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Effects of alkali on protein polymerization and textural characteristics of textured wheat protein

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ABSTRACT

The impact of alkali addition on the degree of gluten polymerization and textural characteristics of textured wheat protein was investigated. Results showed that the extrusion process increased the average molecular weight of gluten as evidenced by SDS-PAGE and SDS extractable protein. The addition of alkali not only promoted the degree of gluten polymerization, but also induced dehydroalanine-derived cross-linking. Alkali addition decreased the content of cystine and increased the contents of dehydroalanine and lanthionine. The obvious decrease of free SH showed that dehydroalanine-derived cross-linking was quantitatively less crucial than disulfide cross-linking. Furthermore, the protein cross-linking induced by alkali improved the texture properties of gluten extrudates. SEM analysis showed extrusion under alkaline condition conferred a more fibrous microstructure as a consequence of a compact gluten network.

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1. Introduction

As one of the most cultivated cereal grain, wheat plays an important role in diets. Their intrinsic viscoelastic behavior takes responsibility for the characteristics of different wheat-based products (Delcour et al., 2012). Wheat gluten is a co-product of wheat starch in the wet processing. The dry solid contains 75–85% protein and 5–10% lipid (Wieser, 2007).

Gluten proteins are of great technological significance in food systems, baking or extrusion steps during food production, thereby meeting the desired cooking taste and flavor (Day, Augustin, Batey, & Wrigley, 2006). However, on the one hand, the increasing production of wheat gluten leads to overproduction, causing the traditional market saturation. On the other hand, the water-insoluble properties of wheat protein, which can mainly be ascribed to a high proportion of hydrophobic amino acids, immensely restrict its applications in food industry (Liu, Zhu, Peng, Guo, & Zhou, 2016; Zayas, 1997). Therefore, deep processing is urgently needed to create added-value of wheat gluten and to enlarge the application area. Considering its poor functional properties such as solubility, emulsification and foaming ability, the structural modification of protein has been usually adopted.

Physical, chemical and enzymatic modification methods are feasible. Compared to chemical and enzymatic methods, physical methods, including mixing (Danno & Hoseney, 1982), high pressure treatment (Apichartsrangkoon, Ledward, Bell, & Brennan, 1998), heating (Hargreaves, Popineau, Meste, & Hemminga, 1995) and microwave drying (Walde, Balaswamy, Velu, & Rao, 2002), are considered to be safe and without impurities, and therefore are widely used. Among them, one promising and emerging technology for transforming vegetable proteins into consumer-acceptable products is extrusion, a mixture process of high-temperature, high-pressure and high-shear. It is capable of producing varieties of cooked food like ready-to-eat cereals, some snacks, pet foods (Lin, Huff, & Hsieh, 2002) and meat analogs more closely resembling muscle food.

Many studies have been conducted on textured vegetable protein as meat analogs especially soy protein, including texture, chemical characteristics of extruded soy protein (Lin, Huff, & Hsieh, 2000), extrusion process parameters under high moisture (Lin et al., 2002) and protein-protein interactions (Liu & Hsieh, 2007). Mechanism of protein-protein interactions during extrusion process and extrusion parameters used to be the focus of traditional extruded wheat protein (Kinsella & Franzen, 1978; Li & Lee, 1996a, 1996b; Li & Lee, 1997). The textural characteristics of gluten extrudate were evaluated by its resilience and hardness, which positively correlated with gluten network formation. In addition, the correlations between protein cross-linking and textural characteristics remain to be studied.

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It was believed that the addition of alkali increased intermolecular cross-linkage, which enhanced the extrudate's textural characteristics (Shiau & Yeh, 2001). Rombouts, Lagrain, Brijs, and Delcour (2012) found that β-elimination of cystine contributed to the gluten network during hot alkaline conditions. Wu, Beta, and Corke (2006) found that effect of alkali was associated with the formation of three-dimensional networks. Accordingly, we hypothesized that alkali could impact product quality by facilitating protein cross-linking.

Against this background, the objective of the present study is to investigate the impact of alkali addition on protein polymerization behaviors during extrusion process of wheat gluten. In addition, to obtain the correlation between protein polymerization and textural characteristics of textured wheat protein, the SE-HPLC and SDS-PAGE were applied to evaluate the degree of protein polymerization of wheat protein during extrusion process under alkaline conditions. Moreover, levels of free -SH and related amino acids (cystine, LAN, DHA) were also determined to evaluate SS cross-linking and DHA-derived cross-linking. Furthermore, the correlation coefficients of extrudates texture profiles and gluten polymerization degree were established. In order to visualize the textural structures of the extrudate, the microstructure of textured wheat protein was observed by SEM.

2. Materials and methods

2.1. Materials

Commercial wheat gluten (brand: ReapSun) was purchased from Anhui Ruifuxiang Food Co. LTD, its moisture and protein content (N × 5.7) were 13.03 ± 0.01% and 78.06 ± 0.08%, respectively. Wheat starch (brand: ShunHe) was purchased from local supermarket. The ingredients (wheat gluten and wheat starch) were blended in a ratio of 9:1 using a mixer. All solvents, chemicals and reagents were at least of analytical grade in the experiments.

