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Modulation of mechanical and surface hydrophobic properties of food protein films by transglutaminase treatment

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Abstract

The effects of microbial transglutaminase (MTGase) treatment on the mechanical and hydrophobic properties of cast films from various food proteins were investigated. SDS–PAGE analyses confirmed that the MTGase treatment led to the formation of insoluble film network for most of food proteins. This enzymatic treatment significantly ($P < 0.05$) increased tensile strength (TS) values of cast films of all food proteins by 13–33% (except whey proteins). Meanwhile, the influence on the elongation at break (EB) values was mainly dependent upon the molecular nature of proteins. For most of food proteins, both increases in TS and EB values of films were obtained after the MTGase treatment. Furthermore, the surface hydrophobicity of most of protein films was also significantly improved. These results suggest that the enzymatic treatment by MTGase could be used as an effective technique to improve mechanical and surface hydrophobic properties of protein films.

Keywords: Protein film; Microbial transglutaminase (MTGase); Food proteins; Mechanical property; Surface hydrophobicity

1. Introduction

Food proteins have been widely applied, alone or in mixtures, to obtain biodegradable and/or edible films, since protein-based films have better gas barrier and mechanical properties compared with those from polysaccharides and lipids (Cuq, Aymard, Cuq, & Guilbert, 1995; Cuq, Gontard, & Guilbert, 1998). However, the moisture barrier properties of these films are poor, due to the inherent hydrophilic properties of most of food proteins and the application of high amounts of hydrophilic plasticizer in film preparation (Gennadios & Weller, 1994). In some cases, e.g. under elevated relative humidity (RH) conditions, even the mechanical and gas barrier properties of protein films (especially those from soy proteins) would become worse (Cho & Rhee, 2002). Thus, improvements in different properties of food protein films are the focus of many researches.

Lots of treatments, including physical, chemical and even enzymatic methods, have been tried to improve the mechanical properties of protein-based edible films, including the addition of chemical cross-linking agents (e.g., aldehydes), heat curing, the transglutaminase-induced cross-linking treatment, ultraviolet or γ-irradiation treatment (de Carvalho & Grosso, 2004; Hernández-Muñoz, Villalobos, & Chiralt, 2004; Kim, Weller, Hanna, & Gennadios, 2002; Larré, Desserme, Barbot, & Gueguen, 2000; Liu, Tellez-Garay, & Castell-Perez, 2004; Mariniello et al., 2003; Rhim, Gennadios, Fu, Weller, & Hanna, 1999; Tang, Jiang, Wen, & Yang, 2005; Vachon et al., 2000). Of these

Abbreviations: MTGase, microbial transglutaminase; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TS, the tensile strength; EB, the elongation at break; MW, molecular weight; C-SPI, commercial soy protein isolates; N-SPI, native soy protein isolates prepared from defatted soy meal in lab; NaCN, sodium caseinate; WPC, whey protein concentrate; G, gelatin; PPI, peanut protein isolate; 2-ME, β-mercaptoethanol.

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treatments, the enzymatic treatment by means of transglutaminase seems to be most potential for improving the mechanical properties of protein films, due to the consideration from safety and effectiveness. Besides, this treatment may also improve surface hydrophobicity of soy protein films (Tang, Jiang, et al., 2005). However, information about this modification of protein films is still limited, and the data obtained by different researchers are inconsistent. On the other hand, the extent of improvement in mechanical and/or hydrophobic properties of protein films by means of transglutaminase seems to be dependent upon the molecular nature of the protein (de Carvalho & Grosso, 2004; Larré et al., 2000; Mariniello et al., 2003; Tang, Jiang, et al., 2005).

Thus, the major objective of this work was to investigate and compare the influence of microbial transglutaminase (MTGase) treatment on mechanical and hydrophobic properties of cast films from various kinds of food proteins. The underlying mechanisms for the modification extent were also illuminated.

