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Phenolics-protein interaction involved in silver carp myofibrilliar protein films with hydrolysable and condensed tannins

Xiaohua Nie [a, *], Liming Zhao [b], Ningning Wang [a], Xianghe Meng [a, *]

[a] Department of Food Science and Technology, Ocean College, Zhejiang University of Technology, Hangzhou, Zhejiang 310014, China
[b] State Key Laboratory of Bioreactor Engineering, R&D Center of Separation and Extraction Technology in Fermentation Industry, East China University of Science and Technology, Shanghai 200237, China

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ABSTRACT

Silver carp myofibrillar protein films were prepared with tannins (tannic acid and apple procyanidins) at various concentrations (30 and 50 g/kg protein) under alkaline and heating condition. Phenolics-protein interaction as well as related properties of incorporated film was evaluated. Available amino and total sulfhydryl groups in film-forming solution (FFS) were decreased especially by tannic acid, whereas protein solubilities in the film were more sharply reduced by apple procyanidin. SDS-PAGE pattern suggested that non-sulfide covalent bonds were formed between tannins and protein molecules, and more protein polymerization was induced by apple procyanidin. On the other hand, the incorporation of tannic acid and apple procyanidin led to an increased tensile strength (TS) with accompanying decreases in elongation at break (EBA) and water vapor permeability (WVP). The film with apple procyanidin had higher TS and EBA as well as lower WVP than that with tannic acid. Tannins especially condense tannin could covalently crosslink with protein molecules to form protein-phenolics- protein polymers, and thus improve some physicochemical properties of the resulting protein films.

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1. Introduction

Edible/biodegradable films from natural biopolymers have attracted the increasing attention as replacement of synthetic polymer films in food industry, due to the health and environmental concerns. Among the natural biopolymers, food proteins have been recognized as potential materials because of their abundance, good film-forming capacity and biodegradability. Myofibrillar protein is the main fraction of fish muscle, and has been successfully used to prepare edible/biodegradable films with good barrier properties against gases, organic volatiles and lipids (Chinabhark, Benjakul, & Prodpran, 2007; Kaewprachu, Osako, Benjakul, & Rawdkuen, 2016; Sobral, Monterrey-Q, & Habitante, 2002). There are large populations of underutilized fish species due to unattractive flavor, color, texture and small size. Silver carp (*Hypophthalmichthys molitrix*) is one of the widely cultured freshwater fish in China, with an annual harvest above 3.80 million tons in 2013 (Fisheries bureau, Ministry of Agriculture of China, 2014). Silver carp is rich in protein but with extremely muddy odour and many tiny-bones. There is a great interest in using silver carp myofibrillar protein to produce edible/biodegradable film.

However protein films are inferior to synthetic polymer films in terms of mechanical strength and water resistance. Chemical crosslinking of protein molecules has been considered as an effective method that leads to stronger and less permeable films (Wihodo & Moraru, 2013). Natural phenolic compounds are well known to react with active groups in protein molecules and thus change the structure and properties of proteins, particularly under oxidation (Le Bourvellec & Renard, 2012; Ozdal, Capanoglu, & Altay, 2013; Rawel, Czajka, Rohn, & Kroll, 2002). They may play the role of crosslinking agents in film preparation to enhance mechanical strength, water resistance and thermal stability, e.g. soy protein film with rutin (Friesen, Chang, & Nickerson, 2015), wheat gluten film with tannic acid (Hager, Vallons, & Arendt, 2012) and gelatin film with curcuma ethanol extract (Bitencourt, Fávaro-Trindade, Sobral, & Carvalho, 2014). However, some authors considered polyphenols as plasticizer in protein films to increase the flexibility (Arcan & Yemenicioğlu, 2011; Insaward, Duangmal, & Mahawanich, 2014; Kavoosi, Dadfar, Mohammadi Purfard, & Mehrabi, 2013). The interaction between phenolic compounds and protein molecules

* Corresponding author.
E-mail addresses: niexiaohua2000@zjut.edu.cn (X. Nie), niexiaohua2000@zjut.edu.cn (X. Meng).

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depends on the type of protein, the nature of phenolics and the process conditions including pH and temperature (Budryn et al., 2015; Ozdal et al., 2013). Hydrogen bonds, hydrophobic binding and covalent crosslinking can be involved in phenolics-protein complexes. Resultant interactions in phenolics-protein complex is an important factor affecting the film properties. Phenolics-protein interaction and its association to film properties have not yet been studied widely.

Tannins are natural phenolic compounds with different molecular weights, and generally classified into hydrolysable and condensed types. Tannic acid is a typical hydrolysable tannin, consisting of 8–10 molecules of gallic acid per molecule of glucose. Procyanidins, generally known as condensed tannin, are oligomers or polymers of flavan-3-ol. The aim of this study was to make silver carp myofibrillar protein films with hydrolysable and condensed tannins (tannic acid and apple procyanidin) under alkaline and heating condition, and evaluate the interaction of the added tannins with protein molecules during the film preparation. The related film properties including mechanical behavior, water resistance and thermal stability were investigated.

