Journal of Cereal Science 60 (2014) 143–150

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Comparison of the conformational and nutritional changes of deamidated wheat gliadin by citric acid and hydrochloric acid

Chaoying Qiu [a], Weizheng Sun [a], Guowan Su [a], Chun Cui [a], Mouming Zhao [a,b,*]

[a] College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China
[b] State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China

ARTICLE INFO

Article history:
Received 21 November 2013
Received in revised form
3 February 2014
Accepted 4 February 2014
Available online 5 March 2014

Keywords:
Wheat gliadin
Deamidation
Conformation
Amino acid composition

ABSTRACT

Deamidated wheat gliadins were prepared using hydrochloric acid (HCl) and citric acid (HDWG and CADWG), respectively. Their secondary structure, protein molecular interaction, thermal properties and nutritional changes were compared by Fourier transform infrared spectroscopy (FTIR), Raman spectrum, atomic force microscopy (AFM), differential scanning calorimetry (DSC), and amino acid analysis, respectively. Secondary structures and molecular vibration model showed slight difference between HDWG and CADWG, but significant difference between control gliadin and deamidated wheat gliadins. HDWG and CADWG had different shapes on the mica surface that the former showed some extent of linear aggregates and fibrils while the latter mainly exhibited globular aggregates. This result was further supported by thermal characteristics that CADWG had higher denaturation temperature than control gliadin and HDWG. Citric acid deamidation could increase the Lysine content and better maintain the total essential amino acids of *in vitro* digests of gliadin compared with HCl.

1. Introduction

Wheat gluten is the main source of plant proteins and sufficiently produced worldwide. It is mainly composed of glutenins and gliadins according to their polymerization properties. Gliadins are one kind of monomeric globular protein with low molecular weight (30 kDa–100 kDa), bonded by intra-molecular disulfide bonds. They are classified into α-, β-, γ- and ω-gliadins according to biochemical and genetic properties (Thewissen et al., 2011). Gliadins are formed by a non-repetitive domain rich in α-helix structure and by a heterogeneous repetitive domain rich in β-reverse turns (Secundo and Guerrieri, 2005). A solution of gliadins was more effective in lowering surface tension than glutenin (Thewissen et al., 2011). However, the use of wheat gliadins is mainly in film materials or encapsulation prepared in alcohol solution. Its use as a functional protein is limited by the low solubility due to many nonpolar amino acids and glutamines (Wang et al., 2006). Chemical and enzymatic modifications have been made to improve the foaming and emulsifying properties of gliadins (Thewissen et al., 2011; Majzoobi et al., 2012). Deamidation is an effective way to increase protein solubility and improve functional properties by reducing intra-intermolecular hydrogen bonding and enhancing electrostatic repulsion between protein molecules (Day et al., 2009; Qiu et al., 2013). The most common method for the deamidation is by HCl treatment. However, considerable hydrolysis of the peptide bonds is inevitable to produce bitter tasting peptides and also reduces the processing property (Liao et al., 2010). Carboxylic acid was also reported to be a better choice to deamidate, which can reduce the potential risk for celiac people and create little proteolysis (Qiu et al., 2013).

Deamidation under acid condition and high temperature caused changes in protein conformation due to increase of electrostatic repulsion. The amphiphilic characteristics of protein increased as a result of increase of negatively charged polar groups. The structure and conformational properties of protein could affect the nutritional and functional properties of the processed foods. A better understanding of the physicochemical, conformational properties and thermal properties of deamidated wheat gliadin can enhance their potential utilization as a kind of new food ingredient. Although deamidated wheat gluten and gliadin by different acids and their functional properties have been reported (Qiu et al., 2013; Wong et al., 2012), the research for deamidated gliadins focuses little on their thermal characteristics and amino acid composition changes. In addition, molecular vibration reflected by Raman spectrum and nanometer images by AFM can offer more structural

* Corresponding author. State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China. Tel./fax: +86 20 87113914.
E-mail address: femmzhao@scut.edu.cn (M. Zhao).

http://dx.doi.org/10.1016/j.jcs.2014.02.003
0733-5210/

information of gliadin (McMaster et al., 1999). Most research focused on the individual purified gliadin (McMaster et al., 1999; Paananen et al., 2006). Although the crude gliadin samples are more practically used for application, the research on the conformation and characteristics of crude gliadins is still limited.

In our recent work, we investigated the properties of the citric acid deamidated wheat gliadins and found that citric acid deamidation remarkably increased the protein solubility and emulsion stability at neutral pH (Qiu et al., 2013). Some differences were observed between the performance of HCl deamidated gliadin and citric acid deamidated gliadin in emulsions (Day et al., 2009; Qiu et al., 2013). The functional properties of the proteins are related with physicochemical and conformational properties. In this paper, citric acid and HCl deamidation were carried out to compare the structural characteristics of gliadin treated with different acids.

