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The effects of microbial transglutaminase on the properties of fish myofibrillar protein film

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ABSTRACT

In this study, the effects of microbial transglutaminase (MTGase) on the properties of fish myofibrillar protein (FMP) films were investigated. As MTGase content increased, the thickness and tensile strength of the FMP films increased. In contrast, their elongation at break (167.49–85.61%), film transparency, water vapor permeability ($2.38–2.02 \times 10^{-9}$ g m^{-1} s^{-1} Pa^{-1}), moisture content, film solubility, and degree of swelling all decreased ($P < 0.05$). Lightness also decreased, and yellowness increased as MTGase content was increased ($P < 0.05$). MTGase action also significantly improved barrier properties and thermal stability of films. Electrophoretic and FT-IR studies revealed that cross-linking and conformational changes were pronounced in film treated with MTGase. Based on these results, the addition of MTGase produced a good alternative method for improving FMP film properties; however, the mechanical and water barrier properties of the resulting films need further development.

1. Introduction

Pollution by non- or slowly-degradable packaging material waste is an increasing environmental concern. In this context, the packaging materials from natural resources have ability to reduce or replace the commercial synthetic packaging films. Biodegradable films can be made from natural resources including carbohydrates, proteins, and lipids. Among these materials, proteins are extensively used as the film-forming materials due to their film-forming ability, abundance, high nutritional value, and biodegradability (Kaewprachu, Osako, Benjakul, Tongdeesoontorn, & Rawdkuen, 2016b). Fish myofibrillar proteins have been used frequently as a polymer for film-forming (Kaewprachu, Osako, Benjakul, & Rawdkuen, 2016a; Kaewprachu et al., 2016b; Prodpran, Benjakul, & Phatcharat, 2012; Rostamzad, Paighambari, Shabanpour, Ojagh, & Mousavi, 2016). Myofibrillar proteins are generally dissolved in the solution that provides the pH away from the isoelectric point (pI ≅ 5) of the proteins (Iwata, Ishizaki, Handa, & Tanaka, 2000). The dissociation and solubilization of myofibrillar proteins are then sufficient for expecting film formation. Previous experiments (Kaewprachu et al., 2016a, 2016b) showed that fish myofibrillar protein based films (FMP) had UV light barrier properties (200–280 nm) better than the synthetic wrap film (polyvinyl chloride; PVC). However, the relatively poor mechanical properties and high water vapor permeability posed significant limitations for their applicability in food packaging. Therefore, protein film network modifications are required to enhance these apparent weaknesses.

Various approaches have been used to enhance the protein based films' properties such as the use of chemicals, irradiation, thermal, and enzymatic modification. There are many researches that studied the enhancement of protein based films via chemical modifications by glutaraldehyde or genipin (0.15, 0.30, and 0.67%, w/v) (Amadori et al., 2015), gamma-irradiation (0–50 kGy) (Xu et al., 2012), and enzymatic reticulation (0–8 units per gram of protein) (Wang, Liu, Ye, Wang, & Li, 2015). In recent years, enzymatic cross-linking treatment has been used extensively over chemical modifications because of the toxicity of chemical synthetic agents, which is clearly unsuitable for real food systems application.

Microbial transglutaminase (MTGase; EC.2.3.2.13) is most commonly used for protein modification. MTGase is a protein-glutamine γ-glutamyltransferase, which can be catalyzed the formation of an isopeptide bond between the group of carboxyl amide (donor) of glutamine residues and the group of ε-amine (acyl acceptor) of lysine residues. These reactions can be induced the formation of the intra- and intermolecular ε-(γ-glutamyl) lysine covalent bonds via cross-linking process (Nielsen, 1995). The cross-linking are commonly induced by MTGase could improve the films' mechanical and physical properties by

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increasing the tensile strength, but lowering the elongation at break and solubility of the protein film. In addition, the MTGase enzymatic activity and biochemical reactions are affected by the pre-treatment of the protein (denaturation), the pH of the film-forming solution, the reaction time, the temperature, as well as the concentration of MTGase (Schmid, Sängerlaub, Wege, & Stäbler, 2014). Particularly, the effect of concentrations of MTGase on the protein based films' properties have been extensively studied on whey protein isolate (Schmid et al., 2014), gelatin-calcium carbonate (Wang et al., 2015), and jellyfish protein (Lee, Lee, Yang, Won, & Song, 2015). Nevertheless, information regarding the effect of MTGase on the protein based films properties, especially in FMP films, remains very scarce. Therefore, the aim of this study was to investigate the effect of MTGase on the mechanical, physical, chemical, barrier, and thermal properties of FMP based film against a commercial wrap control film (low density polyethylene; LDPE).

2. Materials and methods

2.1. Materials

Microbial transglutaminase (MTGase) (Activa TG-K: 100 activity units per gram) was supplied by Ajinomoto Co. Inc. (Tokyo, Japan). Coomassie Blue R-250 was purchased from Merck (Darmstadt, Germany). SDS was purchased from Wako (Osaka, Japan). Glycerol and other analytical grade reagents were obtained from Kokusan Chemical Co., Ltd. (Yokohama, Japan). Low density polyethylene wrap film (LDPE) of 10 μm thickness (Ube Film Company Ltd., Japan) was used as the commercial wrap film in this study.

