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Effects of partial hydrolysis and subsequent cross-linking on wheat gluten physicochemical properties and structure

Kaiqiang Wang [a], Shuizhong Luo [a,b], Jing Cai [a,b], Qiaoqiao Sun [a], Yanyan Zhao [a,b], Xiyang Zhong [a,b], Shaotong Jiang [a,b], Zhi Zheng [a,b,*]

[a] School of Biotechnology and Food Engineering, Hefei University of Technology, Hefei 230009, China
[b] Key Laboratory for Agricultural Products Processing of Anhui Province, Hefei University of Technology, Hefei 230009, China

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ABSTRACT

The rheological behavior and thermal properties of wheat gluten following partial hydrolysis using Alcalase and subsequent microbial transglutaminase (MTGase) cross-linking were investigated. The wheat gluten storage modulus (G') and thermal denaturation temperature (T_g) were significantly increased from 2.26 kPa and 54.43 °C to 7.76 kPa and 57.69 °C, respectively, by the combined action of partial hydrolysis (DH 0.187%) and cross-linking. The free SH content, surface hydrophobicity, and secondary structure analysis suggested that an appropriate degree of Alcalase-based hydrolysis allowed the compact wheat gluten structure to unfold, increasing the β-sheet content and surface hydrophobicity. This improved its molecular flexibility and exposed additional glutamine sites for MTGase cross-linking. SEM images showed that a compact 3D network formed, while SDS–PAGE profiles revealed that excessive hydrolysis resulted in high-molecular-weight subunits degrading to smaller peptides, unsuitable for cross-linking. It was also demonstrated that the combination of Alcalase-based partial hydrolysis with MTGase cross-linking might be an effective method for modifying wheat gluten rheological behavior and thermal properties.

1. Introduction

Wheat flour is known to contain a number of different proteins in abundance. One such example is wheat gluten, which is a byproduct of wheat starch. Wheat gluten can be divided into two main groups, namely the monomeric gliadins and polymeric glutenins, based on their solubility in 70% aqueous ethanol (Joye, Lagrain, & Delcour, 2009). The gliadins provide viscosity and extensibility to dough, whereas the glutenins contribute to dough cohesiveness and elasticity (Kasarda, 1989). Wheat gluten is a good quality and inexpensive protein compared with other vegetable proteins. However, the rheological behavior and thermal stability of raw wheat gluten are relatively poor. As raw wheat gluten is widely underutilized as an animal feed, its current applications are limited; therefore, it is desirable to develop new applications for this product in the food industry.

Microbial transglutaminase (MTGase, γ-glutamyltransferase, EC 2.3.2.13) is known to catalyze the acyl-transfer reaction between the carboxamide group of peptide-bound glutamine residues (acyl donors) and a range of primary amines (acyl acceptors), including the ε-amino group of lysine residues in certain proteins (Motoki & Seguro, 1998). MTGase catalyzes cross-linking between the glutamine and lysine side chains on wheat gluten, resulting in the formation of high-molecular-weight polymers and improvement in the network structure and gelation behavior of wheat gluten (Wang, Zhao, Yang, Jiang, & Chun, 2007). In addition, MTGase has been used to modify the functionality of a number of food proteins in soybeans (Song & Zhao, 2014), meat (Chanarat & Benjakul, 2012), and peanuts (Clare, Gharst, & Sanders, 2007), among others. However, owing to the high glutamine and proline contents in the interior of its structure and the rarely ionized side chains, wheat gluten exhibits poor solubility (Wieser, 2007; Georget & Belton, 2006), and is thus unsuitable for MTGase catalysis. A number of pretreatments have been examined in an attempt to disrupt or unfold the protein structure. These include physical methods (Hu, Zhao, Sun, Zhao, & Ren, 2011), chemical methods (Liao et al., 2010), and enzymatic methods (Sabadin, Villas-Boas, de Lima Zollner, & Netto, 2012), resulting in a greater number of active sites being exposed for MTGase cross-linking. The use of physical methods is generally considered safe for the modification of

* Corresponding author at: School of Biotechnology and Food Engineering, Hefei University of Technology, Hefei 230009, China.
E-mail address: zhengzhi@hfut.edu.cn (Z. Zheng).

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proteins, but its efficiency is limited. In contrast, the use of chemical methods may have an impact on food safety due to the chemical reagents utilized and the by-products generated. Partial proteolysis has been reported to be a well-accepted and safe method for the modification of the behavior and physicochemical properties of chickpea protein (Ghribi et al., 2015), soybean protein (Zhang et al., 2014), sunflower protein (Martinez, Baeza, Millan, & Pilosof, 2005), and hemp protein (Yin et al., 2008), among others. Up till now, information concerning the effect combination of partial proteolysis with MTGase cross-linking on wheat gluten behavior and physicochemical properties is still limited.

Therefore, the objective of this work was to evaluate the impact of combination of Alcalase-based partial hydrolysis with MTGase cross-linking on the rheological behavior and thermal properties of wheat gluten. The storage modulus, loss modulus, thermal denaturation temperature, enthalpy change of denaturation, free SH content, and surface hydrophobicity were examined to interpret changes in the physicochemical properties. In addition, the structure of wheat gluten was studied using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, Fourier transform infrared spectroscopy, and scanning electron microscopy methods. This work may lead to an increase in applications of wheat gluten in the food industry.

