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Heat-induced interaction between egg white protein and wheat gluten

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ABSTRACT

Some wheat-based food systems, such as cakes and cookies, include mixtures of gluten and egg white protein (EWP) and are processed under heating conditions. Changes in these proteins during processing can affect the quality of the end product. This study investigated protein polymerization during heating of (mixtures of) wheat gluten and EWP. Chemical changes were studied by size-exclusion high performance liquid chromatography (SE-HPLC), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), thiol (SH) measurement and Fourier transform infrared (FTIR). During heating, protein polymerization was observed in the mixtures of gluten, glutenin, gliadin and EWP according to SE-HPLC profiles and results of SDS-extractable protein. The results of SDS–PAGE profiles of different proteins were in accordance with SE-HPLC. The number of SH groups in the majority of proteins showed a significant decrease, implying that disulfide (SS) bonds contributed to the extractability loss. In addition, changes of secondary structure tested by FTIR indicated protein aggregation.

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1. Introduction

Wheat is one of the most important components of the human diet due to the whole range of applications in food, such as breads, pasta, noodles and cookies (Dinelli et al., 2007; Veraverbeke & Delcour, 2002). Wheat flour proteins can be classified into albumin, globulin, gliadin and glutenin fractions (Rombouts, Lagrain, & Delcour, 2012). Glutenin and gliadin constitute gluten which is of great technological importance in wheat-based food systems (Delcour et al., 2012). Glutenin proteins, distributed in a wide range of molecular weights, can be classified into high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) (Ma, Li, et al., 2013). Gliadin proteins are a kind of alcohol-soluble, monomeric proteins with an absence of inter-chain disulfide bonds (Balaguer, Gomez-Estaca, Cerisuelo, Gavara, & Hernandez-Munoz, 2014). A heating step during wheat-based food production induces formation of a strong gluten network through intermolecular SS cross-links in glutenin and gliadin, which influences the quality and stability of wheat-based products, such as bread and pasta (Bruneel, Pareyt, Brijs, & Delcour, 2010; Lagrain, Brijs, & Delcour, 2008).

EWP is generally obtained through spray-drying and widely used in the food industry because of its variety of functional properties, such as gelling, foaming and emulsifying characteristics (Baron et al., 2003; Lai et al., 2010). EWP consists of high biological value proteins, such as ovalbumin, conalbumin, ovomucoid and ovomucin. During heat-denaturing, these proteins can be induced to form extensive SH–SS exchanges, resulting in high molecular weight complexes (Onda & Hirose, 2003).

Wilderjans, Luyts, Brijs, and Delcour (2013) found a cross-link between egg protein and gluten protein that coincided with a major decrease in protein extractability during baking in a cake system. Khouryieh, Herald, and Aramouni (2006) showed that a whole-egg can reduce cooking loss, increase hardness and lightness of fresh noodles and hypothesized that egg albumin strengthens the gluten network to hold the starch during cooking. Tachi, Ogawa, Shimoyamada, Watanabe, and Katoh (2004) proposed a significant improvement of EWP on chewing texture, elasticity, stickiness and sensation of Chinese type noodles. Changes in rheological properties and sensory evaluation were considered to be caused by a compact and finer network structure consisting of wheat-EWP. A few studies have been focused on the effect of EWP on the physical properties of wheat-based products as a whole. However, the results were highly susceptible to the cross-linking between EWP and gluten under heating conditions. Generally, the cross-linking of EWP and glutenin was inferred, for glutenin contains most of the cysteine residues and disulfide bonds (Lindsay & Skerritt, 1999). Nonetheless, neither the interactions between gliadin monomers, glutenin polymers and EWP were clear, nor was their interaction degree at a certain temperature.

In this study, EWP was interacted with glutenin and gliadin under different temperature conditions, and changes in protein solubility, molecular weight distribution, –SH content and

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secondary structure were studied in order to delineate the interactions between glutenin, gliadin and EWP. Moreover, the interactions between EWP and gluten were also analyzed in order to prove the inferences in previous studies.

2. Materials and methods

2.1. Materials

Wheat flour was purchased from Xinjiang Tianshan Ltd. Co. Its moisture, starch and protein contents were 13.02%, 74.10% and 10.97%, respectively. EWP was obtained from Kangde Ltd. Co. Its moisture and protein content were 5.04% and 87.35%, respectively. Wheat gluten was provided by Lianhai Weijing Ltd. Co. Its moisture, starch and protein content were 10.00%, 9.40% and 77.76%, respectively.

2.2. Preparation of glutenin and gliadin proteins

Gliadins were extracted using the method described by Shi, Li, Wang, and Zhou (2004) with some modifications. An ethanol-aqueous solution (65%) and wheat gluten were mixed at a ratio of 30:1 (V/W), stirred for three hours and then centrifuged at 1650*g* for 30 min. The supernatants were pooled and gliadin was obtained after freeze-drying. Glutenins were obtained using the method described by Liu et al. (2007) with some modifications. Wheat gluten was mixed with water at a ratio of 1:20 (W/V) and the pH of the mixture was adjusted to 10.0. The solution was stirred for three hours and then centrifuged (1650*g*, 30 min). The supernatants were obtained, and anhydrous ethanol was added until the final concentration of ethanol reached 65% and the pH was adjusted to 7.0 using hydrochloric acid (HCl) aqueous solution. The solution was stewed for four hours and the precipitates were collected after centrifugation (1650*g*, 30 min). Glutenin was obtained after freeze-drying.

