of *in vitro* digests of DWG

The protein recovery at different digestion stage is shown in Fig. 2A. Protein recovery at a neutral pH was improved after deamidation, especially at the pepsin digestion stage. This was

mainly due to the increase of protein solubility at a neutral pH value.

Fig. 2B illustrates the *in vitro* digestibility of DWG. The *in vitro* digestibility of wheat gluten by pepsin was around 50–60%. Further incubation with pancreatin led to a significant increase of digestibility to around 90%. After deamidation, the *in vitro* digestibility of wheat gluten by pepsin decreased, while it increased by pancreatin. This was consistent with the change in amounts of peptides with different molecular weight as shown in Table 2.

From Fig. 2B to Fig. 1D, we can observe that the digestibility by pancreatin showed a similar trend with the α -helix content, while an opposite trend with the β -sheet content was shown. This was consistent with a previous report about correlation between trypsin, α -chymotrypsin activity and the secondary structure of oxidizing sarcoplasmic proteins (Sun et al., 2011). These results also confirmed that the changes of protein structure affected both chemical and physical recognition sites of proteins by proteases (Marmon & Undeland, 2013).

The effect of deamidation on the antioxidant activity of *in vitro* digests is shown in Fig. 2C. Wheat gluten digestion products by pepsin showed higher antioxidant activity than those subsequently digested by pancreatin. It was suggested that more peptides were generated upon pepsin digestion, which benefited the increase of the antioxidant activity. When further digested by pan-

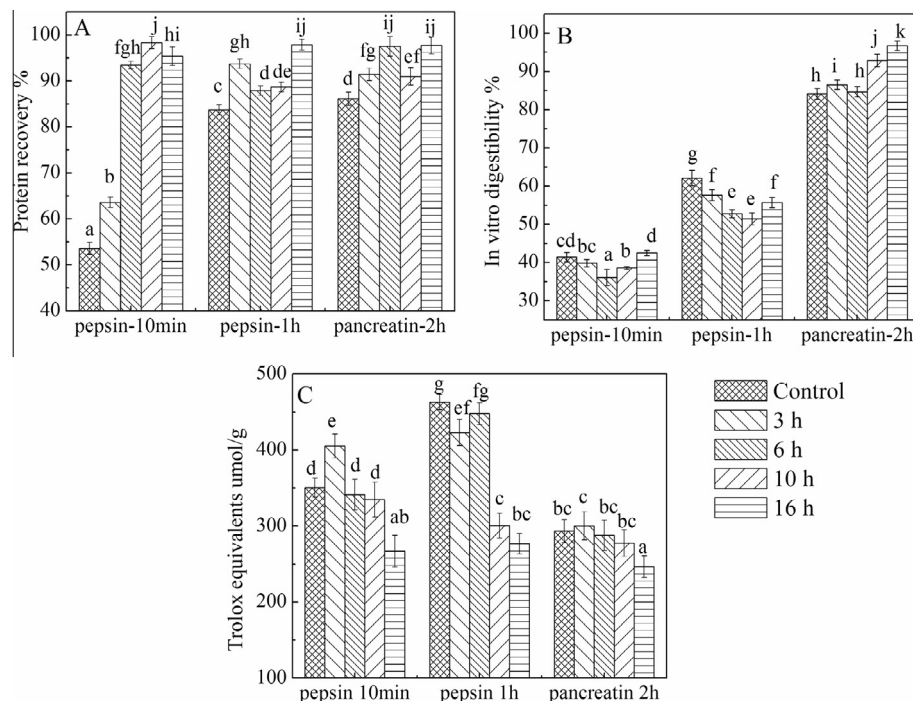


Fig. 2. Changes in the *in vitro* digestion properties of DWG with a different deamidation time: (A) protein recovery of DWG after digestion; (B) *in vitro* digestibility of DWG; (C) ORAC value of *in vitro* digests of DWG. Different letters (a–k) on the top of columns indicate significant differences among samples ($p < 0.05$).

