Food Bioscience 15 (2016) 118–125

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Characterization of surimi slurries and their films derived from myofibrillar proteins with different extraction methods

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ARTICLE INFO

Article history:
Received 29 September 2015
Received in revised form
5 July 2016
Accepted 5 July 2016
Available online 9 July 2016

Keywords:
Films
surimi
Alaska pollock
sodium chloride
pH
sodium tripolyphosphate

Chemical compounds studied in this article:
Sodium chloride (PubChem CID: 5234)
Sodium tripolyphosphate (PubChem CID: 24455)
Magnesium nitrate hexahydrate (PubChem CID: 21871556), Sodium hydroxide (PubChem CID: 14798)
Hydrochloric acid (PubChem CID: 313)
Potassium chloride (PubChem CID: 4873)
Tris buffer [2-amino-2-(hydroxymethyl) propane-1,3-diol] (PubChem CID: 6503)
DTNB [5,5'-dithiobis (2-nitrobenzoic acid)] (PubChem CID: 6254)

ABSTRACT

Characteristics of surimi slurries and their films made from fish myofibrillar protein were investigated. Films made from fish protein slurries consisting of Alaska pollock surimi (15% and 20%) and sodium chloride (0%, 2%, and 5%) were either adjusted to different pH (7, 9, and 11) or mixed with sodium tripolyphosphate (STP) (1.0%, 1.5%, and 2.0%). Increasing pH from 7 to 9 led to higher viscosity and exposed sulfhydryl sites, while further pH increase 9–11) reduced the viscosity values and surface reactive sulfhydryl groups. NaCl and STP addition decreased slurry viscosity. In most treatments involving the roles of pH and either sodium chloride or sodium tripolyphosphate in film formation, sodium chloride addition contributed to lower puncture strength and puncture distance. Meanwhile, there was a tendency for elevated tensile strength and reduced elongation at break as sodium chloride concentration was increased from 0% to 5%. Shifting pH to a more basic condition contributed to higher puncture strength and distance. As STP concentration in the surimi slurries increased up to 2%, puncture and tensile strength increased, but puncture distance and elongation at break decreased.
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1. Introduction

Fish is a very important source of proteins. Interestingly, compared to land animals, fish contains considerably higher quantity of myofibrillar proteins (Lanier et al., 2005). Myofibrillar proteins play significant roles in constructing gel network in foods (Lanier et al., 2005; Shiku et al., 2004). Myofibrillar proteins comprise of myosin (55–60%) that possesses gelling ability and water binding capacity (Lanier et al., 2005).

Surimi is a good source of fish myofibrillar proteins. According to Park and Lin (2005), surimi is produced by mincing fish fillet and then the fish mince is subjected to the washing and dewatering process, which removes sarcoplasmic proteins in order to concentrate myofibrillar proteins. Furthermore, the washed fish mince is refined to remove pin bones, scales and connective tissues, pressed by screw press to remove most of free water before being mixed with cryoprotectants, and then frozen (Park and Lin, 2005). Surimi is classified as an intermediate product because it can be further processed into various foods. In United States and Europe, it is mostly used to produce crabsticks; while in Asia, the products derived from surimi are more varied, for instance fish ball, fried fish cake, and grilled surimi seafood. However, more application of surimi is still expected to increase added values of that material. One of promising applications using surimi can be how to utilize its film-forming ability. Our unpublished research indicated the significant role of surimi's film-forming ability in a way to minimize the migration of oil and moisture in fried surimi seafood.

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http://dx.doi.org/10.1016/j.fbio.2016.07.002
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Fish proteins have been used to produce films, especially as bio-packaging (Cuq et al., 1996, 1997; Iwata et al., 2000; Prodpran and Benjakul, 2005; Shiku et al., 2003; Shiku et al., 2004; Chinabhark et al., 2007; Prodpran et al., 2007; Weng et al., 2007; Weng et al., 2009). According to Cuq (2002), in order to produce films from proteins, three essential steps must be followed: (1) breaking intermolecular bonds that stabilize native protein configuration, (2) rearrangement of protein molecules, and (3) formation of new bonds that promote three-dimensional network formation. The first step can be mediated by shifting pH of film-forming solution to alkali or acid (Prodpran and Benjakul, 2005; Weng et al., 2009), adding salt, or incorporating sodium tripolyphosphate (STP). Salt, especially NaCl, helps dissociate myofibrils and extract actomyosin, myosin, actin, and other myofibrillar proteins and thus improves gelation (Nielsen and Pigott, 2004; Xiong and Delles, 2009). Moreover, salt also increases protein-protein repulsion (Xiong and Delles, 2009). STP dissociates actomyosin as well; starting at both ends of the A-band (Xiong et al., 2000). STP also increases pH (Shults and Wierbicki, 1973), which induces more repulsion force among negatively charged proteins.

