Food Research 6 (2) : 403 - 412 (April 2022)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Characteristics and functional properties of gelatin and gelatin hydrolysate from bigeye snapper (*Priacanthus tayenus*) bone

[1]Janpet, C., [1]Manakit, P., [2]Klinmalai, P., [2]Kaewprachu, P., [2]Jaisan, C., [2]Surayot, U., [2]Chakrabandhu, Y. and [2,*]Wangtueai, S.

[1]*Division of Marine Product Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand*

[2]*College of Maritime Studies and Management, Chiang Mai University, Samut Sakhon 74000, Thailand*

Article history:
Received: 22 May 2021
Received in revised form: 27 June 2021
Accepted: 5 September 2021
Available Online: 25 April 2022

Keywords:
Bigeye snapper,
Fish bone,
Fish gelatin,
Fish gelatin hydrolysate,
Antioxidant activity,
Functional property

DOI:
https://doi.org/10.26656/fr.2017.6(2).344

Abstract

Bigeye snapper bone, a by-product from the surimi industry, was a starting material to prepare gelatin through hot water extraction (HG) and retort extraction (RG). Yields of HG and RG were 0.35 and 0.90% (based on wet weight), respectively. It was high in protein content (72.15-75.36%). Gelatins from both methods showed significant differences in the quantity of chemical compositions ($P \leq 0.05$). The HG showed greater gel strength (384 g) and viscoelastic properties than the RG ($P \leq 0.05$). Both HG and RG were then hydrolyzed by protease (papain) with a degree of hydrolysis of 43.29% and 37.22%, respectively. The functional properties and antioxidant capacities of both gelatin hydrolysates were also investigated. The HG hydrolysate (HGH) showed higher foam expansion (1.33-14.67%), but a lower emulsifying stability index (33.5-5.33 mins) than those of RG hydrolysate (RGH) (0.67-8.00% and 76.8-1.0 min, respectively). Antioxidant capacities showed that both HGH and RGH are good sources of functional ingredients in foods.

1. Introduction

Seafood processing leads to enormous amounts of waste, particularly the solid wastes from surimi processing such as bone, skin, scales and entrails (Wangtueai and Noomhorm, 2009). On the other hand, the demand for processed seafood is highly growing. Hence, the effective utilization of by-products from the fishery product processing industry has gained attention among both academic researchers and industries. Bigeye snapper (*Priacanthus* spp.) has long been an important species for surimi production in Thailand due to the high grade and good properties of surimi products produced (Benjakul *et al*., 2009). Around 50-70% of the raw material weight ends up as waste depending on species and product types. Conversion of those remainders into high value-added products can pave the way for high-efficiency utilization of fish resources, leading to sustainable fishery product production. In addition, the fish by-products rich in protein and collagen potentially be used as raw material for collagen and gelatin production (Wangtueai *et al*., 2016).

Fish gelatin is a substance in the protein hydrolysate group produced from collagenous material, a by-product of the fish processing industry such as fish skin, scale, and bone. Fish gelatin has near-equivalent properties with mammalian gelatin and is widely used because it meets health and religious requirements (Huang *et al*., 2019). However, the obtained fish gelatin generally has a lower molecular weight than native collagenous materials, which influences gelatin properties (Kaewrung *et al*., 2013). Therefore, the purpose of gelatin production is to control the hydrolysis of collagenous material (partial hydrolysis) into a soluble product with desirable properties such as gel strength, viscosity, and melting point (Wangtueai *et al*., 2013). Various factors have also been a concern in the production of fish gelatin, including its raw material, pretreatment, and processing parameter such as temperature, time, pH (Wangtueai and Noomhorm, 2009; Kaewrung *et al*., 2013).

