



Combined effect of squid ink tyrosinase and tannic acid on heat induced aggregation of natural actomyosin from sardine



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ABSTRACT

The mixture of squid ink tyrosinase (SIT) at 300 and 500 U/g protein and tannic acid (TA) at 0.5 and 1% (based on protein) increased the turbidity and surface hydrophobicity of natural actomyosin (NAM) from sardine (*Sardinella albella*) in a dose dependent manner. Total sulphhydryl group content decreased in the NAM solutions with coincidental increase in disulphide bond content, when added with SIT/TA mixture. Ca^{2+} -ATPase activity was also lower in NAM solutions added with SIT/TA mixture. Higher aggregation of protein filaments was noticeable in NAM added with SIT at 500 U/g protein and 1% TA, which also had the highest storage modulus (G') and largest particle size. Negative charge of NAM was decreased when SIT/TA mixture was incorporated. Therefore the mixture of SIT and TA, particularly at higher level, could be used as the novel protein cross-linker in fish mince and surimi.

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1. Introduction

Myofibrillar proteins including myosin and actin, found as actomyosin complex, play an important role in gelation of fish mince and surimi (Niwa, 1992). Gelation is the main parameter determining the quality of surimi. Thermal gelation of fish muscle proteins has been reported to occur in a three-step process including (1) dissociation of myofibril structures by protein solubilisation in the presence of salt; (2) partial unfolding of protein structure induced by heat treatment; and (3) aggregation of unfolded protein via both covalent and non-covalent bonds to form a three-dimensional network (Stone & Stanley, 1992). The conformational change of actomyosin caused by heat correlates with the exposure of functional groups such as sulphhydryl groups and hydrophobic domains (Benjakul, Visessanguan, Ishizaki, & Tanaka, 2001). Subsequently, those groups most likely undergo disulphide bond formation and hydrophobic interaction, respectively. Additionally, gelation is dependent upon temperature (Sano, Ohno, Otsuka-Fuchino, Matsumoto, & Tsuchiya, 1994), heating rate (Yongsawatdigul & Park, 1999), pH and type of actomyosin (Lefevre, Fauconneau, Thompson, & Gill, 2007). Generally lean fish are used for surimi production due to their high gelation and whiteness.

Because of their overexploitation, dark fleshed fish have been used as an alternative source. The dark fleshed mince has high contents of lipid, myoglobin as well as proteases and generally exhibits poor gel forming ability (Chaijan, Benjakul, Visessanguan, & Faustman, 2004). Therefore, various food-grade additives such as microbial transglutaminase (MTGase), bovine plasma protein, porcine plasma protein and egg white have been used to improve the properties of surimi from dark fleshed fish (Benjakul, Phatcharat, Tammatinna, Visessanguan, & Kishimura, 2008; Benjakul, Visessanguan, & Chantarasuwan, 2004; Benjakul, Visessanguan, & Kwalumtharn et al., 2004). However, the use of cross-linking enzymes such as MTGase in surimi may not be economical because of its high cost and other protein additives have been prohibited due to safety concern. Hence there is a need for novel, cheap and effective additives, which are capable of improving the properties of fish mince and surimi.

Tannin belongs to the polyphenol group abundantly found in plants. Tannic acid (TA) is a specific commercial form of tannin consisting of a central carbohydrate (glucose) and 10 galloyl groups (Lopes, Schulman, & Hermes-Lima, 1999). Tannin contains sufficient hydroxyls and other groups such as carboxyls to form strong complexes with the proteins and other macromolecules (Kroll, Rawel, & Rohn, 2003). TA can be oxidised to corresponding quinones by enzymatic and non-enzymatic reaction (Balange & Benjakul, 2010; Hurrell & Finot, 1984). The quinones are extremely reactive and can further react with various amino acid

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side chains such as sulphhydryls, amines, amides, indoles, and tyrosines commonly present in proteins, resulting in the formation of inter and intramolecular crosslinks (Bittner, 2006; Burzio & Waite, 2000; Ito, Kato, Shinpo, & Fujita, 1984; Mattinen, Lantto, Selinheimo, Kruus, & Buchert, 2008).

Polyphenol oxidases (PPOs) are copper containing enzymes, which are found in mammals, plants, fungi, and other microorganisms. Tyrosinase, belonging to the group of PPOs, is a bifunctional enzyme as it catalyses ortho-hydroxylation of monophenols (monophenolase or cresolase activity) and subsequent oxidation of diphenols (diphenolase or catecholase activity) to quinones. Tyrosinase can oxidise tyrosine in the side chain of proteins to the quinone, which can further crosslink with lysyl, tyrosyl, and cysteinyl residues of proteins (Selinheimo, Lampila, Mattinen, & Buchert, 2008). Tyrosinase also reacts on various monophenolic and diphenolic small compounds, such as phenol and catechol or phloretic acid and hydrocaffeic acid (Buchert et al., 2010). Recently, squid ink tyrosinase (SIT) has been extracted from squid melanin-free ink (Vate & Benjakul, 2015). This tyrosinase along with some polyphenols, which act as substrates for the enzyme, can be used as the protein cross-linker in gelly food products. Therefore the objectives of this study were to elucidate the combined impact of SIT and TA on the heat induced aggregation and to investigate physicochemical changes of natural actomyosin from sardine (*Sardinella albella*) during heating at various temperatures.

