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γ-PGA and MTGase improve the formation of ε-(γ-glutamyl) lysine cross-links within hairtail (*Trichiurus haumela*) surimi protein

Yaqin Hu[a], Ying Shao[a], Chunhua Wu[a], Chunhong Yuan[b], Gakushi Ishimura[b], Wenjuan Liu[a], Shiguo Chen[a,*]

[a] National Engineering Laboratory of Intelligent Food Technology and Equipment, Key Laboratory for Agro-Products Postharvest Handling of Ministry of Agriculture, Key Laboratory for Agro-Products Nutritional Evaluation of Ministry of Agriculture, Zhejiang Key Laboratory for Agro-Food Processing, Fuli Institute of Food Science, College of Biosystems Engineering and Food Science Zhejiang University, Hangzhou 310058, China

[b] Department of Food Production and Environmental Management, Faculty of Agriculture, Iwate University, Morioka, Iwate 020-8550, Japan

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ABSTRACT

The present study investigated the mechanism of ε-(γ-glutamyl) lysine cross-links within hairtail (*Trichiurus haumela*) surimi protein via γ-polyglutamic acid (γ-PGA) and MTGase. The results indicated that the addition of MTGase and γ-PGA markedly improved the gelation properties of hairtail surimi protein, including its maximum breaking force and deformation, water holding capacity and gel strength. The maximum improvements were achieved by adding 0.5 units MTGase/g meat paste in combination with 0.06% γ-PGA. SDS-PAGE showed that the band intensity of cross-linked proteins increased, whereas that of myosin heavy chain decreased after treatments. Further scanning electron microscopy (SEM) analysis showed the formation of a denser gel matrix, which was caused by much stronger and more inter- and intra-molecular cross-linking of proteins, via MTGase catalysing ε-(γ-glutamyl) lysine cross-links formed between lysine residues in the gel protein and glutamic residues in the hydrolytic γ-PGA. The results provide reliable guidance for the improvement of hairtail surimi protein gelation properties.

1. Introduction

Hairtail, a commercial marine fish, is very popular in Asian countries and is abundant in the eastern Pacific Ocean. It is a fish rich in protein and unsaturated fatty acids, and various fish products have been developed from it. However, the poor gelation property of hairtail muscle proteins limits its utilization (Liu, Chen, et al., 2014), particularly for making highly valued fish gels. Gelling properties play an important role in the quality of fish-meat-based gelled products, which determines their market price and consumer acceptability. Due to its poor gelling properties, hairtail is one species that has never been fully developed and utilized (Jiang, Hsieh, Ho, & Chung, 2000), resulting in a value of hairtail-meat-based products as low as that of the fish mackerel.

A number of attempts, including ultraviolet irradiation technology, improved heating treatment technology (Cheecharoen, Kijroongrojana, & Benjakul, 2011; Fu et al., 2012), and certain gel enhancers, mainly microbial transglutaminase (MTGase) (Chanarat, Benjakul, & H-Kittikun, 2012), reductase (Hsieh, Tsai, & Jiang, 2002), protease inhibitor (Hsieh et al., 2002), phenolic compounds (Balange & Benjakul, 2009), and carbohydrates (Cardoso, Mendes, Vaz-Pires, & Nunes, 2009), have been investigated for improving the gelling properties and adding value to hairtail muscle protein. However, either these methods are highly expensive, or it is too difficult to adjust and control the operating conditions. In addition, traditional food additives, such as starch (Resconi et al., 2016), calcium lactate (Choi et al., 2014), and carrageenan (Ma, 2012), despite enhancing the gelling effect, have a negative effect on other sensory parameters, which affects the acceptance of the final product. Therefore, it is necessary to develop novel food additives to improve the gelling properties and additional value of hairtail muscle proteins without additional effects on sensory properties.

γ-polyglutamic acid (γ-PGA) is an anionic homo-polyamide composed of D- and L- glutamic acid units connected by γ-amide linkages. It is synthesized via secretion into the extracellular matrix by *Bacillus subtilis* during fermentation. It is a unique polymer with various applications in pharmaceutical manufacturing; food processing; congealment prevention; the post-harvest of vegetables, fruits and marine products; the cosmetic industry; tobacco manufacturing; leather manufacturing; and plant seed protection (Li, Li, Deng, & Liang, 2008). The

* Corresponding author at: College of Biosystems Engineering and Food Science, Zhejiang University, China.
E-mail address: chenshiguo210@163.com (S. Chen).

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characterization of its biological and physicochemical properties, such as a great fibre forming property, film formation property, oxygen barrier property, plasticity, cohesiveness, as well as moisture retention and biodegradability, makes it advantageous for future development and applications (Chen & Sun, 2004; Li et al., 2008). In the food industry, γ-PGA can prevent starch retrogradation, strengthen texture, and maintain appearance. It can also be used as a stabilizer for ice cream, a bitter flavour remover, a thickener for fruit juice, or an additive for taste improvement. Furthermore, γ-PGA can increase the solubility of Ca^{2+} both inside and outside the cell and prompt intestinal absorption; therefore, γ-PGA and its derivatives can be used as nutritional additives in dietary products (Zu & Zhou, 2011). Importantly, γ-PGA is digested into single glutamic acids and is then absorbed by the stomach in the human body. Hence, as an additive, γ-PGA can improve food flavour and increase food nutrition.

MTGase is the transglutaminase (protein-glutamine γ-glutamyltransferase, EC 2.3.2.13) from microbial materials. The formation of non-disulphide covalent bonds in proteins, particularly ε-(γ-glutamyl) lysine cross-links, is catalysed by MTGase via the acyl transfer between the γ-amide groups of a glutamine residue and the ε-amino groups of a lysine residue, thus contributing to enhanced gel quality in the seafood industry (Chanarat et al., 2012).