Bioactive peptides with antioxidant properties derived from various proteins by enzymatic hydrolysis have become a topic of great interest for pharmaceutical, food health and processing and preservation industries. The enzymatic digestion of proteins is a potential method for peptide production with improved bioactivities and functional properties of materials such as papain which have been used to obtain the bioactive peptides from various native protein materials (Wangtueai *et al*., 2013;

*Corresponding author.
Email: *sutee.w@cmu.ac.th*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

Wangtueai *et al*., 2016). Normally, fish gelatin hydrolysate is produced using enzymatic or physicochemical processes to hydrolyse fish gelatin (Wangtueai *et al*., 2020), which has various biological and functional properties derived from its specific peptides such as antioxidant and angiotensin-I-converting enzymes inhibitory activity (Akagündüz *et al*., 2014; Wangtueai *et al*., 2020). Thus, using fish by-products to produce gelatin and gelatin hydrolysates could enhance raw material utilization and develop the high-value product as bioactive ingredients.

Hence, the objective of this study was to obtain gelatin and gelatin hydrolysate from bigeye snapper bone, a by-product of the surimi processing plants. In this work, two extraction methods were used to extract the gelatin. First, rheological and chemical characteristics were determined. Second, fish bone gelatin was subjected to enzymatic hydrolysis for biological and functional properties evaluation.

2. Materials and methods

2.1 Chemicals

Papain (≥3 activity unit/mg), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All reagents used in this experiment were analytical grade.

2.2 Fish bones preparation

Bigeye snapper (*Priacanthus tayenus*) bones were obtained from a surimi processing plant, Samut Sakhon Province, Thailand. The fish bones were cleaned using tap water and drained in a plastic basket at room temperature for 5 mins. They were then stored at -18 to -20°C with a maximum storage period of two months until the need for further experiments.

2.3 Gelatin extraction

Fish bones were pre-treated using the alkali method. In brief, fish bones were thawed and cleaned using running tap water for 3-4 hrs. The thawed fish bones were soaked in 5 volumes (v/w) of 0.8% (w/v) NaOH solution at room temperature for 2 hrs to remove the non-collagen protein and the subcutaneous tissue after they were swollen. Then, they were neutralized by washing in running tap water for 3-4 hrs until about pH 7 is achieved. The pre-treated fish bone was subjected to gelatin extraction using two different methods such as hot water extraction (modified method from Wangtuei and Noomhorm (2009)) and retort extraction (modified method from Yang *et al*. (2009)). The hot water extraction was done in distilled water at 70°C for 3 hrs. The retort extraction was carried out in an autoclave (FD100R, ZEALWAY Instrument Inc., DE, USA) at 121°C, 15 psi for 30 mins. After extraction, the extracted solution from both methods was filtered through double layers of cheesecloth to remove coarse solids, centrifuged at 6000 rpm, and filtered through Whatman No.1 filter paper (Whatman, Maidstone, England) to remove fine residues. The filtered solution was then freeze-dried (Labconco, Kansas, MO, USA). The dried fish bone gelatin was stored in a plastic bag and kept in a desiccator at room temperature until use. The obtained gelatin from hot water extraction and retort extraction were referred to as "HG" and "RG", respectively. Gelatin yield (YD) was calculated using the following equation: YD (%) = [Dried fish bone gelatin weight (g)/ Pre-treated fish bone weight (g)] × 100.

2.4 Gelatin hydrolysate preparation

Fish bone gelatin hydrolysate was prepared according to the method described by Wangtueai *et al*. (2016). The 5 g of gelatins were dissolved in 100 mL of 0.1 M sodium phosphate buffer pH 7.0 and treated with 2% (w/w based on gelatin content) papain at 55°C for 3 hrs. Then, the enzyme was inactivated by heating it at 90°C for 10 mins in a water bath. Finally, the gelatin hydrolysate solutions were dried using a freeze dryer (Labconco). The obtained gelatin hydrolysate powders were packed into a zip-lock plastic bag and stored in a desiccator until further use. The obtained gelatin hydrolysates from HG and RG were referred to as "HGH" and "RGH", respectively.

2.5 Characteristics and functional properties of gelatin and gelatin hydrolysate

2.5.1 Proximate compositions

Moisture, protein, and ash content of bigeye snapper bone gelatins were determined according to the AOAC methods 934.01, 954.01, and 942.05, respectively (AOAC, 2000).