2. Materials and methods

2.1. Chemicals

Adenosine-5'-triphosphate (ATP), 8-anilino-1-naphthalenesulphonic acid (ANS), guanidine thiocyanate, sodium hydrogen sulphite, β -mercaptoethanol (β -ME) and Tris-maleate were obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Potassium chloride, sodium chloride, calcium chloride, trichloroacetic acid, potassium dihydrogen phosphate and ammonium molybdate were procured from Merck (Darmstadt, Germany). 5,5-Dithiobis (2-nitrobenzoic acid) (DTNB) was purchased from Wako Pure Chemical Industries (Tokyo, Japan). Bovine serum albumin (BSA) was obtained from Fluka (Buchs, Switzerland).

2.2. Collection and preparation of fish

Sardines (*S. albella*) with an average weight of 50–60 g were caught from Songkhla coast along the Gulf of Thailand. The fish, off-loaded approximately 12 h after capture, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 1 h. The fish were headed, gutted and washed with water. The flesh was separated manually from skin and bone and kept on ice not longer than 24 h.

2.3. Preparation of natural actomyosin (NAM)

NAM was prepared according to the method of Balange and Benjakul (2010) with a slight modification. Sardine mince was homogenised in chilled 0.6 M KCl, pH 7.0 at a ratio of 1:10 (w/v) using a homogeniser (IKA, Labortechnik, Selangor, Malaysia). To avoid overheating, the sample was placed in ice and homogenised for 20 s, followed by a 20 s rest interval for a total extraction time of 4 min. The homogenate was centrifuged at 5000 g for 30 min at 4 °C using a refrigerated centrifuge (Avanti J-E Centrifuge; Beckman Coulter, Fullerton, CA, USA). To the supernatant, three volumes of chilled deionised water (0–2 °C) were added to precipitate NAM. The NAM was collected by centrifuging at 5000 g for 20 min at 4 °C.

The NAM pellet was dissolved in chilled 0.6 M KCl, pH 7.0 for 30 min at 4 °C and then centrifuged at 5000 g for 20 min at 4 °C. The supernatant was collected and used as NAM.

2.4. Preparation of squid ink tyrosinase

2.4.1. Preparation of melanin-free ink

Melanin-free ink was prepared according to the method of Vate and Benjakul (2013). Squids were purchased from a local market in Hat Yai, Thailand, stored in ice using a squid/ice ratio of 1:2 (w/w), and transported to the Department of Food Technology, Prince of Songkla University, Thailand. Upon arrival, ink sac was separated from the squid by cutting the ink duct and ink was squeezed out from the ink sac. The squid ink was diluted ten-fold using the cold deionised water (2–4 °C). Then it was subjected to centrifugation at 18,000 \times g for 30 min at 4 °C to remove the melanin using a refrigerated centrifuge (Allegra 25 R centrifuge, Beckman Coulter, Palo Alto, CA, USA). The supernatant obtained was used as melanin-free ink (MFI).

2.4.2. Fractionation of tyrosinase

Tyrosinase from MFI was fractionated as per the method of Simpson, Marshall, and Otwell (1987) with a slight modification. MFI (50 mL) was mixed with 50 mL of 0.05 M sodium phosphate buffer (pH 7.2), containing 1.0 M NaCl and 0.2% Brij 35. The mixture was stirred continuously at 4 °C for 30 min. Solid ammonium sulphate was added into the mixture to obtain 60% saturation. The mixture was allowed to stand at 4 °C for 30 min. The precipitate was collected by centrifugation at 12,500 \times g at 4 °C for 30 min. The pellet obtained was dissolved in a minimum volume of 0.05 mM sodium phosphate buffer (pH 7.2) and dialysed with 15 volumes of the same buffer with three changes overnight. The fraction containing tyrosinase referred to as 'squid ink tyrosinase, SIT' was kept at –40 °C until used.

2.4.3. Measurement of tyrosinase activity

Tyrosinase activity was assayed using l-DOPA (3,4-Dihydroxy-L-phenylalanine) as a substrate according to the method of Simpson et al. (1987) with a slight modification. Reaction mixtures consisted of 600 μ L of 15 mM l-DOPA in deionised water, 400 μ L of 0.05 M phosphate buffer (pH 6.0) and 100 μ L of deionised water. To initiate the reaction, 100 μ L of SIT was added and the reaction was run for 3 min at room temperature. The formation of dopachrome was monitored by reading at 475 nm using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan). One unit of activity was defined as the enzyme causing an increase in the absorbance at 475 nm by 0.001/min. Enzyme and substrate blanks were prepared by excluding the substrate and enzyme, respectively, from the reaction mixture and deionised water was used instead.

2.5. Study on the effect of SIT/TA mixture at different concentrations on heat-induced aggregation of NAM

2.5.1. Preparation of SIT/TA mixtures

TA solution (2% w/v) was firstly prepared in deionised water and pH was adjusted to 7 using 1 M NaOH. TA solution was mixed with SIT (2500 U/mL) to obtain the different TA (0.5 and 1%) and SIT (300 and 500 U) as the working concentrations in NAM.

2.5.2. Effect of heat treatment on aggregation and physicochemical changes of NAM added with SIT/TA mixtures

NAM was diluted to 1 mg/mL with chilled 0.6 M KCl (pH 7.0). NAM solutions added with the prepared SIT/TA mixtures to obtain various TA (0.5% and 1% based on protein) and SIT (300 and 500 U/g protein) levels, were heated at a heating rate of 0.65 °C/min from 20

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