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Effect of bambara groundnut protein isolate on autolysis and gel properties of surimi from threadfin bream (*Nemipterus bleekeri*)

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ABSTRACT

Impact of bambara groundnut protein isolate (BGPI) on autolysis and gel properties of surimi from threadfin bream (*Nemipterus bleekeri*) was investigated. BGPI with trypsin inhibitory activity of 6356.3 ± 6.02 unit/g markedly increased ($P < 0.05$) the breaking force and deformation of modori gel as the levels used (0–3 g/100 g) increased. Nevertheless, only 0.25 g/100 g BGPI increased breaking force and deformation of kamaboko gel and increasing BGPI levels showed detrimental effect on gelation. Myosin heavy chain (MHC) was more retained when BGPI concentration increased, especially in modori gel. However, whiteness slightly decreased ($P < 0.05$) with increasing BGPI levels. Microstructure of kamaboko gel added with 0.25 g/100 g BGPI had finer and more ordered fibrillar structure than that without BGPI addition. Therefore, BGPI at an appropriate level could be an alternative food-grade inhibitor to improve gel properties of surimi.

1. Introduction

Proteolysis induced by heat-activated and heat-stable indigenous proteases bound with muscle fibres has been known to be associated with the substantial decrease in gel strength of surimi. This phenomenon known as “modori” is the most detrimental factor, causing gel weakening with the loss in market value (Morrissey, Wu, Lin, & An, 1993). The presence of indigenous proteases in fish mince or surimi results in a decreased gel strength, especially at temperatures close to 50–60 °C (Alvarez, Couso, & Tejada, 1999). Heat-activated proteases associated with fish muscle can be classified as sarcoplasmic and myofibril associated proteases based on extractability (Kinoshita, Toyohara, & Shimizu, 1990). Sarcoplamic proteases can be removed to some extent during washing process, while myofibril associated proteases still remain in surimi, resulting in gel weakening (Benjakul, Seymour, Morrissey, & An, 1996). Myofibril associated serine protease in carp muscle showed the highest autolytic activity at 55 °C (Osatomi, Sasai, & Cao, 1997). Myofibril associated serine protease in lizardfish (*Saurida wanieso*) with a molecular weight of 60 kDa was able to hydrolyze myosin heavy chain at 55–60 °C (Cao et al., 1999).

Threadfin bream has been widely used in Thailand and other countries in the Southeast Asia as an important raw material for surimi production. However, the surimi from this species is susceptible to modori due to the presence of heat-activated proteases, which were active at 50–60 °C (Kinoshita et al., 1990). To minimize the weakening of surimi gels, food-grade inhibitors have been used to protect myofibrillar proteins from proteolysis caused by indigenous proteases. Those include beef plasma protein (BPP), egg white and whey protein concentrate (Chang-Lee, Lampila, & Crawford, 1990; Hamann, Amato, Wu, & Jones, 1990; Morrissey et al., 1993; Weerasinghe, Morrissey, & An, 1996). Additionally, soy protein isolate has been used in surimi gel as non-muscle protein additive, owing to its safety and reasonable price (Luo, Shen, & Pan, 2006). Legume seeds are rich in protease inhibitors (Liener & Kakade, 1980), which can be used to prevent “modori” in surimi. Bambara groundnut is an indigenous legume in the southern part of Thailand. Production of protein isolate from this legume is able to increase its value and can be used as protein additive in surimi. The purpose of this study was to investigate the preventive effects of protein isolate from bambara groundnut on autolysis of surimi from threadfin bream and to study its impact on gel properties of surimi.

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2. Materials and methods

2.1. Chemicals and surimi

Sodium chloride and trichloroacetic acid were purchased from Merck (Darmstadt, Germany). Sodium dodecyl sulphate (SDS), *N,N,N′,N′*-tetramethyl ethylene diamine (TEMED) and Coomassie Blue R-250 were procured from Bio-Rad Laboratories (Hercules, CA, USA). *N*-α-Benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) and trypsin from bovine pancreas (BAEE 10,200 units/mg) were obtained from Sigma. Frozen surimi grade A from threadfin bream (*Nemipterus bleekeri*) was purchased from Man A Frozen Foods Co., Ltd. (Songkhla, Thailand) and kept at −20 °C until use. The storage time was not more than 2 months.