2.5.2 Viscosity

The viscosity of gelatin was determined using a Brookfield viscometer (Brookfield DV-II+, USA). Bigeye snapper bone gelatin was dissolved in distilled water with a concentration of 6.67% (w/v), stirred at 60° C for 30 mins, shaken in a water bath until completely dissolved, and the viscosity was measured using the Brookfield viscometer with a control temperature at 27±1°C.

2.5.3 Viscoelastic properties

Dynamic studies were performed Rheometer AR2000 (TA Instruments, DE, USA) using a cone-plate geometry (diameter 60 mm, cone angle 1°, gap 0.1 mm). Fish bone gelatin solution was prepared by stirring the mixture for about 30 mins at 40°C. The viscosity measurement was performed at a scan rate of 1°C/min, frequency of 1 Hz, oscillating applied stress of 3.0 Pa and gap of 0.15 mm (Liu *et al*., 2009). During the testing, the gelatin solutions were firstly cooled from 35 to 5°C and heated back to 35°C to study gelation and subsequent melting. The elastic modulus (G', Pa), viscosity modulus (G", Pa), and the phase angle (δ) were evaluated as a function of temperature.

2.5.4 Gel strength

The gel strength of gelatin was done using the method of Wangtueai and Noomhorm (2009). Gelatin was dissolved in distilled water (6.67%, w/v). The 30 mL of gelatin solution was filled in glass measuring bottles (40.1 mm dia. × 52 mm height, flat bottom), sealed with plastic film and kept at 4°C for 16-18 hrs before measurement. The gel strength was measured using a texture analyzer (TA.XT Plus, Stable Micro Systems Ltd., Surry, England) while the sample was still at 9-10° C. A plunger with a 12.7 mm diameter had a penetration depth of 4 mm into the gelatin gels with a speed of 1 mm/s. The force in g at this temperature is the gel strength.

2.5.5 Degree of hydrolysis

The degree of hydrolysis of gelatin hydrolysates (HGH and RGH) were determined according to the method of Benjakul and Morrissey (1997). The 63 µL of 5% (w/v) gelatin hydrolysate solution was mixed with 1 mL of 0.2 M phosphate buffer and 0.5 mL of 0.01% trinitrobenzenesulfonic acid (TNBS) solution. The mixtures were then placed in a water bath at 50°C for 30 mins in the dark. The reaction was terminated by adding 1 mL of 0.1 M sodium sulfite. The mixtures were cooled down at ambient temperature for 15 mins. The absorbance was measured at 420 nm using a spectrophotometer (SPECORD 50 PLUS, Analytikjena, Jena, Germany), and α-amino acid was expressed in terms of *L*-leucine. The degree of hydrolysis (DH) is defined as follows:

$$\text{Degree of hydrolysis (DH)} = [(L_s\text{-}L_0)/(L_{max}\text{-}L_0)] \times 100$$

Where L_s is the amount of α-amino acid of gelatin hydrolysate sample, L_0 is the amount of α-amino acid in original bigeye snapper bone gelatin and L_{max} is the maximum number of α-amino acid in bigeye snapper bone gelatin obtained after acid hydrolysis using 6N HCl at 100°C for 24 hrs.

2.5.6 Protein solubility

Protein solubility of fish bone gelatin hydrolysate was determined according to the method of Wangtueai *et al*. (2016). The gelatin hydrolysate (0.2 g) was dispersed in 20 mL of distilled water, and then the pH of the mixture was adjusted to 2, 4, 6, 8, and 10 with either 0.5N NaOH or 0.5N HCl. The mixture was stirred at room temperature (about 25°C) and centrifuged at 4,500×*g* for 30 mins. The protein solubility was calculated as follows:

$$\text{Protein solubility index (\%)} = (A/B) \times 100$$

Where A is protein content in the supernatant and B is the total protein content in the sample.