creatin, the peptide was cleavage to more free amino acids, which might lead to a decrease of antioxidant activity (Zhu et al., 2008). Additionally, the ORAC value decreased with increasing of deamidation degree upon pepsin digestion. But this discrepancy was reduced at the digestion stage by pancreatin. This effect was consistent with a previous report on the deamidated C-hordein and maize zein (Chiue, Kusano, & Iwami, 1997). The deterioration of the antioxidant activity of C-hordein and maize zein was mainly related to the collapse of the β -turn structure of the repeat sequence and protein fragmentation (Kawase et al., 2003). However, in this work, the β -turn structure increased after deamidation (Fig. 1D), and the molecular weight was not influenced by deamidation (Fig. 1C). So the decrease of the antioxidant activity of wheat gluten hydrolysates was supposed to be not related to the collapse of the β -turn of DWG.

3.5. Free amino acids composition of *in vitro* digests of DWG

As shown in Table 3, the two digestion stages substantially increased the content of FAAs, especially at the pancreatin digestion stage. The pepsin digestion released the FAAs content to around 70 mg/100 g protein, indicating pepsin digestion mainly caused peptide bonds cleavage (Zhu et al., 2008). FAAs levels increased about 10-folds after pancreatin digestion compared with the pepsin digestion stage, but total FAAs were only around 600 mg/100 g protein after pancreatin digestion. Thus, most of the final digests still existed as a form of peptide. The digests after pancreatin digestion showed a high level of Arg, Tyr, Leu, Ile and Phe, which was in accordance with an earlier report (Goodman, 2010; Liao et al., 2010). After deamidation, *in vitro* digests showed increase of total FAAs in the pancreatin digestion stage. This correlated well with the above results, which showed an increased digestibility by pancreatin (Fig. 2B) and increased molecular weight less than 5 kDa of digests molecules after deamidation (Table 2). Both hydrophobic amino acids and essential amino acids increased after deamidation. Free Lys content of the digests decreased at the pepsin digestion stage but showed little difference at the pancreatin digestion stage. It can be observed in Table 3 that after deamidation, release of Glu, Asp and Asn was less compared with

Table 4

Amino acid composition (mol %) of DWG digests.

	mol %				
	Control	3 h	6 h	10 h	16 h
Asx (Asp + Asn)	3.37	3.40	3.16	3.01	3.42
Thr	3.06	3.12	2.97	2.87	3.14
Ser	5.72	5.84	6.20	5.62	5.76
Glx (Glu + Gln)	26.2	27.1	26.5	26.0	26.0
Gly	5.89	5.67	5.49	5.54	5.48
Ala	3.81	4.07	4.00	3.93	3.54
Cys	2.23	2.20	2.16	2.26	2.05
Val	4.05	4.04	3.92	4.16	3.84
Met	1.02	1.04	0.65	0.64	0.61
Ile	3.38	3.53	3.41	3.60	3.41
Leu	6.16	6.27	6.11	6.30	6.08
Tyr	2.07	1.92	1.92	1.85	1.80
Phe	3.70	3.34	3.50	3.48	3.42
His	4.29	3.64	3.98	4.22	4.25
Lys	1.98	2.07	3.16	3.52	3.52
Arg	3.62	3.36	3.73	4.04	4.11
Pro	18.8	18.8	18.6	18.4	19.1
Trp	0.61	0.61	0.62	0.66	0.66
Essential AAs	24.0	24.0	24.3	25.2	24.7

control. This might be caused by the rearrangement of protein molecules that changed the enzyme action sites (Liao et al., 2010).

3.6. Total amino acids composition of *in vitro* digests of DWG

The total amino acid composition (mol %) of final *in vitro* digests (after pepsin digestion for 1 h and pancreatin digestion for 2 h) of unmodified and deamidated wheat gluten is shown in Table 4. Glu (Gln) and Pro are the main amino acids of wheat gluten. The Lys amount evidently increased from 1.98% for 3 h deamidated products to 3.52% for 16 h deamidated products. Deamidation by citric acid caused unfold of protein molecules to let buried Lys exposed, which could explain the improved Lys release after digestion (Chan & Ma, 1999). Furthermore, the total amounts of essential AA showed an increase from 24.0% to 24.7%. Wheat gluten was not considered to be a good source of protein with nutritional properties for its low content of Lys compared with other