Therefore, the present study investigates the effects of combining MTGase with γ-PGA on the gelation properties of hairtail surimi proteins. Gel properties, including breaking force and deformation, water holding capacity, whiteness, and textual profiles (chewiness, gumminess, cohesiveness, springiness, and hardness) were investigated. The mechanism of the change of hairtail muscle protein gelation properties was clarified using chemical interactions, sulfhydryl, non-disulphide covalent bonds, and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) patterns and scanning electronic microscopy (SEM) images.

2. Materials and methods

2.1. Materials

MTGase (activity of 100 units/g), γ-PGA (food grade), frozen hairtail surimi muscle (AA grade), and broad protein marker were purchased from Ajinomoto Co., Ltd. (Tokyo, Japan), Donglin Chemical Co., Ltd. (Guangzhou, China), Maruha Nichiro Co., Ltd. (Tokyo, Japan), and Bio-Rad Co., Ltd. (Hercules, USA), respectively.

2.2. Preparation of fish surimi protein gel

The frozen hairtail surimi protein, which was stored for 30 d, was thawed with tap water (approximately 20 °C). The final concentration of added NaCl and the final moisture content were maintained at 2.5 and 80%, respectively. γ-PGA (0, 0.2, 0.4, 0.6, 0.8, and 1‰) and MTGase (0, 0.1, 0.3, 0.5, 0.6, and 0.8 units/g) were added accordingly. Then, the final concentration of added starch and the final moisture content were adjusted to 10 and 80%, respectively. A mortar and pestle were used to grind the acquired paste at 4 °C for 30 min, which was then packed into a stainless-steel ring (3.0 cm in diameter, 3 cm in length). After a two-step water bath heating process (preheating at 40 °C for 90 min and then heating at 90 °C for 25 min), the gel was obtained and then cooled with iced water for 20 min (Benjakul, Visessanguan, Riebroy, Ishizaki, & Tanaka, 2002). Finally, it was stored at 4 °C for 12 h for further use.

2.3. Determination of gelation properties

2.3.1. Breaking force and deformation

The breaking force and deformation of hairtail surimi protein gels were determined according to the method described by Benjakul et al. (2002). The 4 °C gels were thawed at room temperature and then cut into 2.5-cm lengths. All samples were placed in a texture analyser (Model CT3, Brookfield, Middleboro, USA), and a spherical plunger (diameter 5 mm) was used to press the cut surface at a pre-test speed of 2 mm/s, a depression speed of 1 mm/s, and a post-test speed of 2 mm/s under the compression mode (deformation 20 mm; induction force 5 g) in a basic single test.

2.3.2. Water holding capacity

The water holding capacity (WHC) of hairtail surimi protein gels was measured based on the method described by Benjakul et al. (2002) with some modifications. A piece of gel sample (5 mm thick) cut from the central section of the cylindrical gel was weighed (m_1) and then placed between 3 layers of Whatman filter paper (lower and upper, 9 cm in diameter). Force with a 10-kg weight was applied to press the sample and papers for 2 min, and the sample was weighed (m_2) again. The WHC was expressed as the percentage of sample weight and calculated using the following Eq. (1):

$$\mathrm{WHC}(\%) = m_2/m_1 \times 100 \quad (1)$$

2.3.3. Whiteness

The whiteness of hairtail surimi protein gels was measured using a colour difference metre (Model WSC-S, Tokyo, Japan). Whiteness was calculated using the following Eq. (2):

$$\mathrm{W} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2} \quad (2)$$

where b∗ = yellowness/blueness; a∗ = redness/greenness; and L∗ = lightness.

2.3.4. Textural profile analysis

The textural profile analysis (TPA) of hairtail surimi protein gels was determined using a texture analyser (Model CT3, Brookfield, Middleboro, USA) according to the method described by Benjakul et al. (2002). A cylindrical plunger (5 mm in diameter) was perpendicularly pressed into the cutting plane of the gel specimens (2.5 cm in length) at a 1 mm/s constant depression speed in a two-cycle test. Textural parameters, including chewiness, gumminess, cohesiveness, springiness, and hardness, were measured eight times.

2.4. Mechanism of promoting effects on gelation properties

2.4.1. Determination of chemical interactions

Chemical interactions of hairtail surimi protein gels were determined according to the method of Gómez-Guillén, Borderías, and Montero (1997). Samples of 2 g were incubated with 10 mL of 0.05 mol/L NaCl (SA), 0.6 mol/L NaCl (SB), 0.6 mol/L NaCl + 1.5 mol/L urea (SC) and 0.6 mol/L NaCl + 8 mol/L urea (SD) in 20 mmol/L Tris-HCl buffer solution (pH 8.0), respectively. After incubation at 4-5 °C with agitation for 1 h, the mixture was centrifuged at 10,000*g* for 15 min. The protein concentration of the supernatants of the SA, SB, SC, and SD solutions was measured using the biuret method. Chemical interactions were expressed as g protein/L solutions and calculated from the following Eqs. (4)–(7):

$$\text{Nonspecfic bonds} = c(SA) \quad (4)$$

$$\text{Ionic bonds} = c(SB - SA) \quad (5)$$

$$\text{Hydrogen bonds} = c(SC - SB) \quad (6)$$

$$\text{Hydrophobic interactions} = c(SD - SC) \quad (7)$$

where c(SA) is the protein concentration of SA solution; c(SB-SA) is the difference in the value of the protein concentration of the SB and SA solutions. Additionally, c(SC-SB) and c(SD-SC) were calculated in the same way.

2.4.2. Determination of reactive sulfydryl and total sulfhydryl group

The reactive and total sulfhydryl groups (SH) of hairtail surimi