LWT - Food Science and Technology 50 (2013) 511–518

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

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

Effects of protein isolates from black bean and mungbean on proteolysis and gel properties of surimi from sardine (*Sardinella albella*)

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ARTICLE INFO

Article history:
Received 4 March 2012
Received in revised form
29 July 2012
Accepted 21 August 2012

Keywords:
Sardine
Surimi gel
Degradation
Black bean
Mungbean
Protein isolate
Protease
Inhibitor

ABSTRACT

Effects of protein isolates from black bean (BBPI) and mungbean (MBPI) on proteolysis and gelling properties of surimi from sardine (*Sardinella albella*) were investigated. Both BBPI and MBPI showed inhibitory activity against proteolysis in kamaboko (40/90 °C) and modori (65/90 °C) gels in a concentration-dependent manner. Myosin heavy chain (MHC) was more retained in both gels when the concentration of both protein isolates increased up to 1 g 100 g^{-1}. This was associated with the increased breaking force and deformation as well as lowered degradation as evidenced by the decrease in trichloroacetic acid-soluble peptide content ($p < 0.05$). Whiteness of kamaboko and modori gels slightly decreased with increasing BBPI or MBPI levels ($p < 0.05$). However, water-holding capacity of both gels increased with increasing levels of both protein isolates. Microstructure of kamaboko and modori gels added with 1 g 100 g^{-1} BBPI or MBPI was finer and denser with more ordered structure than that of the control. Generally, BBPI showed slightly higher gel strengthening effects and inhibition against proteolysis of surimi gels than MBPI. Therefore, proteolysis of sardine surimi, associated with endogenous proteases, could be retarded by the addition of BBPI or MBPI, leading to the increased gel strength.

1. Introduction

Sardine (*Sardinella albella*) is a small coastal pelagic fish species, which can be used for surimi production in Thailand. Apart from the darken colour, surimi from sardine has poorer gel properties than those produced from lean fish. This leads to the less demand of surimi from sardine. Proteolytic disintegration of myofibrillar proteins has an adverse effect on gel-forming properties of surimi. The breakdown of myofibrillar proteins inhibits the development of three-dimensional gel network (An, Peter, & Seymour, 1996). When the gel is heated at 50–70 °C, an irreversible proteolytic degradation of myofibrillar proteins occurs, resulting in the disintegration of the gel structure named 'modori' (Alvarez, Couso, & Tejada, 1999). Modori gel is mainly due to the proteolysis caused by heat-stable proteases such as cathepsins, alkaline proteases, and calpains (An et al., 1996). Although some proteases are leached out during washing process, some proteases, especially those bound with myofibrillar proteins are retained and play an important role in degradation of myofibrillar proteins, leading to gel weakening.

To alleviate the problems associated with protein degradation caused by the endogenous proteases, several protein additives have been used in surimi to improve the properties of surimi gels. Whey protein concentrate (WPC), chicken plasma protein (CPP), beef plasma protein (BPP), porcine plasma protein (PPP), and egg white (EW) can be used as food grade protease inhibitors in surimi (Rawdkuen & Benjakul, 2008; Rawdkuen, Benjakul, Visessanguan, & Lanier, 2007). These inhibitors act competitively or non-competitively against endogenous cysteine and serine proteases, which were responsible for hydrolysis of myofibrillar proteins in surimi (Rawdkuen et al., 2007; Weerasinghe, Morrissey, & An, 1996). BPP has shown as the most effective inhibitor towards several proteases via 'trap mechanism' (Weerasinghe et al., 1996). However, the use of BPP and CPP has been forbidden, because of bovine spongiform encephalopathy (BSE) and outbreak of avian influenza (AI), respectively. Egg white is expensive and has an undesirable egg-like odour, whilst blood plasma is associated with off-colour and off-flavour of resulting surimi gel. Plant protein isolate, particularly soy protein isolate, has been used in surimi industry owing to its safety and reasonable price (Luo, Kuwahara, Kaneniwa, Murata, & Yokoyama, 2004).

Due to a variety of legume seeds in Thailand, those seeds can be used for production of protein isolates, which can be used as an alternative protein additive for surimi gel improvement. It has been

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http://dx.doi.org/10.1016/j.lwt.2012.08.018

reported that several legume seed extracts contained trypsin inhibitors and were able to inhibit proteases of fish muscle and surimi (Benjakul, Karoon, & Suwanno, 1999; Benjakul, Visessanguan, & Thummaratwasik, 2000). Luo et al. (2004) stated that vicilin and legumin, two major legume seed storage proteins, acted as co-gelling agents or binders in surimi gels. Protein isolates from selected legume seeds can be a source of protease inhibitors which could lower modori phenomenon in surimi, and/or act as binder or filler, thereby improving gel property. The purpose of this study was to investigate the preventive effects of protein isolate from black bean and mungbean seeds on the hydrolysis of myofibrillar protein by endogenous proteases in surimi made from sardine (*S. albella*) and to study the associated effects on surimi gel improvement.

2. Materials and methods

2.1. Chemicals and surimi

N-α-Benzoyl-DL-arginine-*p*-nitroanilide (BAPNA), trypsin from bovine pancreas (BAEE 10,200 units/mg), β-mercaptoethanol (β-ME) and wide range molecular weight protein markers were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Sodium dodecyl sulphate (SDS), Coomassie Blue R-250, *N*,*N*,*N*′,*N*′-tetramethyl ethylene diamine (TEMED) and all chemicals for electrophoresis were procured from Bio-Rad Laboratories (Hercules, CA, USA).