2.3. Thermal treatment

Protein samples (gluten, EWP, glutenin, gliadin, or gluten-EWP, glutenin-EWP, gliadin-EWP mixture of equal mass proportion) were mixed with distilled water at a ratio of 1:1 (W/V). They were heated at a constant temperature (65, 75, 85, 95, 105 °C and boiling temperature, respectively) for four hours. After heat-treatment, they were freeze-dried and ground to a fine powder.

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

Molecular weight distribution and proteins solubility of samples were determined by SE-HPLC (LC-20AT, Shimadzu, Kyoto, Japan) using the method described by Bean and Lookhart (2001) with some modifications. Protein samples were mixed with sodium phosphate buffer (50 mM, 1% SDS, pH 7.0) at a ratio of 1:1 (W/V, gluten, glutenin, gliadin, EWP) and 2:1 (W/V, gluten-EWP, glutenin-EWP, gliadin-EWP), respectively. All samples were stirred for 10 min, centrifuged (5220*g*, 5 min) and the supernatant was collected and then filtrated (Millex-HP, 0.45 μm, polyethersulfone). A 20 μl aliquot of each supernatant was loaded onto a TSK G4000-SWXL analytical column (Tosoh Biosep, Shanghai), and elution achieved with sodium phosphate buffer (50 mM, 1% SDS, pH 7.0) at a flow rate of 0.7 ml/min. The elution curve was monitored at 214 nm. Peak areas were calculated and SDS-extractable protein was expressed as the percentage of peak area of SDS-extractable protein in non-reducing sodium phosphate buffer (50 mM, 1% SDS, pH 7.0) to peak area of SDS-extractable protein in reducing sodium phosphate buffer (50 mM, 1% SDS, 1% dithiothreitol, pH 7.0).

2.5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) analysis

Non-reduced SDS–PAGE was performed using a 12% separating gel (pH 8.8) and a 5% stacking gel (pH 6.8). A 7 mg amount of each sample was stirred in 1 ml sample buffer (pH 6.8, 0.01 M Tris–HCl, 10% (W/V) SDS, 10% (v/v) glycerol, 0.1% (W/V) bromphenol blue). The dispersions were centrifuged (5220*g*, 5 min) after heating for 5 min in boiling water. A 10 μl aliquot of the supernatants was loaded onto the instrument and the voltage of electrophoresis was 100 V during the run.

2.6. Free –SH group determination

Total contents of free –SH were determined using the method described by Beveridge, Toma, and Nakai (1974) with some modifications. The following solvents were used: Tris–glycine–EDTA buffer (10.4 g Tris, 6.9 g glycine and 1.2 g EDTA per liter, pH 8.0, defined as TGE), 2.5% SDS in Tris–glycine–EDTA buffer defined as SDS-TGE. Ellman's reagent (4 mg/ml) was dissolved in TGE. A mass of 60 mg (gluten, glutenin, gliadin) or 20 mg (EWP, gluten-EWP, glutenin-EWP, gliadin-EWP) of the samples was incubated with 4 ml of SDS-TGE for one hour with vortexing every 10 min in ambient temperature. The reaction tubes were centrifuged at 1650*g* for 20 min. Absorbance of moderate supernatant was adjusted to a total volume of 5 ml with SDS-TGE. After adding 0.04 ml Ellman's reagent and incubating for 30 min, the reaction system was measured at 412 nm against the blank (without Ellman's reagent).

2.7. Secondary structure analysis

Secondary structures of samples were studied by FTIR. The protocol used in this study has previously been described by Meziani et al. (2011) with some modifications. FTIR scans were obtained with a Nicolet iS 10 spectrometer (Thermo Nicolet, America). The scanning rate was 10 kHz and 256 scans were used for reference and samples between 4000 and 400 cm^{-1}. The nominal instrument resolution was 4 cm^{-1}. References were recorded for air. The data was studied by analyzing the amide I band after deconvolution and secondary-derivation using Omnic (version V6.2, Thermo Nicolet, America) and Peak Fit 4.12 software.

2.8. Statistical analysis of data

All tests were performed in at least triplicate. SPSS software (version 13.0, SPSS Inc., Chicago, IL, USA) was used to evaluate the significant differences ($p < 0.05$) and analyze variance between data via the ANOVA procedure.

3. Results and discussions

3.1. Effect of EWP on SDS solubility and the molecular weight distribution profiles of gluten, glutenin and gliadin proteins

SE-HPLC is an important method for protein size fractionation and protein solubility demonstration, which are good indications of the degree of cross-linking. The SE-HPLC profiles from protein samples are shown in Fig. 1. According to Wang et al. (2012), the SE-HPLC profiles of all proteins except for EWP can be divided into four fractions, induced large glutenin polymers (P1), medium glutenin polymers (P2), monomeric proteins (P3) and peptides and amino (P4). Both peak area and peak height unmistakably

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