2. Materials and methods

2.1. Chemical

Tannic acid (food grade, 92%) and apple procyanidin (food grade, phenolic content 90%) were given by Qingyuan Tech Company (Hangzhou, China). The content of tannic acid is 930.5 mg/g powder using ultraviolet spectrophotometry. Apple procyanidin preparation is composed mainly of procyanidin oligomers (524 mg/g powder), anthocyanins (45 mg/g powder), flavan-3-ols (81 mg/g powder), chlorogenic acid (108 mg/g powder) and other flavonoids (120 mg/g powder). 2,4,6-trinitrobenzenesulphonic acid (TNBS) and 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma- Aldrich (St. Louis, MO, USA). All the other reagents were of analytical grade.

2.2. Myofibrillar protein preparation

Fresh silver carp was purchased from a local market (Hangzhou, Zhejiang, China). Fish meat was homogenized in five volumes of 50 mmol/L NaCl at 10,000 rpm for 2 min, and filtered through a double-layer cheesecloth. The washing process was carried out four times. The resulting mince was termed as myofibrillar protein and stored at −18 °C for not more than one month. Before the film preparation, protein content was measured by the Kjedahl method (Method 920.123, AOAC, 2000).

2.3. Preparation of film-forming solution (FFS) and film

Frozen myofibrillar protein was thawed at 4 °C overnight, and dispersed in distilled water to obtain a final protein content of 20 g/L. Glycerol was added as a plasticizing agent at 350 g/kg protein. Tannic acid or apple procyanidin were added at 30 and 50 g/kg protein. The solution was adjusted to pH 11.0 with 2 mol/L NaOH, and then heated at 70 °C for 30 min with continuous stirring. The heated solution was termed film-forming solution (FFS). The control FFS was prepared without the addition of tannic acid and apple procyanidin. After centrifugation (3000 g, 10 min) to remove air bubbles, the FFS (50 mL) was spread onto a silicone resin plate (140 × 140 mm) and subsequently dried at 60 °C for 6 h. Finally, films were manually peeled off and kept in a chamber at 25 °C and 50% relative humidity for 48 h.

2.4. Available amino group content

The content of available amino groups was measured using 2,4,6-trinitrobenzenesulphonic acid (TNBS) method (Balange & Benjakul, 2009). FFS sample (0.25 mL) was mixed with 4 mL of 0.2 mol/L sodium phosphate buffer (pH 8.2, containing 0.6 mol/L NaCl) and 2 mL of 1 g/L TNBS solution, followed by incubation in the dark at 50 °C for 30 min. The reaction was terminated by addition of 0.1 mol/L Na_2SO_3 (2 mL). The absorbance was read at 420 nm using a UV720 spectrophotometer (Meipuda Co., Shanghai, China). The content of available amino groups was calculated from the standard curve using L-lysine and expressed as μmol NH_2/g protein.

2.5. Total sulfhydryl group content

The content of total sulfhydryl groups was measured using 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) method (Insaward et al., 2014). FFS sample (1 mL) was mixed with 9 mL of 0.2 mol/L Tris-HCl buffer (pH 8.0) containing 8 mol/L urea, 20 g/L SDS and 10 mmol/L EDTA. The mixture (4 mL) was added with 0.4 mL of 1 g/L DTNB solution, followed by incubation at 40 °C for 25 min. The absorbance was read at 412 nm using a UV720 spectrophotometer (Meipuda Co., Shanghai, China). The content of total sulfhydryl groups was calculated from the standard curve using L-cysteine and expressed as μmol SH/g protein.

2.6. Protein solubility in various extraction solution

Protein solubility of each film was measured in various extraction solutions according to the method of Nuthong, Benjakul, and Prodpran (2009). Film sample (0.1 g) was suspended in 5 mL of extraction solution (S1: distilled water; S2: 8 mol/L urea; S3: 8 mol/L urea plus 20 g/L β-mercaptoethanol) at room temperature for 24 h with continuous stirring, followed by centrifugation at 10,000 g for 15 min. Protein content in the supernatant was determined using Coomassie brilliant blue method (Pierce & Suelter, 1977). To obtain the total protein, the film sample was extracted with 1 mol/L NaOH. Protein solubility was expressed as the percentage of soluble protein with respect to total protein.

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

Before the test, a film extract solution was prepared as described by Nie, Gong, Wang, and Meng (2015). Film sample (0.1 g) was extracted with 5 mL of 0.2 mol/L Tris-HCl solution (pH 8.0) including 8 mol/L urea, 20 g/L SDS and 20 g/L β-mercaptoethanol at room temperature for 24 h with continuous stirring. After centrifugation at 10,000 g for 15 min, the supernatant was referred to as film extract solution and used for SDS-PAGE electrophoresis.

SDS-PAGE electrophoresis of FFS and film extract solution was performed on a mini Protean III apparatus (Bio-Rad Laboratories, Hercules, CA, USA), using 40 g/L stacking gel and 100 g/L resolving gel. FFS or film extract solution was mixed with an equal volume of loading buffer (0.2 mol/L Tris–HCl, 200 g/L glycerol, 40 g/L SDS, 100 g/L β-mercaptoethanol, 0.05 g/L bromophenol blue, pH 6.8), and then heat-denatured at 100 °C for 5 min. Electrophoresis was carried out at a constant voltage of 120 V. The gel was stained with Coomasie Brilliant Blue R-250 (1 g/L), and washed in destaining solution including methanol (250 mL/L) and acetic acid (100 mL/L).

2.8. Fourier transform infrared (FTIR) analysis

FTIR spectra of films were performed on a Tensor 27 FTIR spectrometer (Brucker Optics, Ettlingen, Germany) equipped with

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