2.5.7 Foaming properties

Foaming expansion (FE) and foam stability (FS) of fish bone gelatin hydrolysate were determined as described in Giménez *et al*. (2009) with slight modification. Fish bone gelatin hydrolysate solutions were prepared at the concentration of 0.1, 0.5, 1.0, and 3.0% (w/v). Protein hydrolysates (20 mL) with each concentration were transferred into a 50 mL cylinder and homogenized at 10,000 rpm for 10 mins. The total volume was measured at 0, 30, and 60 mins after whipping. FE and FS were calculated according to the following equation:

$$\text{FE (\%)} = [(A\text{-}B)/B] \times 100$$

Where: A is the volume after whipping (mL) and B is the volume before whipping (mL)

$$\text{FS (\%)} = [(A\text{-}B)/B] \times 100$$

Where A is the volume at 30 and 60 mins after whipping (mL) and B is the volume before whipping (mL)

2.5.8 Emulsifying properties

Emulsifying activity index (EAI) and emulsion stability index (ESI) were determined according to the method described in Giménez *et al*. (2009) with a slight modification. Soybean oil (10 mL) and gelatin hydrolysate solution (30 mL) at different concentrations (0.1, 0.5, 1.0 and 3.0% (w/v)) were mixed and homogenized at a speed of 10,000 rpm for 10 mins. Aliquots of the emulsion (100 µL) were taken from the bottom of the container at 0 and 10 mins after homogenization and diluted with 0.3% SDS solution (1:200 dilution). The absorbance of the diluted solution was measured at 500 nm. The absorbances measured

immediately (A_0) and 10 min (A_{10}) after emulsion formation were used to calculate the EAI and the ESI as follows:

$$EAI\ (m^2/g) = (2\times2.303\times DF\times A)/(0.25\times P)$$

$$ESI\ (min) = A_0\times\Delta t/\Delta A$$

Where DF is the dilution factor (200), A is the absorbance at 500 nm, P is the fish bone gelatin concentration (g/mL) and $\Delta A = A_0 - A_{10}$ and $\Delta t = 10$ min

2.5.9 DPPH radical scavenging activity

The DPPH radical scavenging activity was determined using the method of Ahn *et al*. (2014). The 1.5 mL of fish bone gelatin hydrolysate solutions were added to 1.5 mL of 0.05 mM DPPH in 95% ethanol. This mixture was then shaken vigorously using a Vortex mixer (IKA®Labortechnik, Janke and Kunkel, Germany) and kept in the dark at room temperature for 30 mins. The absorbance was measured at 517 nm. The reaction blank was prepared in the same manner, except that distilled water was used instead of the sample.

DPPH radical scavenging activity (%) = $[(A_{blank} - A_{sample}) \times 100] / A_{blank}$

Where A_{sample} is the absorbance of sample and A_{blank} is the absorbance of the blank.

The DPPH scavenging activity was plotted against concentration and the IC_{50} (fish bone gelatin hydrolysate concentration providing 50% of DPPH radicals scavenging activity) was calculated from the graph using linear regression analysis.

2.5.10 ABTS radical scavenging activity

The ABTS radical scavenging activity of fish bone gelatin hydrolysates was determined using the method of Ahn *et al*. (2014). The stock solution of ABTS radical was prepared by mixing with 7 mM ABTS in potassium persulphate 2.45 mM in the ratio of 1:1(v/v) and kept in the dark at room temperature (RT) for 12-16 hrs. The 95% ethanol solution was used for dilution of the stock solution in order to prepare the ABTS working solution with obtained an absorbance of 0.70±0.02 at 734 nm. The 150 µL sample solution was mixed with the 2850 µL of ABTS working solution and kept in the dark at RT for 8 min. The absorbance of the mixture was then measured at 734 nm. The distilled water was used instead of the sample for the reaction blank.

ABTS radical scavenging activity (%) = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$

Where: A_{sample} is the absorbance of sample and A_{blank} is the absorbance blank.

The ABTS scavenging activity was plotted against concentration and the IC_{50} (fish bone gelatin hydrolysate concentration providing 50% of ABTS radicals scavenging activity) was calculated from the graph using linear regression analysis.

2.5.11 Ferric reducing antioxidant power (FRAP)

The FRAP value was done according to the method described by Wangtueai *et al*. (2016). FRAP solution contained 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ in 40 mM HCl, and 2.5 mL 10 mM $FeCl_3\cdot6H_2O$ (ratio 10:1:1). This mixture was incubated in a water bath (Memmert GmbH, Schwabach, WNB, Germany) at 37°C for 30 mins. The FRAP solution was always freshly prepared. The 25 mg/mL of fish bone gelatin hydrolysate solution (200 µL) was added to 1300 µL of FRAP solution, and incubated in the dark for 30 mins. The absorbance of the mixture was measured at 595 nm. The $FeSO_4\cdot7H_2O$ was used to prepare a standard curve and the FRAP assay was expressed as mmol $FeSO_4$/g fish bone gelatin hydrolysate.

