LWT - Food Science and Technology 65 (2016) 122–127

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Trypsin inhibitor from yellowfin tuna (*Thunnus albacores*) roe: Effects on gel properties of surimi from bigeye snapper (*Priacanthus macracanthus*)

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ARTICLE INFO

Article history:
Received 26 June 2015
Received in revised form
22 July 2015
Accepted 30 July 2015
Available online 4 August 2015

Keywords:
Tuna
Surimi
Trypsin inhibitor
Gel properties
Proteinases

ABSTRACT

Effects of partially purified trypsin inhibitor from the roe of yellowfin tuna (*Thunnus albacores*) (TIYTR) at different levels (0–3.0 g/100 g) on gelling properties of bigeye snapper (*Priacanthus macracanthus*) surimi were investigated. TIYTR showed inhibitory activity against proteolysis in kamaboko (40/90 °C) and modori (60/90 °C) gels in a concentration-dependent manner. Myosin heavy chain (MHC) was more retained in both gels when the level of TIYTR increased up to 3.0 g/100 g. This was associated with the increased breaking force and deformation as well as lowered protein 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 TIYTR levels ($p < 0.05$). However, water-holding capacity of both gels was improved as TIYTR level increased ($p < 0.05$). Incorporation of TIYTR, beef plasma protein (BPP) and egg white (EW) at a level of 3.0 g/100 g resulted in the increased breaking force and deformation of surimi gels. Nevertheless, TIYTR and BPP showed the higher gel strengthening effect than EW. Therefore, TIYTR could be used as an alternative cheap proteinase inhibitor to improve gel strength of surimi.

1. Introduction

Gel-forming ability of myofibrillar proteins is the most important functional requirement to provide the superior quality of surimi-based products, which can be affected by both intrinsic and extrinsic factors. The rapid and severe breakdown of myofibrillar proteins, particularly myosin, at higher temperature inhibits the development of three dimensional gel network. This leads to gel weakening of surimi-based products (Kudre & Benjakul, 2013). Gel weakening phenomenon or "modori" is a major concern in surimi gel manufacture and is induced by endogenous heat-activated proteinases, which are able to degrade myofibrillar proteins (Benjakul, Visessanguan, & Thummaratwasik, 2000). Gel softening varies with species but is generally caused by serine and cysteine proteinases (Rawdkuen & Benjakul, 2008).

Thailand is one of the most important surimi producing countries in Southeast Asia. Bigeye snapper has become more economically important as a raw material for surimi production due to its white color and large availability. Also, it is not consumed directly due to its appearance and thick skin. Bigeye snapper caught in Thailand normally includes two species, *Priacanthus tayeuns* and *Priacanthus macracanthus*. *P. macracanthus* has a much poorer gel quality compared to *P. tayeuns* (Benjakul, Visessanguan, & Leelapongwattana, 2003). Thus, *P. macracanthus* surimi is considered to be of low value because of its poor gel-forming ability. *P. macracanthus* surimi typically undergoes textural degradation by endogenous proteinases, especially trypsin-like serine proteinases (Benjakul et al., 2003). To improve gel quality of bigeye snapper surimi, some means of inhibiting proteolytic activity must be

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http://dx.doi.org/10.1016/j.lwt.2015.07.074
0023-6438/

sought. Food-grade proteinase inhibitors, commonly used in surimi, include beef plasma (BPP), egg white, potato powder and whey protein concentrate (Morrissey, Wu, Lin, & An, 1993; Yongsawatdigul & Piyadhammaviboon, 2004). Food-grade proteinases inhibitors have been used to protect myofibrillar proteins from proteolysis caused by indigenous proteinases. However, the application of BPP in surimi has been prohibited by the outbreak of bovine spongiform encephalopathy or mad cow disease. Additionally, some BPP preparations result in off-flavors and off-color. Egg white is high cost and has an undesirable egg-like odor, white off-color problems may be encountered when potato powder is used (Rawdkuen & Benjakul, 2008). Hence, alternative food-grade proteinase inhibitors for surimi production are still needed.

The roe of tuna is an abundant by-product from the commercial processing of canned tuna and a large amount of roe is produced each year. The roe is normally sold and used as animal feed. Based on our previous study, yellowfin tuna roe contained high trypsin inhibitory activity (Klomklao, Benjakul, & Kishimura, 2014). Recently, Klomklao, Benjakul, and Simpson (2015) reported that partially purified trypsin inhibitor from yellowfin tuna roe (TIYTR) inhibited sarcoplasmic proteinases and autolysis of bigeye snapper mince and its washed mince at 60 °C in a concentration-dependent manner. Therefore, the addition of TIYTR possessing the trypsin inhibitory activity should pave the way for gel improvement of bigeye snapper surimi. The objective of this study was to investigate the preventive effects of TIYTR on gelling properties of bigeye snapper (*P. macracanthus*) surimi.

2. Materials and methods

2.1. Chemicals

*N*α-Benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (BAPNA), trypsin from porcine pancreas, β-mercaptoethanol (βME), sodium chloride, trichloroacetic acid, tris (hydroxymethyl) aminomethane, dimethylsulfoxide, sodium caseinate, ammonium sulfate, sodium dodecyl sulfate (SDS), Coomassie Blue R-250 and *N*,*N*,*N*′,*N*′-tetramethyl ethylene diamine (TEMED) and bovine serum albumin were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Beef plasma protein (BPP) and EW powder were obtained from Food EQ Co., Ltd. (Bangkok, Thailand).