2.2. Extrusion and sample preparation

The extrusion was carried out on a FMHE36-24 twin-screw extruder (FUMACH, Hunan, China) with kneading block. The screw configuration used in the experiments was modular construction which consisted of forwarding elements (L/D = 24). Meanwhile, the barrel was equipped with five independent heating zones and cooling devices. Furthermore, the cooling water was circulated at controlled flow rates to prevent overheating of barrel. The die was cylindrical appearance with a rectangular orifice (33 mm × 3 mm). The raw materials and water were added respectively.

The extruder barrel temperatures were set at 60, 90, 110, 145 and 165 °C from the first (feeding zone) to the fifth, respectively. When the die temperature arrived at 100 °C, starting the machine. The moisture level was maintained at around 55% by dosing water at ambient temperature through a positive displacement pump. The feed rate and screw speed rose gradually, which were finally kept at 25 kg/h and 380 rpm, respectively. Additions of $NaHCO_3$ (0.1 up to 1.5 wt% on wheat gluten basis), Na_2CO_3 (0.1 up to 1.0 wt%) or NaOH (0.1 up to 0.5%) were mixed with raw materials before feeding. All the extruded samples of each treatment were dried in a convection oven at 40 °C for about 3 h.

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

To deliver further insight into the protein molecular weight distribution and the protein extractability in sodium dodecyl sulfate (SDS) containing medium, SDS-HPLC was conducted according to a previously reported method (Wagner, Morel, Bonicel, & Cuq, 2011). Freeze dried samples [8.0 mg protein/mL] were dissolved in 1.0 mL of sodium phosphate buffer (0.05 M, pH 6.8) containing 2.0% (w/v) sodium dodecyl sulfate (SDS). The mixture was shaken for 1 h at room temperature. All resulting samples were centrifuged 10 min at 8000*g*, and then filtered over 0.45 μm polyethersulfone membrane. The protein extracts (20 μL) were loaded onto a Tskgel-G4000-SWXL column (7.8 mm × 300 mm). The elution solvent was 0.05 M sodium phosphate buffer (1% SDS, pH 7.0) at the flow rate of 0.7 mL/min and column temperature 28 °C. The elution curve was monitored at 214 nm. SDS extractable protein (SDSEP) of unheated wheat gluten and extruded wheat gluten with or without alkali treatment (under non-reducing or reducing conditions) was always expressed as a percentage of total protein extractability. The SDSEP content of textured wheat protein (under nonreducing or reducing conditions) is the ratio of the peak area under the SE profile of samples compared to the peak area under the SE profile of the reference sample. The reference is unheated wheat gluten which is extracted under reducing conditions (SDS-phosphate buffer in the presence of 1.0% (w/v) dithiothreitol (DTT)).

2.4. SDS–PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) analysis

SDS-PAGE was performed using 12% separating gel (pH 8.8) and 5% stacking gel (pH 6.8). The extraction buffer of protein was 0.01 M Tris–HCl (pH 6.8), including 10% (w/v) SDS, 5% (v/v) 2-mercaptoethanol (2-ME), 10% (v/v) glycerol, 0.1% (w/v). 2-ME was not existent in the non-reduced protein. Each sample of 10.4 mg was stirred in 1.0 mL of extraction buffer for 30 min and left for 1 h at room temperature. The sample solution was centrifuged at 7690*g* for 10 min after boiling in water for 5 min. Then sample volumes of 15 μL were injected into each well. The voltage of electrophoresis was stable at 100 V in the whole procedure. Standard proteins as molecular weight markers were: phosphorylase B (97,400), serum albumin (66,200), actin from rabbit muscle (43,000), carbonic anhydrase (31,000), trypsin inhibitor (20,100), lysozyme (14,400), respectively.

2.5. Dehydroalanine (DHA) Determination

The DHA contents of samples were determined following a method reported by Rombouts, Lagrain, Brijs, and Delcour (2010) with some modifications. Accurately weigh the sample (about 80 mg protein) in a test tube with screw cap. Add 0.50 mL of the HCl solution (1.5 N) to each sample, which was heated (oil bath or heating block) for 120 min at 110 °C. DHA was converted into pyruvic acid after acid hydrolysis. Series of clarification process were carried out, followed by colorimetric quantification under 340 nm.

2.6. Determination of cystine and LAN

The determinations of cystine and LAN were conducted according to the method described by Rombouts et al. (2009) with some modifications. Amino acids were liberated by hydrolyzing (22 h, 110 °C) freeze-dried samples (15.0 mg protein) with 1.0 mL 6.0 mol/L HCl in evacuated sealed tubes after flushing the samples with nitrogen to prevent amino acid oxidation. After complete hydrolysis, the mixtures were put in a vacuum desiccator to remove hydrochloric acid. After that, samples were transferred into a 25-mL volumetric flask, shaken vigorously and filtered. The filtrate was then stored at 4 °C before injection. Amino acid compositions were determined using a Hitachi L-8900 automatic amino acid analyzer. Separation of 20 μL samples was performed at

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