2.6 Statistical analysis

A one-way analysis of variance was done. Mean comparisons were performed using Duncan's multiple range test. Paired sample t-test was applied to compare the means of parameters between two treatments. Significant differences among mean values were evaluated for comparing treatments at 95% confidence intervals ($P\leq0.05$). The statistical package used in this study was SPSS software version 17 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1 Gelatin yield

The yield of gelatin extracted from Bigeye snapper bone using the hot water extraction (HG) and the retort extraction (RG) method is shown in Table 1. The HG and RG yielded 0.35±0.11 and 0.90±0.11% (based on a wet weight basis), respectively. The higher yield was found in RG, which might be due to the effects of extraction temperature and pressure. The optimum and sufficient temperature in the denaturation of soluble collagen during the extraction would result in a good yield obtained (Koli *et al*., 2012; Kaewrung, *et al*., 2013). However, the yields of gelatin vary depending on raw materials (fish skin, scale, head, or bone), pre-treatment methods, extraction conditions as well as fish species (Jongjareonrak *et al*., 2006; Wangtueai and Noomhorm, 2009).

Table 1. Proximate compositions and properties of bigeye snapper bone gelatin

Chemical composition and properties	HG	RG
Yield (%)	0.35±0.11[b]	0.90±0.11[a]
Ash (%)	5.89±0.86[a]	1.86±0.23[b]
Moisture (%)	7.58±0.32[b]	8.49±0.12[a]
Protein (%)	72.15±0.01[b]	75.36±0.24[a]
Viscosity (cP)	5.04±0.06[a]	4.12±0.01[b]
Gel strength (g)	384±43[a]	281±73[b]

Values are presented as mean±SD. Values with different superscripts within the same row are significantly different (P≤0.05). HG: bigeye snapper bone gelatin from the hot water extraction, RG: bigeye snapper bone gelatin from the retort extraction.

3.2 Proximate composition and properties of gelatin

The moisture, protein, and ash content of HG and RG are shown in Table 1. Proximate compositions of the obtained gelatin were affected by the extraction method (P≤0.05). Both the HG and the RG had moisture content lower than 10%. The low value in moisture content of gelatin can prevent the sticky effects and can increase the shelf life. Protein content ranged between 72.15 and 75.36%. There are various reports on the protein content in different fish bone gelatin ranging from 70 to 82% (Muyonga *et al*., 2004; Koli *et al*., 2012; Shakila *et al*., 2012). The ash content in HG was higher RG (P≤0.05). This might be possibly affected by the different extraction times of each using extraction methods. The HG was extracted for 3 hrs while the RG was extracted in 30 min, which the longer extraction time might allow some minerals such as calcium carbonate to dissolve into extracted gelatin solution resulting in higher ash content. There are several researchers that reported the extracted gelatin with higher ash content such as 8.4-11.2% in Nile perch bone gelatin (Muyonga *et al*., 2004), 6.58-10.3% in red snapper and grouper bone gelatin (Shakila *et al*., 2012), and 2.7-2.8% in pink perch and tiger-toothed croaker bone gelatin (Koli *et al*., 2012). However, the ash content in high-quality gelatin should not exceed 2% (Wangtueai and Noomhorm, 2009). Islam *et al*. (2021) also reported that the sturgeon head gelatin had 17-19% ash content, it categorized as low-quality gelatin.

