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Emulsifying and foaming properties of transglutaminase-treated wheat gluten hydrolysate as influenced by pH, temperature and salt ☆

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Abstract

Hydrolyzed wheat gluten (GH, 77–85% protein) was prepared by limited hydrolysis with chymotrypsin at 37 °C for 4 h (degree of hydrolysis = 6.4%) and 15 h (degree of hydrolysis = 10.3%). The effect of microbial transglutaminase (MTGase) treatment (55 °C for 1 h, or 5 °C for 18 h) on the emulsifying and foaming properties of GH was evaluated under selected food processing conditions (pH 4.0 and 6.5, 0 and 0.6 M NaCl, and temperature 20 and 5 °C). At pH 4.0 and 0 M NaCl the MTGase treatment substantially increased foaming capacity (FC) of GH compared with their respective control GH samples, as a result of enhanced peptide adsorption to the air–water interface, but FC was similar for both control and MTGase-treated GH at pH 6.5. In contrast, foam drainage stability (FS) of MTGase-treated GH decreased at pH 4.0, but increased significantly ($P<0.05$) at pH 6.5 when compared with their respective control GH samples. The FC and FS were affected by 0.6 M NaCl in a pH-dependent manner. The MTGase treatments increased emulsion activity index up to 15-fold at pH 6.5, while emulsion stability index was influenced by emulsion temperature and ionic strength conditions. The MTGase-induced changes in functional properties of GH were attributed to pH-dependent solubility changes, the amphiphilic nature of gluten peptides and increased electrostatic repulsion resulting from deamidation.
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Keywords: Hydrolyzed wheat gluten; Emulsifying properties; Foaming properties; Microbial transglutaminase

1. Introduction

Wheat gluten is a co-product from the wet processing of wheat flour, and it is available in large quantities and at relatively low cost (Popineau, Huchet, Larré, & Bérot, 2002). Gluten is used mainly as an improver of the rheological properties of breadmaking flours and to texturize food, due to its viscoelastic properties when hydrated. It may also be utilized as a functional protein additive in various non-bakery applications because of its desirable structure-enhancing properties (Gontard & Ring, 1996; Janssen, de-Baaij, & Hagele, 1994).

However, gluten utilization in formulated food systems is limited by its low solubility in aqueous solutions (Mimouni, Raymond, Merle-Desnoyers, Azanza, & Ducastaing, 1994; Popineau et al., 2002). The low solubility of gluten is due to the high concentrations of nonpolar amino acid residues (such as proline and leucine) and the polar but non-ionizable glutamine residue (Krull & Wall, 1966).

It is possible to extend gluten's utilization in food applications through chemical and enzymatic modifications. In particular, limited enzyme hydrolysis may improve the solubility and enhance the foaming and emulsifying properties of wheat gluten (Agyare, Xiong, & Addo, 2008; Babiker, Fujisawa, Matsudomi, & Kato, 1996; Kato, Shimokawa, & Kobayashi, 1991; Linarès, Larré, Lemeste, & Popineau, 2000; Mimouni, Azanza, & Raymond, 1999; Mimouni et al., 1994; Popineau et al., 2002). In addition, Babiker et al. (1996) reported that functionality of gluten can be improved by protease digestion followed by microbial transglutaminase (MTGase) treatment.

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Transglutaminase (glutaminyl-peptide:amine γ-glutamyltransferase, EC 2.3.2.13) is an enzyme capable of modifying the functional properties of food proteins (Ando et al., 1989; Kuraishi, Yamazaki, & Susa, 2001; Yokoyama, Nio, & Kikuchi, 2004). It catalyzes an acyl transfer reaction between γ-carboxyamide of peptide or protein-bound glutamine residue (acyl donor) and a primary amine (acyl acceptor) (Kuraishi et al., 2001). The enzyme also catalyzes hydrolysis of the γ-carboxyamide group of glutaminyl residues, resulting in deamidation (Ando et al., 1989; De Jong & Koppelman, 2002).

However, there is limited information on the effect of specific food processing parameters (e.g., temperature, ionic and pH conditions) on the emulsifying and foaming characteristics of transglutaminase-modified hydrolyzed gluten. Such an investigation is important because emulsifying and foaming properties (which are closely related to protein solubility) of the resulting gluten fragments may be influenced by environmental factors such as pH, temperature and ionic strength (Linarès et al., 2000; Linarès, Larré, & Popineau, 2001; Popineau et al., 2002; Popineau, Pineau, Evon, & Bérot, 1999). In addition, emulsifying properties have been positively correlated with surface hydrophobicity of proteins (Kato & Nakai, 1980).