2.2. Preparation of bambara groundnut protein isolate

Mature bambara groundnut (*Vigna subterranean*) with the moisture content of 1.67 g/100 g was obtained from a market, Hat Yai, Songkhla, Thailand. The sample (1 kg) was dehulled and ground using a blender (Moulinex, Type AY46, Shenzhen, Guangdong, China) to obtain about 420 g fine powder. Bambara groundnut protein isolate (BGPI) was prepared according to the methods of Pastor-Cavada, Juan, Pastor, Alaiz, and Vioque (2009) with a slight modification. Bambara groundnut powder was suspended in 10 volumes of 2 g/L 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 using a Beckman Model Avanti J-E centrifuge (Beckman Coulter, Inc., Fullerton, CA, USA). The supernatant was collected and pH was adjusted to 4.5 using 6 mol equi/L HCl. The precipitate formed was recovered by centrifugation at 8000×*g* for 30 min at 25 °C. 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 'Bambara groundnut protein isolate, BGPI'. BGPI was placed in polyethylene bag and stored at 4 °C until use.

2.3. Trypsin inhibitory activity assay

Trypsin inhibitory activity of BGPI was measured by the method of Benjakul, Visessanguan, and Thummaratwasik (2000). A solution containing 200 μL of inhibitor solution (2 g/L), 200 μL of bovine pancreas trypsin (1 g/L) and 1000 μL of 50 mmol/L Tris–HCl, pH 7 containing 10 mmol/L $CaCl_2$ was pre-incubated at 37 °C for 15 min. To initiate the reaction, 200 μL of BAPNA (0.4 g/L in DMSO) (pre-warmed to 37 °C) were added and vortexed immediately to start the reaction. After incubating for 10 min, 200 μL of 300 mL/L acetic acid was added to terminate the reaction. The reaction mixture was centrifuged at 8000×*g* for 5 min. Residual activity of trypsin was determined by measuring the absorbance at 410 nm. One unit of trypsin 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 threadfin bream surimi

Surimi (3 g) with the 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 trichloroacetic acid. The mixture was homogenised for 1 min at 11,000 rpm using an IKA homogeniser (IKA Labortechnik, Selangor, Malaysia). The homogenate was centrifuged at 7500×*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 μmol tyrosine/g sample.

To determine the autolytic pattern, another set of samples was terminated with 27 mL of 50 g/L SDS (85 °C) after incubation for 60 min. 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 7500×*g* for 10 min. The supernatant was subjected to SDS-PAGE analysis.

2.5. Effect of BGPI on gel properties of threadfin bream surimi

Frozen surimi was tempered in running water (25 °C) until the core temperature reached 0–2 °C. The surimi was then cut into small pieces with an approximate thickness of 1 cm. The surimi was placed in a mixer (National Model MKK77, Tokyo, Japan). The moisture was adjusted to 80 g/100 g and NaCl at a level of 2.5 g/100 g was added. BGPI was added to obtain the final concentrations of 0, 0.25, 0.5, 1, 2 and 3 g/100 g. The mixture was chopped for 4 min at 4 °C to obtain a homogenous sol. Air bubbles in the sol were removed manually by spreading the sol into the thin layer using a spatula. The sol was then stuffed into a polyvinylidene chloride casing with a diameter of 2.5 cm and both ends were sealed tightly. During stuffing, the generation of air bubble was also avoided. Kamaboko gels were prepared by incubating the sol at 40 °C for 30 min, followed by heating at 90 °C for 20 min. To prepare modori gels, the sol was incubated at 65 °C for 30 min, followed by heating at 90 °C for 20 min. All gels were cooled in iced water suddenly. Prepared gels were stored for 24 h at 4 °C before analyses.

3. Analyses

3.1. Textural analysis

Textural analysis of all surimi gels was carried out using a model TA-XT2 texture analyser (Stable Micro System, Surrey, UK). Gels were equilibrated at room temperature (25–30 °C) for 2 h before analysis. Ten cylindrical samples (2.5 cm in length) were prepared and tested. Breaking force (strength) and deformation (cohesiveness/elasticity) were measured by the texture analyser equipped with a spherical plunger (5-mm diameter) with a cross-head speed of 60 mm/min and 60% compression.

3.2. Determination of whiteness

All gels were subjected to whiteness measurement using a HunterLab (ColorFlex, Hunter Associates Laboratory, Reston, VA, USA). Illuminant C was used as the light source of measurement. L^*, a^* and b^* values were measured. Whiteness was calculated using the following equation (NFI, 1991):

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

3.3. Determination of expressible moisture content

Expressible moisture content was measured according to the method of Benjakul, Visessanguan, and Chantarasuwan (2004). Cylindrical gel samples were cut into a thickness of 5 mm, weighed (X) and placed between two pieces of Whatman paper (No. 1) (Whatman International Ltd., Maidstone, England) at the bottom