The gel strength of gelatin is one of the most important qualities to evaluate the quality of the produced gelatin. Gelatin price in the markets is principally based on the gel strength values of the gelatins (Wangtueai *et al*., 2010). The HG had higher gel strength than the RG (P≤0.05). This might be due to a higher temperature in the extraction process used for RG. The use of high extraction temperature resulted in a low molecular weight (MW) distribution of the extracted gelatin structure (Karim and Bhat, 2009). The higher temperature might destroy the structure of α- and β-chain of gelatin resulting in the lowered MW of gelatin (Benjakul *et al*., 2009). This leads to a non-forming of the strong gel due to the lower inter-junction zones in the gel systems and finally leads to decreasing in gel strength (Zhou *et al*., 2006). The viscosity is the second most commercially important physical property of gelatin (Wangtueai and Noomhorm, 2009). The viscosity of the HG was slightly higher than the RG (P≤0.05) .This also might be because the high extraction temperature was used for RG production. Koli *et al*. (2012) reported that viscosity is particularly controlled by the MW distribution of gelatin. In general, the commercial gelatins are produced from mammalian animals, which have a range of 2-7 cP of viscosity (Shakila *et al*., 2012).

3.3 Viscoelastic properties

The viscoelastic properties of fish bone gelatin (HG and RG) solutions (6.67%, w/v) were evaluated through the programs of cooling and heating. The viscoelastic properties upon cooling and heating from 5°C to 35°C are shown in Figure 1 (A-F). Storage modulus (G') and loss modulus (G") of HG was higher than those of RG (Figure 1A-D) in both during cooling from 35 to 5°C and heating from 5 to 35°C. The G' of HG increased greatly in a short time during cooling program as well as G" (Figure 1A and 1C). This was in accordance with the results of gel strength that the gel of gelatin was stored at 4°C for 16-18 h. Analysis of variation of phase angle (δ) can provide a gelling and melting point of gelatin by the point of the sharp increase or decrease of phase angles (Liu *et al*., 2008). These showed that the onset of gelling in HG and RG solutions were at about 15 and 11°C, respectively (Figure 1E), while the melting points were at 22 and 19°C, respectively (Figure 1F). The rate of gelling and melting of RG was higher than HG (Figure 1E and 1F). The gelling temperature of the present study was lower than the gelatin of hybrid kalamtra sturgeon heads type A (13°C) (Islam *et al*., 2021), channel catfish head bones (18°C) (Liu *et al*., 2009), and sea bream bones and scale gelatin (14-20°C) (Akagündüz *et al*., 2014). While, the melting temperature of the present study was lower than the gelatin of hybrid Kalamtra sturgeon heads (23°C) (Islam *et al*., 2021), channel catfish head bones (25°C) (Liu *et al.,* 2009), and sea bream bones and scales gelatin (22-26°C) (Akagündüz *et al*., 2014). Previous research reported the relationship between melting point and gelatin MW as higher the MW, higher melting temperature (Liu *et al*., 2009). However, the fish gelatins normally have a lower gel modulus, and gelling and melting temperatures than the gelatins from mammalian due to fish gelatins has lower imino acid content (proline and hydroxyproline) (Haug *et al*., 2004).