Table 3

Free amino acid composition (mg/100 g) of DWG digests during *in vitro* digestion.^a

	mg/100 g protein					
	Control-pep ^b -1 h	10 h-Pep-1 h	16 h-Pep-1 h	Control-pan ^c -2 h	10 h-Pan-2 h	16 h-Pan-2 h
Asp	4.33	3.04	2.46	11.5	7.50	8.42
Thr	1.32	1.66	1.55	10.8	8.67	9.79
Ser	1.61	1.74	1.56	8.00	9.08	9.82
Asn	1.87	1.11	0.96	12.4	11.8	13.3
Glu + Gln	5.67	4.03	3.61	69.1	50.6	56.3
Gly	1.38	1.35	1.19	3.52	3.35	3.60
Ala	2.15	1.91	1.60	4.31	11.7	12.3
Val	1.89	3.52	2.50	23.4	22.5	23.1
Cys	7.05	7.30	7.11	39.8	39.3	41.3
Met	4.67	5.32	4.88	21.7	22.7	22.2
Ile	2.41	3.13	2.74	29.1	28.6	29.0
Leu	3.72	6.57	4.46	90.6	93.2	93.1
Tyr	2.09	4.13	4.21	55.4	60.5	58.9
Phe	2.42	4.49	3.96	53.9	59.1	57.7
His	2.09	3.11	1.59	18.0	18.4	18.2
Trp	0.57	1.64	0.24	13.4	13.2	11.4
Lys	21.8	13.9	11.4	35.0	32.4	34.8
Arg	1.50	0.82	0.81	91.7	89.5	95.5
Pro	5.71	4.32	2.47	10.6	29.5	22.6
Hydrophobic AAs	21.0	29.7	22.2	281	318	308
Essential AAs	38.8	40.2	31.7	278	280	281
Total AAs	74.3	73.1	59.2	602	612	621

^a DWG digestion samples are the supernatants (soluble) of the hydrolysates.

^{b,c} Pep and pan are abbreviations of pepsin and pancreatin.

protein source like soybean. Thus, the increase in the amount of Lys and total essential AAs in digests, indicated that citric acid deamidation might benefit the nutritional properties of wheat gluten. Meanwhile, Cys in the digests slightly decreased after deamidation for 16 h which could be explained by the loosened structure of wheat gluten after deamidation.

The antioxidant activity and radical scavenging activity was reported to be correlated with the presence of Tyr, Val, Met, His, Ala, and Leu residues that both in free forms and as residues in peptides and proteins (Hernández-Ledesma, Amigo, Recio, & Bartolomé, 2007; Zhang et al., 2011). Especially, His exhibits a strong antioxidant activity due to the decomposition of its imidazole ring (Sarmadi & Ismail, 2010; Wang et al., 2007). As shown in Table 4, the amounts of Met, Tyr, Cys, and His exhibited decreased trends with increasing deamidation time. The decrease of these amino acids in the final digests seemed to be associated with the changes of antioxidant activity of wheat gluten digests after deamidation (Fig. 2C). Apart from the above reasons, other factors like peptide structure as well as amino sequence might also influence the antioxidant activity of hydrolysates (Wang et al., 2007; Zhu et al., 2008).

4. Conclusions

In conclusion, citric acid deamidation significantly increased the solubility and surface hydrophobicity of wheat gluten at a neutral pH and caused little protein hydrolysis. Deamidation increased the *in vitro* digestibility of wheat gluten at the pancreatin digestion stage. This improvement of digestibility can be explained by the significant increase of surface hydrophobicity and change of the protein secondary structure. After deamidation, the ORAC values of *in vitro* digests decreased, and this appears to be related to the lower release of amino acids that contribute to the antioxidant activity. In addition, amounts of Lys and essential AAs of DWG digests increased, indicating deamidation might be able to improve the nutritional characters of wheat gluten. These findings indicated the unique effect of deamidation on the digestion process of wheat gluten and may facilitate the novel applications of citric acid deamidated wheat gluten.

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