2. Materials and methods

2.1. Materials

Wheat gluten was obtained from Ruifuxiang Food Co., Ltd. (Bozhou, China). Alcalase (150000 U/g) was purchased from Pangbo Biological Engineering Co., Ltd. (Nanning, China). MTGase (1000 U/g) was purchased from Yiming Biotechnology Co., Ltd. (Weifang, China). 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) and 1-anilino-8-naphthalenesulfonate (ANS) were purchased from Sigma Aldrich (St. Louis, MO, USA). All chemicals used were at least of analytical grade.

2.2. Preparation of partially hydrolyzed wheat gluten

Wheat gluten (6.0°g) was dissolved in distilled water (100 mL), and the resulting mixture was adjusted to pH 8.0 using 1 M NaOH. After stirring for 30 min at room temperature, the desired quantity of Alcalase (0.02%, 0.04%, 0.06%, 0.08%, and 0.10%, w/w) was added. Each resulting dispersion was incubated at 55 °C with stirring for 10 min, and transferred to a bath of boiling water for a further 20 min. Finally, each hydrolysate was collected and freeze-dried. The raw wheat gluten is referred to as WG, and the partially hydrolyzed samples are referred to as HWG-2, HWG-4, HWG-6, HWG-8, and HWG-10, based on the percentage of Alcalase used in their preparation. The degree of hydrolysis (DH) was determined according to the method reported by Adler-Nissen (1986), and was calculated using the following equation:

$$\mathrm{DH} = \frac{BN_b}{\alpha h_{tot} M_p} \times 100\%$$

where B is the amount of NaOH in mL, N_b is the normality of NaOH, α is the average degree of dissociation of the α-NH_2 groups, h_{tot} is the total number of peptide bonds in the protein substrate (8.38 mequiv/g wheat gluten), and M_p is the mass of protein in g. The determined DH values are shown in Supplementary Fig. S1.

2.3. Preparation of composite modified wheat gluten

The partially hydrolyzed wheat gluten samples (10.0 g) were dissolved in distilled water (100 mL) and the pH of each dispersion was adjusted to pH 7.0 using either 1 M NaOH or 1 M HCl. The resulting dispersions were then stirred for 30 min at 25 °C, MTGase (20 U/g) was added, and the mixture incubated at 45 °C for 1 h. After this time, the reactions were stopped by transferring to an ice/water bath, and the samples were then freeze-dried. The composite modified wheat gluten samples are referred to as CWG, CHWG-2, CHWG-4, CHWG-6, CHWG-8, and CHWG-10. Raw wheat gluten was used as a control.

2.4. Evaluation of hydrated wheat gluten rheological behavior

The rheological behavior of the samples was studied according to the modified Wang, Xu, et al. (2014) procedure. Each sample (1.0 g) was dispersed in distilled water (10 mL) and centrifuged at 5488*g* for 5 min at 4 °C. Dynamic rheological evaluations were performed using a DHR-3 rheometer (TA Instruments, New Castle, DE, USA) at 25 °C with a frequency of 0.1–10 Hz, deformation of 0.1%, and a 1 mm gap width or close to the linear region of viscoelasticity. The hydrated wheat gluten samples were shaped into disks, relaxed for 10 min at 25 °C, and fixed to the parallel plates (40 mm).

2.5. Differential scanning calorimetry (DSC)

The thermal properties of wheat gluten were analyzed using a Q200-DSC instrument (TA Instruments, New Castle, DE, USA) according to the method reported by León, Rosell, and de Barber (2003). Each freeze-dried wheat gluten sample (8–10 mg) was weighed into an aluminum pan, and heated from 20 to 100 °C at a rate of 10 °C/min under a flow of nitrogen gas (80 mL/min). An empty aluminum pan was used as a reference. The denaturation peak temperature (T_g) and enthalpy (ΔH) were analyzed using the Muse TA Rheology System Software.

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

SDS–PAGE experiments were performed according to the modified method of Laemmli (1970). The discontinuous system consisted of separating gel (12%, pH 8.6) and stacking gel (5%, pH 6.8). Wheat gluten samples were solubilized in Tris–HCl buffer (0.0625 M, pH 6.8) containing SDS (2.3%), 2-mercaptoethanol (5%), glycerol (10%), and bromophenol blue (0.1%). The resulting solutions were heated for 10 min in boiling water, cooled to 25 °C, and centrifuged at 10976*g* for 10 min. A portion of sample (10 μL) was then loaded into each well. After electrophoresis was carried out, the gel was dyed with Coomassie Brilliant Blue-R250 (0.25%) in trichloroacetic acid (50%), and was destained with a mixture of methanol/acetic acid/water (1:1:8, v/v/v).

2.7. Free sulfhydryl (SH) determination

The number of free SH groups in each sample was determined according to the modified method of Chan and Wasserman (1993). Wheat gluten samples (100 mg) were dissolved in phosphate buffer (PBS; 10 mL, 1 mM EDTA, 1% SDS, pH 8.0), and were centrifuged at 10976 *g* for 10 min. A sample of the suspension (3 mL) was added to PBS (3 mL), DTNB reagent (100 μL, 4 mg/mL) was added, and the samples were shaken for 1 h at 25 °C. The absorbance of each supernatant was measured at 412 nm against the blank. The absorbance measurements were converted to free SH values using a calibration curve with reduced glutathione.