2.2. Preparation of partially purified trypsin inhibitor from yellowfin tuna roe (TIYTR)

The roes of yellowfin tuna (*Thunnus albacores*), with a size of 51.8 ± 2.4 g/roe, were obtained from Tropical Canning (Thailand) Public Co. Ltd., Songkhla, Thailand. The samples (5 kg) were placed in ice using a roe:ice ratio of 1:2 (g:g) and transported to the Department of Food Science and Technology at the Thaksin University (Phatthalung, Thailand) within 2 h of roe removal. The whole roes were cleaned using cold water (4 °C), vacuum-packed and immediately frozen at −20 °C until needed for use.

Frozen roes were thawed using running tap water (26–28 °C) until the core temperature reaches −2 to 0 °C. The samples were cut into pieces with thickness ranging from 1 to 1.5 cm and homogenized with three volumes of cold acetone at −20 °C for 30 min according to the method of Klomklao, Benjakul, and Kishimura (2010). The homogenate was filtered under vacuum through Whatman No. 4 filter paper. The residue obtained was then homogenized with two volumes of cold acetone (−20 °C) for 30 min, and then the residue was collected by filtering under vacuum through Whatman No. 4 filter paper. The defatted roe was air-dried at room temperature (28–30 °C) until dry and free of acetone odor.

To prepare the extract, roe powder was mixed with 10 mmol/L Na-phosphate buffer (pH 7.0) containing 0.5 mol/L NaCl at a ratio of 1:9 (g:mL) and shaken (BW 201 Shaking bath, Tokyo, Japan) for 30 min at 150 rpm at room temperature (28–30 °C). The roe extract was recovered by centrifuging at 10,000 × g for 30 min at 4 °C.

Next, the roe extract was partially purified by heat-treatment at 60 °C for 10 min (Klomklao et al., 2015) and then cooled with ice water. This treatment produced coagulated debris which was subsequently removed by centrifugation at 10,000 × g for 10 min at 4 °C. The supernatant was freeze-dried using a freeze-dryer (CoolSafe 55, ScanLaf A/S, Lynge, Denmark). The dried powder obtained (TIYTR) was placed in polyethylene bag and kept at 4 °C until used.

2.3. Trypsin inhibitory activity assay

Trypsin inhibitory activity was measured by the method of Klomklao, Benjakul, Kishimura, and Chaijan (2011) using BAPNA as substrate. A solution containing 200 μL of inhibitor solution and 200 μL (2 g/L) porcine pancreas trypsin was preincubated at 37 °C for 15 min. Then, 1 mL of the mixtures containing 800 μL of 0.5 mmol/L BAPNA and 200 μL of distilled water (prewarmed to 37 °C) was added and vortexed immediately to start the reaction. After incubating for 10 min, 900 μL of 300 mL/L acetic acid was added to terminate the reaction. The reaction mixture was centrifuged at 8000 × g for 5 min (Eppendorf Micro Centrifuge). Residual activity of trypsin was measured by the absorbance at 410 nm due to *p*-nitroaniline released. One unit of trypsin activity was defined as an increase of 0.01 absorbance unit/ml min under the assay condition. One unit of trypsin inhibitory activity was defined as the amount of inhibitor, which reduces the enzyme activity by one unit.

2.4. Bigeye snapper preparation

Bigeye snapper (*P. macracanthus*) were caught from the Songkhla Coast along the Gulf of Thailand, stored in ice and off-loaded approximately 24–36 h after capture. Fish were transported in ice to the Department of Food Science and Technology, Thaksin University, Phatthalung within 2 h. Fish were then filleted and kept in ice until used for surimi gel preparation.

2.5. Effect of TIYTR on gel properties of bigeye snapper surimi

2.5.1. Surimi gel preparation

Surimi was prepared according to the method of Benjakul, Visessanguan, Tueksuban, and Tanaka (2004). Fresh bigeye snapper were washed with tap water. The flesh was removed manually and minced into the uniformity. The mince was then washed with cold water (5 °C) at a mince/water ratio of 1:2 (g:g). The mixture was stirred gently for 3 min and washed process was repeated twice. Finally the washed mince was subjected to centrifugation using a Model CE 21 K basket centrifuge (Gradniumpiant, Belluno, Italy) with a speed of 700 × g for 15 min. The washed mince referred to as 'surimi' was kept in ice until used.

2.5.2. Effect of TIYTR on gel-forming ability of bigeye snapper surimi

Surimi prepared was added with 2.5 g/100 g salt and TIYTR was added at level of 0, 0.5, 1, 2 and 3 g/100 g. The moisture content was then adjusted to 80 g/100 g. The mixture was chopped for 5 min at 4 °C to obtain the homogenous sol. The sol was then stuffed into polyvinylidine casing with a diameter of 2.5 cm and both ends of casing were sealed tightly. Kamaboko and modori gels were prepared by incubating the sol at 40 and 60 °C for 30 min, respectively, followed by heating at 90 °C for 20 min in a water bath (Memmert, Schwabach, Germany). After heating, all gels were immediately cooled in iced water for 30 min and stored at 4 °C overnight prior to