The association/entanglement of proteins can be achieved by heating film-forming solutions or adjusting the pH of sample solution. Protein molecules unfold upon heating and pH adjustment. Therefore, it exposes active sites that play important roles in creating a three dimensional network (Lanier et al., 2005). This step is indicated by extensive interaction between protein molecules through hydrogen, ionic, hydrophobic, and covalent bonds (Shiku et al., 2003; Weng et al., 2007). Various fish was tried to measure film-forming characteristics. Application of fish protein isolate slurry as a coating material to reduce oil and moisture migration in foods during frying had been studied and patented by Kelleher and Williamson (2007). However, they only used acid extraction method to obtain the fish protein isolates. Authors believe that the use of commercially available surimi (instead of fish protein isolate, which is not commercially available yet) with other common extraction methods would provide users with more convenient and effective process. But the effect of commercially refined fish protein (surimi) on the film-forming ability was not extensively evaluated.

The objectives were to explore the feasibility of refined fish myofibrillar protein (surimi) as a resource for film, to evaluate the extraction methods for myofibrillar proteins, and further to understand the relationship between viscosity and protein interaction in film-forming.

2. Materials and methods

2.1. Preparation of fish protein-based films

Alaska pollock (*Gadus chalcogrammus*, formerly *Theragra chalcogramma*) frozen surimi (FA grade) (NOAA, 2016) was obtained from Glacier Fish, (Seattle, WA, USA). The surimi contained moisture (74–75%), protein (16–17%), and cryoprotectants (9.0–9.3%). Slurries for fish protein films were prepared by two methods: (1) pH adjustment combined with NaCl addition and (2) STP incorporation combined with NaCl addition. Each method comprised of 18 different combinations of surimi content, NaCl content and pH/STP level. A detailed description of composition of surimi slurries was given in Table 1.

For the first method, surimi was homogenized with deionized water and NaCl to produce surimi slurries. The pH of the surimi slurries was adjusted to 7 by adding HCl (1 N) or to 9 and 11 by adding NaOH (5 N). Meanwhile, for the second method, surimi was added with a particular quantity of deionized water, NaCl, and STP (Table 1).

Table 1
Composition of surimi slurries.

Treatment Number	pH adjustment treatment			STP addition treatment		
	Surimi (%)	NaCl (%)	pH	Surimi (%)	NaCl (%)	STP (%)
1	15	0	7	15	0	1.0
2	15	0	9	15	0	1.5
3	15	0	11	15	0	2.0
4	15	2	7	15	2	1.0
5	15	2	9	15	2	1.5
6	15	2	11	15	2	2.0
7	15	5	7	15	5	1.0
8	15	5	9	15	5	1.5
9	15	5	11	15	5	2.0
10	20	0	7	20	0	1.0
11	20	0	9	20	0	1.5
12	20	0	11	20	0	2.0
13	20	2	7	20	2	1.0
14	20	2	9	20	2	1.5
15	20	2	11	20	2	2.0
16	20	5	7	20	5	1.0
17	20	5	9	20	5	1.5
18	20	5	11	20	5	2.0

In order to remove air pockets, surimi slurries were centrifuged in a Beckman J6-M1 (Beckman Coulter, Brea, CA, USA) at 5 °C for 20 min at 1780 × g followed by air removal using a high vacuum pump (E2M2, Edwards High Vacuum Pump, Edwards High Vacuum Intl., Sussex, UK) for up to 2 h. Then the slurry was subjected to centrifugation again at 5 °C at 1780 × g for 5 min. The slurries were then heated in a water bath at 90 °C for 25 min to promote more protein unfolding and inactivate potential endogenous proteases.

Subsequently, the slurries were kept in an ice bath for about 15 min to cool down. Finally, aliquots of the slurries (5 g) were casted in each well (2.54×10.16 cm^2) on specially prepared polytetrafluoroethylene (PTFE) plates and dried in a shaker (Lab-Line Orbiz Environ-Shaker, Lab-Line Instruments, Inc., Melrose Park, IL, USA) at 50 °C for 2.5 h.

2.2. pH measurement

The pH of surimi slurries from STP and NaCl addition method was measured by directly submerging the pH probe attached to Accumet Research AR 15 pH-meter (Fisher Scientific, St Louis, MO, USA) in a cold room. At least two measurements were recorded.

2.3. Mechanical tests

Films were conditioned at 50% relative humidity (RH) and 25 °C for 2 days prior to mechanical tests (ASTM, 2008). RH was controlled using saturated magnesium nitrate hexahydrate solution (1.25 g/ml). The compound was purchased from Spectrum (Gardena, CA, USA). Both puncture and tensile properties were measured using a texture analyzer (TA-XT plus, Texture Technologies Corp, Hamilton, MA, USA). Puncture tests were conducted following the procedures described in Park and Zhao (2004) and ASTM D6241 (ASTM, 2009) with slight modification. Films were placed in an extensibility fixture with 10 mm openings (TA-108S-1, Texture Technologies Corp) (Fig. 1(a)). A spherical probe (5 mm diameter) moved at a crosshead speed of 1 mm/s to puncture the film. Puncture strength was the maximum force a film could withstand before rupture while puncture distance was the distance the probe moved from the point where the film was touched by the probe to the point where the film ruptured. To eliminate the effect of film thickness variation among treatments, puncture

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