2. Materials and methods

2.1. Materials

Wheat gluten isolate was prepare by hand washing dough using distilled water from the strong wheat cultivar Jinan17 supplied by Jinhe flour Co., Ltd. (Foshan, Guangdong, China) after extraction of the lipid with chloroform by overhead agitator at 1500 rpm for 30 min. Then the gluten was freeze-dried. Protein content was 82.5% as determined by the Kjeldahl method (N × 5.7). Pepsin from porcine gastric mucosa (400 U/mg solid) and pancreatin from porcine pancreas (8 × USP) were purchased from Sigma Chemical Co. Ltd. (St. Louis, USA). All other chemicals and solvents were of analytical grade.

2.2. Preparation of deamidated wheat gliadin

Wheat gluten (8%, w/v) was mixed with HCl (0.09 M) or citric acid (0.2 M) to form suspensions, respectively. The suspensions were hydrated in a shaking water bath at 70 °C for 10 h and 20 h to get deamidated samples with similar deamidation degree levels (20% and 40%) by the two acids. The deamidation degree was determined according to Kato et al. (1987). Then the suspensions were neutralized with sodium hydroxide (1 M), dialyzed for 36 h at 4 °C against distilled water and freeze-dried. Gliadin and deamidated gliadins were extracted according to the method of Thewissen et al. (2011). Wheat gluten or deamidated wheat gluten was mixed with two quantities (ten-fold) of 70% (v/v) ethanol. After stirring (30 min at 20 °C) and centrifugation at 10,000 *g* for 10 min at 20 °C (Hitachi Koki Co. Ltd., Tokyo, Japan), the supernatants were pooled. Before the second extraction step, the cohesive glutenin residue was mechanically disrupted with a spatula. Ethanol in the supernatants was removed by rotary evaporation (50 °C), and the gliadin fractions were freeze-dried. Control gliadin, HDWG and CADWG with deamidation degrees of 20% and 40% (C, H-1, H-2, CA-1 and CA-2) were used for the following research.

2.3. Nitrogen soluble index (NSI) and protein surface hydrophobicity (S_0)

Protein dispersions (10 mg/mL, dissolved in deionized water) were adjusted to a specific value within the range of pH 3–10 by 1 M HCl or NaOH. The dispersions were agitated with a magnetic stirrer for 1 h at room temperature, and then centrifuged at 12,000 *g* for 20 min. Protein content of the supernatant was determined according to Lowry et al. (1951). Bovine serum albumin was used as the standard. Protein solubility was calculated as nitrogen solubility index (NSI) = (protein content of supernatant/amount of proteins added) × 100%.

Surface hydrophobicity (S_0) was determined by the hydrophobicity fluorescence probe 1-anilino-8-naphthalenesulfonate (ANS) using an F7000 fluorescence spectrophotometer (Hitachi Co., Japan). 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, 1.0 mg/mL. Then, 4 mL protein dispersion was mixed with 20 μL of 8 mM ANS^-. Fluorescence intensity (FI) was measured at the wavelengths of 390 nm (excitation) and 470 nm (emission), with a constant excitation and emission slit of 5 nm. The FI for each sample was then computed by subtracting the FI attributed to protein in buffer without ANS^-. The initial slope of the FI versus protein concentration plot was calculated by linear regression analysis and used as an index of S_0.

2.4. Size exclusion high performance liquid chromatography (SE-HPLC)

The molecular weight of control gliadin, HDWG and CADWG was analyzed using size exclusion chromatography conducted according to Wong et al. (2012) with modifications. The chromatographic apparatus consisted of a Waters HPLC 600 system (Waters, Division of Millipore, Milford, MA, USA). Samples were prepared in 50 mM sodium phosphate buffer (pH 7.2) containing 0.5 wt% SDS. These samples (20 μL) were then 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 200 mM NaCl. The elution profiles were monitored at 214 nm with a UV detector. 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. The estimation of molecular weight was based on the elution profiles of protein standards.

2.5. Differential scanning calorimetry (DSC)

The thermal characteristics of proteins were determined by DSC measurements performed using a TA Q100-DSC thermal analyzer (TA Instruments, New Castle, DE 19720, USA) according to Falcão-Rodrigues et al., (2005). The calorimeter was calibrated using an indium standard. Samples were equilibrated at water activity values of 0.79 (about 50% moisture). Samples of 5.0 ± 0.1 mg were weighed into aluminum pans and covers were hermetically sealed into place. An empty, hermetically sealed pan was used as reference. The samples were analyzed at a rate of 10 °C/min from 30 to 100 °C. Denaturation temperature (T_d) and denaturation enthalpy (ΔH) were analyzed from the thermograms by the Universal Analysis 2000 software, Version 4.1D (TA Instruments-Waters LLC).

2.6. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of samples were recorded using a Nicolet 8210E FTIR spectrometer (Nicolet, WI) equipped with a deuterated triglycine sulfate detector. The sample powder (maintained at ambient temperature) included 1 mg sample per 200 mg of KBr. FTIR spectra were obtained from wave numbers 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 band (1580–1700 cm^{-1}) components was made possible by deconvolution using Peak-Fit v 4.12 software (SPSS Inc., Chicago, IL). Full-width at half-maximum (fwhm) was 16 cm^{-1} and kept constant for all peaks during deconvolution. Protein secondary structures were determined as percentages of α-helix, β-sheets, β-turn and random coils according to Secundo and Guerrieri (2005).