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Potential use of gelatin hydrolysate as plasticizer in fish myofibrillar protein film

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ABSTRACT

The effect of fish gelatin hydrolysates having different degree of hydrolysis (DH: 23, 61 and 95%) as plasticizer at various levels (30–60%) in fish myofibrillar protein (FMP) film was investigated, in comparison with glycerol. Regardless of DH, FMP films incorporated with gelatin hydrolysates at 50 and 60% of FMP generally had higher elastic modulus (E) and tensile strength (TS) but lower water vapour permeability (WVP), compared with those added with glycerol at the same level ($p < 0.05$). At the same DH, both E and TS of film decreased, while elongation at break (EAB) and WVP increased with increasing levels of gelatin hydrolysate ($p < 0.05$). When gelatin hydrolysate at the same level (30, 40, 50 or 60%) was used, the decreases in TS and E but the increases in WVP were found as DH increased. Nevertheless, FMP film added with gelatin hydrolysate exhibited the higher b^* value, compared to film containing glycerol ($p < 0.05$). Thus, gelatin hydrolysate could be potentially used as a plasticizer in FMP film and its plasticizing effect was governed by DH and levels used.

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1. Introduction

Biodegradable packaging from biopolymer has been received the increasing attention as an alternative to synthetic plastic counterpart due to the environmental friendly aspect (Kester & Fennema, 1986; Krochta & De Mulder-Johnston, 1997). Among all biopolymers, proteins have been empirically used as packaging materials because of their relative abundance, good film formation ability, biodegradability and nutritive value (Krochta, 2002). In addition, protein films exhibit the better oxygen and carbon dioxide barrier properties and mechanical properties, compared with polysaccharide films (Baldwin & Baker, 2002).

Fish myofibrillar proteins (FMP) have been known for their good film forming ability (Arthan, Benjakul, Prodpran, & Tanaka, 2007; Chinnabhark, Benjakul, & Prodpran, 2007; Prodpran & Benjakul, 2005). However, FMP film alone is very brittle due to the extensive protein–protein interactions mediated by disulfide bond, hydrogen bond, electrostatic force and hydrophobic interactions (Krochta & De Mulder-Johnston, 1997). To improve flexibility of protein film, the addition of plasticizer such as glycerol, sorbitol, polyethylene glycol and sucrose is required. However, those common synthetic plasticizers are highly hydrophilic and hygroscopic, leading to dramatic increase in water-vapour adsorption and permeability of film. Moreover, some plasticizers could migrate out from the matrix of the film due to the lack of compatibility with proteins. Migration of plasticizer from the film matrix to the surface as a result of weak interactions between polymer molecules and plasticizer, was found with PEG 400-plasticized β-Lactoglobulin films (Sothornvit & Krochta, 2000). Generally, the differences in composition, size, structure and shape of plasticizers directly affect their ability to function in film matrix (Orliac, Rouilly, Silvestre, & Rigal, 2003).

Protein hydrolysates can be defined as proteins that are chemically or enzymatically broken down into peptides of different sizes and free amino acids (Adler-Nissen, 1986). Apart from nutritive value, protein hydrolysates also possess antioxidative and antimicrobial activities (Jia et al., 2009; Mendis, Rajapakse, Byun, & Kim, 2005; Yan, Ho, Chu, & Chow, 2008). Protein hydrolysate has been known to have the improved functional properties such as emulsifying properties, etc (Nalinanon, Benjakul, Kishimura, & Shahidi, 2011). Hydrolysates from fish or squid skin gelatins have been reported to contain biologically active peptides with high antioxidant activity (Kim et al., 2001; Lin & Li, 2006; Mendis et al., 2005; Yan et al., 2008). In addition, the short chain peptide of gelatin

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hydrolysate with high mobility might act as plasticizer in protein based film, particularly from FMP. The gelatin hydrolysate incorporated might reduce interaction between chains of proteins and increase the free volume in the protein network of film. However, very rare information regarding the use of gelatin hydrolysate as plasticizer in protein film especially in FMP film has been reported. Peptides in gelatin hydrolysate with different chain lengths and hydrophilicity/hydrophobicity ratios might exhibit varying plasticizing effect in films.

Therefore, the objective of this study was to investigate the effect of gelatin hydrolysate with different degree of hydrolysis (DH) at various levels on the properties of FMP film.

2. Materials and methods

2.1. Chemicals

Glycerol, sodium chloride (NaCl), sodium hydroxide (NaOH), hydrochloric acid (HCl), sodium sulphite, L-Leucine, 2,4,6-trinitrobenzenesulphonic acid (TNBS), di-sodium hydrogen orthophosphate and sodium dihydrogen orthophosphate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Malondialdehyde bis(dimethyl acetal) and β-mercaptoethanol (β-ME) were obtained from Sigma (St. Louis, MO, USA). Acrylamide, *N,N,N′,N′*-tetramethylethylenediamine (TEMED) and bis-acrylamide were procured from Fluka (Buchs, Switzerland).