The present study investigated the efficacy of MTGase in altering the emulsifying and foaming properties of hydrolyzed gluten. The effect of selected food processing conditions (pH, salt concentration and temperature) on the enzyme-induced protein/peptide changes was examined.

2. Materials and methods

2.1. Materials

Commercial untreated, soft wheat flour obtained from ADM Milling Company (Chattanooga, TN, USA) was used for making wheat gluten. Chymotrypsin (52 units/mg) was purchased from Sigma Chemical Company (St. Louis, MO, USA). MTGase used in all treatments was a crude enzyme preparation (Activa®-TI, 99% maltodextrin and 1% MTGase) donated by Ajinomoto Inc. (Teaneck, NJ, USA). The crude enzyme was used without further purification. All chemicals used were at least reagent grade.

2.2. Wheat gluten preparation

Gluten was prepared according to Method 38-10 of AACC (2008), as detailed in our previous report (Agyare et al., 2008). The gluten ball was lyophilized, and the freeze-dried gluten was pulverized into a fine powder and stored in sealed containers at 4 °C until use. Protein content of the gluten powder (76.1%) was determined by Method 986.06 of AOAC (2005) using a Leco Nitrogen Analyzer (Model CN-2000, Leco Corp., St. Joseph, MI, USA).

Gluten was partially hydrolyzed with chymotrypsin. Chymotrypsin was selected among three proteases (chymotrypsin, papain and alcalase) because it was found to be most effective in producing peptide fragments (Agyare et al., 2008). Briefly, a freeze-dried sample of gluten (16 g) was suspended in 400 mL of 0.05 M Tris–HCl (pH 8.0) containing 0.05% sodium azide, and then 160 mg of chymotrypsin was added. The mixture was incubated at 37 °C for 4 or 15 h and then heated at 80 °C for 20 min to inactivate the enzyme. The chymotrypsin-digested gluten was centrifuged at 10,500*g* for 10 min, to obtain the soluble fraction (supernatant). The supernatant was dialyzed (3500 molecular weight cut-off) against distilled water or 0.1 M phosphate buffer (pH 7.0) (Babiker et al., 1996). The former (supernatant dialyzed against distilled water) was freeze-dried (designated as control GH), and the latter (supernatant dialyzed against phosphate buffer) was used for transglutaminase treatment. The degree of hydrolysis (DH) was determined by the trinitrobenzenesulfonic acid method of Adler-Nissen (1979),

2.3. Transglutaminase treatment

The supernatant of chymotrypsin-digested gluten (4 or 15 h hydrolysis) that had been dialyzed against 0.1 M phosphate buffer (pH 7.0), was adjusted to 10 mg/mL protein concentration and then reacted with MTGase (E/S = 1/20) at 5 °C for 18 h, or at 55 °C for 1 h, corresponding to low temperature (5 °C) and optimum temperature (55 °C) conditions of MTGase enzyme activity, respectively. For all the MTGase-treated samples, 0.1% *N*-ethylmaleimide was added after the specific incubation times to inactive the enzyme. The MTGase-treated samples were dialyzed against distilled water, freeze-dried, pulverized with mortar and pestle, and stored in sealed containers at 4 °C until used (designated as MTGase-treated GH). Determination of protein in control and MTGase-treated GH was done by the Biuret method (Gornall, Bardawill, & David, 1949) using bovine serum albumin as standard. The production of carboxyl groups in control and MTGase-treated GH was monitored according to Kobayashi and Chiba (1994), because MTGase catalyzes deamidation in addition to producing lysine-glutamine cross-linking.

2.4. Determination of surface hydrophobicity

The surface hydrophobicity of control and MTGase-treated GH samples was determined by the method of Hayakawa and Nakai (1985). A specific amount of freeze-dried sample was dispersed in 0.01 M phosphate buffer (pH 7.0) to obtain 0.05% protein, in the presence of 0 or 0.6 M NaCl. The sample solutions were centrifuged at 10,000*g* for 15 min (4 °C). Protein concentration in the supernatants was measured by the biuret procedure. A series of dilutions of each supernatant were made with the same buffer to obtain a range of protein concentrations (0.008–0.05%). Then 4 mL of each supernatant was reacted with 20 μL of 1-anilino-8-naphthalenesulfonate magnesium salt (8 mM in 0.1 M phosphate buffer, pH 7.0) for 15 min.