2.2. Preparation of fish myofibrillar protein (FMP)

Firstly, minced fish (fresh tilapia; *Orcochromis niloticus*) was added with five volumes of 50 mM NaCl and then homogenized for 2 min at 11,000 rpm. The mixtures were centrifuged at 10,000 × *g* for 10 min at 4 °C. These processes were repeated two times (Kaewprachu et al., 2016a). Finally, FMP was subjected to freeze drying, packed in plastic bag with zipper and kept at −20 °C until use.

2.3. Preparation of FMP films with MTGase

FMP was added with the distilled water to obtain the final protein concentrations of 1% (w/v). The mixtures were homogenized at 11,000 rpm for 1 min, and then the pH of the mixtures was adjusted to 11 by using 1 N NaOH. After, the solutions were centrifuged at 3000 × *g* for 10 min at room temperature. The obtained supernatants was mixed with glycerol (25% w/w, protein content) and referred to as the film-forming solution (FFS; Kaewprachu et al., 2016b). The FFS was subjected to an additional 30 min of stirring at room temperature. After stirring, the different concentrations (0, 1, 2, 3, and 4% w/w, based on protein content) of MTGase were added into the FFS. Then, the mixtures were stirred for 30 min at room temperature. The FFS was used for film casting by adding 4 g of FFS onto a rimmed silicone resin plate (50 × 50 mm), evaporated, and dried in a ventilated oven environmental chamber (EYELA, Environmental Chamber, model KCL-2000A, Tokyo Rikakikai Co., Ltd., Chuo-ku, Tokyo, Japan) at 25 ± 0.5 °C and 50 ± 5% relative humidity (RH) for 24 h. Finally, the dry film was peeled and its properties were determined.

2.4. Determination of film properties

2.4.1. Film thickness

Film thickness was determined by using a hand-held micrometer (Bial Pipe Gauge, Peacock Co., Tokyo, Japan). The film samples were randomly measured at six locations around the film. Experiments were repeated ten times.

2.4.2. Mechanical properties

A film sample was cut into 2 cm wide and 5 cm long, and then conditioned at 50 ± 5% RH at 25 °C for 48 h prior to testing. Tensipresser (TTP-50BX II, Takemoto Electric Inc., Tokyo, Japan) was used to measure tensile strength and elongation at break according to the ASTM standard method D882–97 (American Society for Testing and Materials, 1999). The conditions of testing were 30 mm of initial grip separation with the cross-head speed at 1 mm/s. Measurement was performed until the films were broken.

2.4.3. Film appearance and color

After the films were dried and conditioned at 50 ± 5% RH at 25 °C for 48 h, the visual aspect of film samples were examined by using a digital camera (Fujifilm Finepix S4900; acquired from Fujifilm Thailand Co. Ltd., Bangkok, Thailand).

Color Reader (CR-13, Konica Minolta, Inc., Japan) was used to determine color attributes of the film and expressed as L^*, a^*, and b^*.

2.4.4. Water vapor permeability (WVP)

A modified ASTM standard method (American Society for Testing and Materials, 1989) was used to evaluate the WVP as described in Kaewprachu et al. (2016b). Measurements were conducted at 30 °C at 50 ± 5% RH, and recorded the weight gain of the cup at an hour interval to 8 h. Experiments were repeated three times and expressed as $g\ m^{-1}\ s^{-1}\ Pa^{-1}$.

2.4.5. Differential Scanning Calorimetry (DSC)

A differential scanning calorimeter (DSC-50, Shimadzu Co., Kyoto, Japan) was used for examining the thermal properties of the films (Kaewprachu et al., 2016b). Each film sample (10–12 mg) contained in an aluminum DSC pan were heated in the temperature range of 25–180 °C at a heating rate of 10 °C/min in a nitrogen atmosphere (20 ml/min).

2.4.6. Moisture content, degree of swelling, and film solubility

Moisture content of FMP films was analyzed following the methods of AOAC (Association of Official Analytical Chemists, 2000). The difference between the initial and final weighing was used to evaluate moisture content.

The swelling of films was examined according to the method presented by Mayachiew, Devahastin, Mackey, and Niranjan (2010) with a slight modification. A film sample was cut into 2 cm wide and 2 cm long and then dried at 105 °C for 24 h in an oven (Advantec, Electric Drying Oven, model DRA430DA, Toyo Seisakusho Kaisha Ltd., Chuo-ku, Tokyo, Japan). After, the film was weighed, left in 30 ml of distilled water at 25 °C for 24 h, and then blotted with a filter paper. The mass of the swollen film was weighed and recorded. The degree of swelling was calculated using the following equation:

$$\text{Degree of swelling} = [(m_f - m_i)/m_i] \times 100 \quad (1)$$

where m_f and m_i are the swollen film and the mass of dried film, respectively.

The films' solubility was analyzed as described by Sai-Ut, Benjakul, and Rawdkuen (2014) and calculated by the weight of the dry matter of un-dissolved debris subtracted from the initial weight of the dry matter. The films' solubility was expressed as the percentage of total weight. Experiments were repeated three times.

2.4.7. Light transmission and transparency of the film

A UV spectrophotometer (UV-1800, Shimadzu Co., Kyoto, Japan) was used for measuring the light transmission of the films. A film sample was cut to a rectangular shape (40 × 40 mm). Measurement was performed at the wavelength between 200 and 800 nm.

A film sample was cut to a rectangular shape (40 × 40 mm) and measured at wavelength of 600 nm using a spectrophotometer. The