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Properties of red tilapia (*Oreochromis niloticus*) protein based film as affected by cryoprotectants

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ABSTRACT

Edible films based on red tilapia (*Oreochromis niloticus*) washed mince in the presence of cryoprotectants at different levels were prepared by casting method using glycerol as plasticiser. Films containing cryoprotectants, except for 4% sucrose, had lower tensile strength (TS) accompanied with higher elongation at break (EAB), compared with the control film ($P < 0.05$). Water vapour permeability (WVP) of films increased ($P < 0.05$) but no changes in transparency value were observed in the presence of cryoprotectants, compared with the control film ($P < 0.05$). Light transmission of films in UV range (200–280 nm) was lowered in films containing cryoprotectants, but increased in the visible range (350–800 nm). Intermolecular interactions between cryoprotectants and protein molecules were found in the film as determined by FTIR spectroscopy. Thermo-gravimetric analysis revealed that films containing 2% sucrose and 2% sorbitol exhibited the lower degradation temperatures with the lower weight loss, compared with other samples. Film with cryoprotectants had a smoother and more homogeneous surface without cracks. However, cryoprotectants at higher level caused the gritty surface. Thus, cryoprotectants present in the fish mince or surimi acted as plasticising agent and directly affected properties of films.

1. Introduction

Increasing use of synthetic packaging films has led to serious ecological problems due to their non-biodegradability. Considerable efforts are being employed worldwide to develop new biodegradable packaging materials from natural polymers. During the last decade, there has been growing interest in edible or biodegradable films based on biopolymers. Edible films and coatings are of interest since they have potential to improve shelf-life, maintain quality and prevent microbial deterioration and physical damage of foods (Ahmad, Benjakul, Prodpran, & Agustini, 2012). The main biopolymers used in the edible film preparation are derived from polysaccharides (Nisperos-Carriedo, 1994) and proteins (Gennadios, McHugh, Weller, & Krochta, 1994). Edible films prepared from protein materials have been paid more attention for the use in the food protection and preservation, owing to their biodegradable, environmental characteristics (Tanaka, Ishizaki, Suzuki, & Takai, 2001) and their ability to form films with satisfactory mechanical and gas barrier properties (Cuq, Gontard, & Guilbert, 1998). Fish proteins including myofibrillar and sarcoplasmic proteins have been used as film-forming materials (Artharn, Benjakul, Prodpran, & Tanaka, 2007). Surimi was also shown as a potential proteinaceous material for film making (Chinabhark, Benjakul, & Prodpran, 2007). Generally, myofibrillar proteins, the major constituent in surimi, are susceptible to freeze damage, leading to poor functional properties. These changes could be reduced remarkably by adding the so-called "cryoprotectants" into washed fish mince (Sultanbawa & Li-Chan, 1998). Sucrose, sorbitol and their mixture are popular cryoprotectants used in frozen surimi. Those cryoprotectants in surimi might affect the properties of film prepared from surimi to some degree. Plasticisers significantly affect mechanical and water vapour barrier properties of protein films (Irissin-Mangata, Bauduin, Boutevin, & Gontard, 2001). Polyols, such as sorbitol, etc. could act as plasticiser in biopolymer-based films due to their ability to reduce intermolecular hydrogen bonding, thereby increasing intermolecular spacing (Irissin-Mangata et al., 2001). Sorbitol is a water-soluble, polar, non-volatile, protein-miscible, polyhydric alcohol with a high boiling point and can act as a suitable plasticiser or cryoprotectant with a compatible water-soluble polymer and proteins (Barreto, Pires, & Soldi, 2003). Hence, the cryoprotectants (sorbitol or sucrose) in surimi more likely have the impact on surimi based

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film. However, there is no information regarding the impact of cryoprotectants in surimi on the properties of resulting films. Therefore, the present study was undertaken to elucidate the impact of cryoprotectants (sorbitol and sucrose) in red tilapia washed mince, simulating frozen surimi, on the properties of films.

2. Materials and methods

2.1. Chemicals

High molecular weight protein markers were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Glycerol and tris (hydroxymethyl) aminomethane were obtained from Merck (Darmstadt, Germany). Sodium dodecyl sulphate (SDS), Coomassie Blue R-250 and *N,N,N′,N′*-tetramethylethylenediamine (TEMED) were procured from Bio-Rad Laboratories (Hercules, CA, USA). Food grade sucrose and sorbitol were purchased from High Science Limited Partnership (Hat Yai, Songkhla, Thailand). All chemicals were of analytical grade.