2. Materials and methods

2.1. Materials

Commercial soy protein isolates (C-SPI, containing 85.2% nitrogen) was obtained from Wonderful Tech. Co. (Shandong Province, China). Native SPI (N-SPI, containing 92.1% nitrogen) was prepared by alkaline extraction (pH 8.0) and acid precipitation (pH 4.3) from defatted soy meal, according to the method described by Seung and Chul (2002). Sodium caseinate (NaCN, containing 88.3% nitrogen) was obtained from New Zealand dairy board. Whey protein concentrate (WPC, containing 84.7% nitrogen) was a product of Proliant Inc. (USA). Gelatin (G, containing 96.8% nitrogen) was purchased from Gold Arrow Gelatin Co. Ltd. (Guangzhou, China). Wheat gluten (WG, containing 58.0% nitrogen) was purchased from WENLIU Wheat Starch Plant (Puyang City, Henan Province, China). Peanut protein isolate (PPI, containing 90.2% nitrogen) was prepared by alkaline extraction (pH 9.0) and acid precipitation (pH 4.5) from defatted peanut meal according to the method of (Jangchud & Chinnan (1999)).

Commercial MTGase enzyme was obtained from Chanshou Biological Co. Ltd. (Jiangsu province, China), and stored in the freezer (−20 °C) before use. The purification and the activity measurement of this enzyme were according to the method of Tang, Jiang, et al. (2005). Other chemical reagents were of analytical or better grade.

2.2. Film preparation

Control films: The film-forming solutions (FFS) were prepared by dissolving 5 g protein in 100 mL of constantly stirred 0.05 M Tris–HCl buffer (pH 8.0) containing 2 g glycerol, and preheated as follows: SPI, at 70 °C for 20 min; NaCN, WPC and PPI, at 80 °C for 30 min; G and WG, at 45 °C for 30 min. After heat pretreatment, the solutions were centrifuged at low speed (100*g*) to remove air bubbles, then cooled to room temperature (25 ± 1 °C) and cast onto leveled glass plates (21 × 35 cm) covered with polyethylene films. The film thickness was controlled by casting the same volume solution (80 mL) on each plate. The castings were air-dried at room temperature (25 ± 1 °C) for 24 h. The dried films were peeled off the plates and various specimens for property testing were cut. Specimens of 2.5 × 10 cm rectangular strips were for tensile testing, and 2 × 2 cm squares for MC and TSM testing.

MTGase-treated films: The film-forming solutions were prepared and preheated as the control. Prior to casting, 8 units per gram of substrate protein (U g^{-1}) of MTGase was added into the FFS pre-cooled to room temperature, and mixed well, then followed by the same process as the control.

2.3. Conditioning

All film specimens were conditioned at 25 °C for 2 days in a desiccator with 50% RH before testing (ASTM, D618-61, 1995).

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

About 5 mg of film samples were suspended in 1 mL of the electrophoresis sample buffer, namely 0.125 M Tris–HCl (pH 6.8), containing 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol (2-ME), 10% (v/v) glycerol and 0.05% (w/v) bromophenol blue. The suspensions were placed at room temperature for more than 24 h with occasional shaking, and then heated for 5 min in boiling water and centrifuged at 10,000*g* for 10 min before electrophoresis. The electrophoresis was carried out on a slab gel consisting of 3% stacking gel and 12.5% separating gel with the SDS-Tris-glycine discontinuous buffered system described by (Laemmli (1970)). Lastly, the electrophoresis gels were stained with 2.25% Coomassie brilliant blue in 50% trichloroacetic and de-stained in 7% acetic acid (methanol:acetic:water was 227:37:236).

2.5. Mechanical characteristics

A TA-XT2i texture analyzer (SMS Co. Ltd., England) was used to assess tensile strength (TS) and elongation at break (EB) of films. Initial grip separation and cross-head speed were set to 50 mm and 1 mm s^{-1}, respectively. TS value was calculated by dividing the maximum load by the initial cross-sectional area of the specimen. EB value was calculated as the percentage of change of the initial gage length of a specimen (50 mm) at the point of a sample failure. Film thickness was measured with a hand-held micrometer to the nearest 0.001 mm. Five measurements