2.2. Fish sample

Fresh red tilapia (*Oreochromis niloticus*) with an average weight of 400–500 g/fish were purchased from a local market in Hat Yai, Songkhla province, Thailand. Fish were kept in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Material Product Technology, Prince of Songkla University within 30 min. Upon the arrival, fish were immediately washed, filleted and minced to uniformity, using a mincer with a hole diameter of 0.5 cm.

2.3. Preparation of fish myofibrillar protein (FMP)

FMP from red tilapia mince was prepared according to the method of Benjakul, Chantarasuwan, and Visessanguan (2003). Fish mince was homogenized with 3 volumes of cold distilled water (4 °C) at a speed of 13,000 rpm for 2 min using an IKA Labortechnik homogenizer (Selangor, Malaysia), followed by filtering through a layer of nylon cloth. The retentate was further mixed with 5 volumes of 50 mM NaCl for 5 min and filtrated through a layer of nylon cloth. The washing process was repeated twice. Then, the resulting washed mince referred to as "FMP" was stored on ice until used for film preparation.

2.4. Preparation of gelatin hydrolysate

Preparation of gelatin hydrolysate with different degree of hydrolysis (DH) was carried out by acid hydrolysis process as described by Tsugita and Scheffler (1982). Fish gelatin powder from tilapia skin (~240 bloom) (Lapi Gelatine S.p.A, Empoli, Italy) was dissolved in 1 M HCl to obtain the protein concentration of 10% (w/v). The mixture was incubated at 50 °C for 15 min. The gelatin solution was then placed in a temperature controlled oil bath at 100 °C. At hydrolysis time designated (1, 6 and 12 h), the sample was taken and cooled suddenly with iced water. The pH of the resulting hydrolysates was adjusted to 7.0 using 2 M NaOH. The obtained hydrolysates were subjected to determination of DH.

DH of gelatin hydrolysates obtained was analysed according to the method of Benjakul and Morrissey (1997). Prior to the analysis, the gelatin hydrolysate was diluted with DDI-water to obtain the protein concentration of 5%. The diluted sample (100 μl) was added with 1.6 ml of 0.2 M phosphate buffer, pH 8.2 and 0.8 ml of 0.01% TNBS solution. The solution was mixed thoroughly and placed in a temperature-controlled water bath at 50 °C for 30 min in the dark. The reaction was terminated by adding 1.6 ml of 0.1 M sodium sulfite. The mixtures were cooled at room temperature for 15 min. The absorbance was read at 420 nm and α-amino group was expressed in terms of L-leucine. The DH was defined as follows (Benjakul & Morrissey, 1997):

$$DH = [(L_s - L_0)/(L_{max} - L_0)] \times 100$$

where L_s is the amount of α-amino groups of gelatin hydrolysate sample. L_0 is the amount of α-amino groups in the original gelatin solution. L_{max} is the total α-amino groups in the original gelatin solution obtained after complete acid hydrolysis (6 N HCl at 100 °C for 24 h).

2.5. Preparation of fish myofibrillar protein (FMP) film

The film-forming solution (FFS) was prepared according to the method of Prodpran and Benjakul (2005). FMP was added with distilled water to obtain the final protein concentration of 2% (w/v). The mixture was homogenized at 13,000 rpm for 1 min. The pH of the mixture was adjusted to 3 using 1 N HCl to solubilize the protein. The solution was filtered through a layer of nylon cloth to remove undissolved debris. Gelatin hydrolysates with different DH (23, 61 and 95%) were added to the FFS at various levels (30, 40, 50 and 60% based on protein of FMP) without glycerol addition. The solution was stirred gently for 30 min at room temperature. The control FFSs (without gelatin hydrolysate) were also prepared by using glycerol as plasticizer at different levels (30, 40, 50 and 60% based on protein of FMP). It was noted that these three DH levels of gelatin hydrolysate were chosen in this study since our preliminary observation revealed that the addition of gelatin hydrolysate with these different DH ranges resulted in FMP films with significant differences in properties especially the mechanical properties.

To prepare the film, 4 g of FFS was cast onto a rimmed silicone resin plate (5 × 5 cm^2) and air blown for 12 h at room temperature prior to further drying at 25 °C and 50 ± 5% relative humidity (RH) for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). Finally, films were manually peeled-off and stored at 25 °C and 50% RH until used for analyses.

2.6. Determination of film properties

2.6.1. Film thickness

The thickness of film was measured using a micrometer (Mitutoyo Absolute, Tokyo, Japan). Five random positions of each film of ten film samples were used for average thickness determination.

2.6.2. Mechanical properties

Prior to the measurement of mechanical properties, the films were conditioned for 48 h in a ventilated oven at 25 °C and 50 ± 5% RH. Elastic modulus (E), tensile strength (TS) and elongation at break (EAB) of films were determined as described by Iwata, Ishizaki, Handa, and Tanaka (2000) with a slight modification using a Universal Testing Machine (Lloyd Instruments, Hampshire, UK) equipped with tensile load cell of 100 N. Ten samples (2 × 5 cm^2) with initial grip length of 3 cm were used for testing.

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