2.2. Fish sample

Fresh red tilapia (*Oreochromis niloticus*) (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 Food 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 washed mince

Washed mince was prepared according to the method of Toyohara, Sakata, Yamashita, and Shimizu (1990) with a slight modification. Mince was homogenised with five volumes of 50 mM NaCl using an IKA Labortechnik homogeniser (Selangor, Malaysia) at a speed of 11,000 rpm for 2 min, followed by centrifuging using a Beckman Model Avanti J–E centrifuge (Beckman Coulter, Inc., Fullerton, CA) at 9600× *g* at 4 °C for 10 min. The washing process was repeated twice. Washed mince containing 77.56% moisture was stored on ice until used for analysis or for film preparation.

2.4. Preparation of film-forming solution

Film forming solution (FFS) was prepared as described by Shiku, Hamaguchi and Tanaka (2003) with a slight modification. Washed mince was mixed with the distilled water to obtain the final protein concentrations of 3% (w/v). The mixture was homogenised at 13,000× *g* for 1 min, followed by addition of cryoprotectants based on washed mince weight as follows: 4% sucrose, 4% sorbitol, 2% sucrose + 2% sorbitol and 4% sucrose + 4% sorbitol. Subsequently, glycerol at 50% (w/w) based on protein content was added as a plasticiser and the mixture was stirred gently for 30 min at room temperature. Finally, the pH of mixture was adjusted to 3 using 1 N HCl to solubilise the protein by electrostatic repulsion. FFS having total solid content of 4.65% (w/v) was subjected to centrifugation at 3000× *g* for 10 min at room temperature to remove air bubbles and aggregated proteins. The supernatant obtained was used for film casting.

2.5. Film casting, drying and conditioning

FFS (4 g) was cast onto a rimmed silicone resin plate (50 × 50 mm^2) and air blown for 12 h at room temperature prior to further drying in an environmental chamber (WTB Binder, Tuttlingen, Germany) at 25 °C and 50% relative humidity (RH) for 24 h. The resulting films were manually peeled off and subjected to analyses. Prior to testing, film samples were conditioned for 48 h at 50 ± 5% relative humidity (RH) at 25 ± 0.5 °C. For ATR-FTIR, SEM and TGA studies, films were dried in a desiccators containing P_2O_5 gel for 2 weeks at room temperature (28–30 °C) to obtain the most dehydrated films.

2.6. Analyses

2.6.1. Film thickness

The thickness of film samples was measured using a digital micrometre (Mitutoyo, Model ID-C112PM, Serial No. 00320, Mituyoto Corp., Kawasaki-shi, Japan). Ten random locations around each film sample were used for determination of thickness.

2.6.2. Mechanical properties

Tensile strength (TS) and elongation at break (EAB) of film samples were determined as described by Iwata, Ishizaki, Handa and Tanaka (2000) using the Universal Testing Machine (Lloyd Instrument, Hampshire, UK). The test was performed in the controlled room at 25 °C and ~50 ± 5% relative humidity. Ten film samples (2 × 5 cm^2) with the initial grip length of 3 cm were used for testing. The film samples were clamped and deformed under tensile loading using a 100 N load cell with the cross-head speed of 30 mm/min until the samples were broken. The maximum load and the final extension at break were used for calculation of TS and EAB, respectively.

2.6.3. Water vapour permeability (WVP)

WVP was measured using a modified ASTM (American Society for Testing and Materials) method (ASTM, 2002) as described by Shiku, Hamaguchi, Benjakul, Visessanguan, and Tanaka (2004). The film samples were sealed on an aluminium permeation cup containing dried silica gel (0% RH) with silicone vacuum grease and rubber gasket. The cups were placed at 30 °C in a desiccators containing the distilled water, followed by weighing after every 1 h intervals for up to 8 h. Five film samples were used for WVP testing. WVP of the film was calculated as follows:

$$\mathrm{WVP}\left(\mathrm{gm^{-1}\ s^{-1}\ Pa^{-1}}\right) = wlA^{-1}t^{-1}(P_2 - P_1)^{-1}$$

where w is the weight gain of the cup (g); l is the film thickness (m); A is the exposed area of film (m^2); t is the time of gain (s); $(P_2 - P_1)$ is the vapour pressure difference across the film (4244.9 Pa at 30 °C).

2.6.4. Light transmission and transparency

Light transmission of films against ultraviolet (UV) and visible light was measured at selected wavelengths between 200 and 800 nm, using a UV–visible spectrophotometer (model UV-160, Shimadzu, Kyoto, Japan) according to the method of Jongjareonrak, Benjakul, Visessanguan, and Tanaka (2011). The transparency value of the film was calculated by the following equation:

$$\text{Transparency value} = (-\log T_{600})/x$$

where T_{600} is the fractional transmittance at 600 nm and x is the film thickness (mm). The higher transparency value represents the lower transparency of films.

2.6.5. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein patterns of washed mince sample and the films were analysed by SDS-PAGE according to the method of Laemmli (1970) using a 10% running gel and 4% stacking gel. Washed mince (3 g) was solubilised in 27 ml of 5% SDS (85 °C) as described by Benjakul, Visessanguan, and Srivilai (2001). To solubilise the films, the