Frozen surimi grade A from sardine (*S. albella*) was obtained from Pacific Fish Processing Co., Ltd. (Songkhla, Thailand) and kept at −20 °C until use, but not longer than one month.

2.2. Preparation of protein isolates from black bean and mungbean

Black bean (*Phaseolus vulgaris* L.) and mungbean (*Phaseolus aureus*) were purchased from Thai Cereals World Co., Ltd. (Bangkok, Thailand). Black bean and mungbean protein isolates were prepared according to the methods of Hoque, Benjakul, and Prodpran (2011) with a slight modification. Black bean and mungbean powder was suspended in 10 volumes of 2 g L^{-1} NaOH solution (pH 12). The mixture was stirred continuously for 2 h at room temperature (28–30 °C), followed by centrifugation at 8000 × *g* for 30 min at 25 °C (Avanti J-E centrifuge, Beckman Coulter, Inc., Fullerton, CA, USA). The supernatant was collected and pH was adjusted to 4.5 using 6 N HCl. The precipitate formed was recovered by centrifugation at 8000 × *g* for 30 min. The pellet was washed with 10 volumes of distilled water (pH 4.5), followed by centrifugation at 8000 × *g* for 30 min. The resulting pellet was freeze-dried. Dried powder obtained was referred to as black bean protein isolate (BBPI) and mungbean proteins isolate (MBPI). Both BBPI and MBPI were placed in polyethylene bag and stored at −40 °C until use. BBPI and MBPI had the protein content of 88.73 and 87.80%, respectively. L^*, a^* and b^* values of BBPI and MBPI were 66.28 ± 0.30, 4.11 ± 0.11 and 21.13 ± 0.06, and 74.20 ± 0.26, 4.85 ± 0.09 and 30.51 ± 0.17, respectively.

2.3. Trypsin inhibitory activity assay

Trypsin inhibitory activity of BBPI and MBPI was measured by the method of Benjakul et al. (2000). Activity of trypsin in presence or absence of inhibitor solution was determined by measuring the absorbance at 410 nm due to *p*-nitroaniline released. One unit of proteolytic activity was defined as an increase of 0.01 absorbance unit ml^{-1} min^{-1} under the assay condition. One unit of trypsin inhibitory activity (TIU) was defined as the amount of inhibitor, which reduced trypsin activity by one unit.

2.4. Autolysis study of sardine surimi

Defrosted surimi (3 g) with the physiological pH of approximately 7.0 was incubated at different temperatures (45, 50, 55, 60, 65, 70, 75 and 80 °C) in a temperature-controlled water bath (Memmert, Schwabach, Germany) for 60 min. The autolytic reaction was terminated by addition of 27 ml of cold 50 g L^{-1} trichloroacetic acid. The mixture was homogenised for 2 min at 11,000 rpm (IKA homogeniser, Labortechnik, Selangor, Malaysia). The homogenate was centrifuged at 8000 × *g* for 10 min using a microcentrifuge (Model MIKRO20, Hettich ZENTRIFUGEN, Tuttlingen, Germany). TCA-soluble peptide content in the supernatant was determined by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) using L-tyrosine as a standard and was expressed as μmole tyrosine g^{-1} sample.

To determine the autolytic patterns of sardine surimi, another set (3 g) of samples incubated at different temperatures for 60 min was mixed with 27 ml of 50 g L^{-1} SDS (85 °C) to terminate the autolytic reaction. The mixture was then homogenised at 11,000 rpm for 1 min. The homogenate was then incubated at 85 °C for 60 min to solubilise the sample. To remove undissolved debris, the mixture was centrifuged at 8000 × *g* for 10 min. The supernatant was subjected to SDS-PAGE analysis.

2.5. Effect of BBPI and MBPI on gel properties of sardine surimi

Frozen surimi was partially thawed at 4 °C for 2–3 h, cut into small pieces with an approximate thickness of 1 cm and then placed in a mixer (National Model MKK77, Tokyo, Japan). The mixture was chopped for 1 min, followed by addition of NaCl at a level 2.5 g 100 g^{-1}. The final moisture content of mixture was adjusted to 80 g 100 g^{-1} by adding iced water. BBPI and MBPI, containing trypsin inhibitors at level of 5916 ± 119 and 5504 ± 153 units g^{-1}, respectively, at different final concentrations (0, 0.25, 0.5, 1 and 1.5 g 100 g^{-1}), were added. Chopping was continued for additional 3 min. Temperature was maintained at below 7 °C during chopping. The sol was stuffed into a polyvinylidene chloride casing with a diameter of 2.5 cm and both ends were sealed tightly. Kamaboko and modori gels were prepared by incubating the sol at 40 and 65 °C for 30 min, followed by heating at 90 °C for 20 min, respectively. Subsequently, all gels were cooled in iced water for 30 min and stored at 4 °C overnight prior to analyses.

2.6. Analyses

2.6.1. Textural analysis

Textural analysis of surimi gels was carried out using a texture analyser (Model TA-XT2, Stable Micro Systems, Surrey, UK). Three cylindrical samples (2.5 cm in length) were prepared and subjected to determination. Breaking force and deformation were measured using the texture analyser equipped with a spherical plunger (diameter 5 mm; depression speed 60 mm min^{-1}).

2.6.2. Determination of whiteness

All gels were subjected to whiteness measurement using a colorimeter (HunterLab, ColorFlex, Hunter Associates Laboratory, Reston, VA). Illuminant C was used as the light source of measurement. L^*, a^* and b^* values were measured and whiteness was calculated using the following equation (Park, 1994):

$$\text{Whiteness} = 100 - \left[\left(100 - L^*\right)^2 + a^{*2} + b^{*2}\right]^{1/2}$$