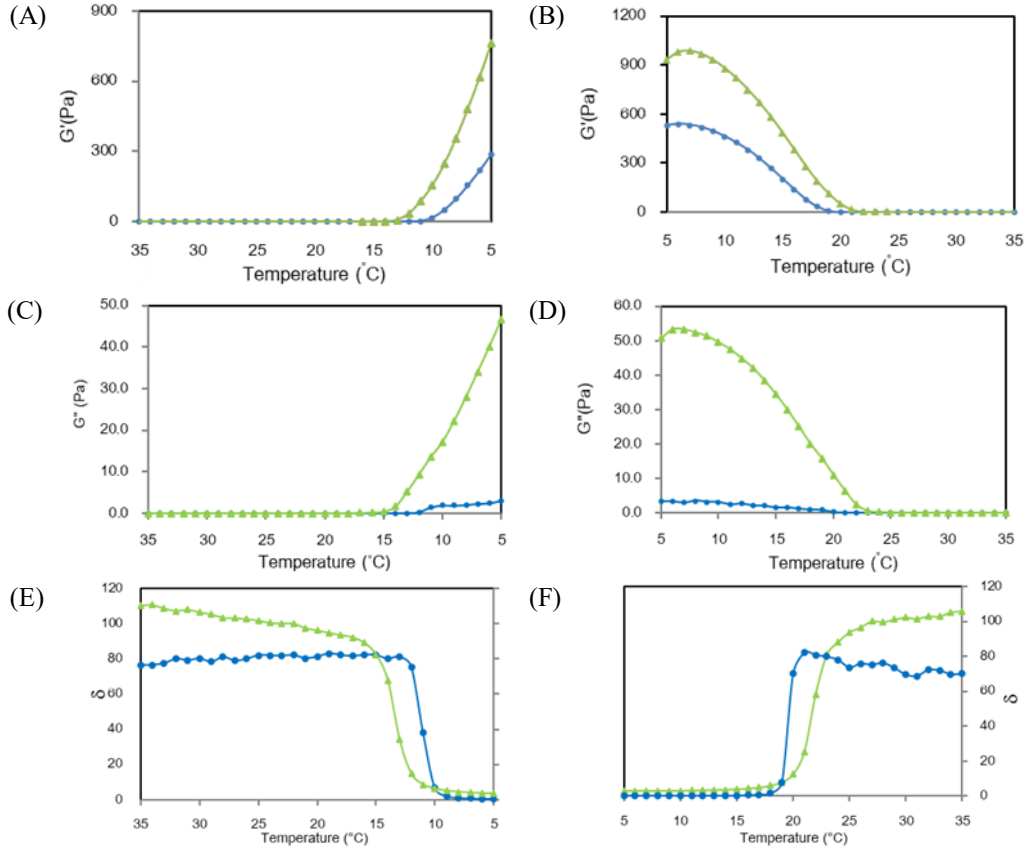

Figure 1. Viscoelastic properties of bigeye bone gelatin upon cooling from 5°C to 35°C, storage modulus (G'; A, B), loss modulus (G''; D, E), and phase angle (δ; E, F). Bigeye bone gelatin using hot water extraction (HG; ▲) and retort extraction (RG; ●).

3.4 Fish bone gelatin hydrolysate

3.4.1 Degree of hydrolysis

The degree of hydrolysis (DH) is usually applied as a parameter of the progress of the protein hydrolysis, which is also widely used as an indicator of comparison among different protein hydrolysates as well as the ability of the enzyme for breaking peptide bonds (Giménez *et al*., 2009; Mongkonkamthorn *et al*., 2020). Bigeye snapper bone gelatins (HG and RG) were hydrolysed using papain in order to obtain the antioxidative peptides. These were obtained the HG hydrolysate (HGH) and the RG hydrolysate (RGH) with the DH of 43.29±2.67 and 37.22±2.30%, respectively. This is possibly due to the differences in size and structure of the protein in both gelatin. Previous research has explained that sole and squid gelatin hydrolysates were obtained from the same hydrolysis process; the difference observed in DH between both of them should be attributed to a different composition of size and structure of the protein chains of gelatins (Giménez *et al*., 2009). The DH of fish gelatin hydrolysate effects on antioxidant activity increased with increasing DH (Sai-Ut *et al*., 2015).

3.4.2 Functional properties of fish bone gelatin hydrolysates

3.4.2.1 Protein solubility

Solubility is one of the most important physicochemical and functional properties of protein hydrolysates (Ktari *et al*., 2012). The solubility of the HGH and the RGH were evaluated over a wide pH range of 2-10 as shown in Figure 2. The protein solubility of the HGH was ranging from 69.75-83.65%, while the RGH ranged from 71.54-84.09%. These pHs ranged, the HGH was similar solubility with the RGH ($P>0.05$), except for a significant difference at pH 4 and 6 ($P\leq0.05$). The solubility of RGH hydrolysates was slightly low at pH 6, it might be due to a pH affecting the charge on the weakly acidic and basic side-chain groups as described by Giménez *et al.* (2009). Protein hydrolysates generally show low solubility at their isoelectric points and high solubility at maximal change (Giménez *et al.*, 2009; Naqash and Nazeer, 2013). Protein solubility is one of the benefits of hydrolysis (Wangtueai *et al*., 2016). Dhanabalan *et al*. (2020) reported that good solubility results from a high DH of obtained protein hydrolysate that it has a high amount of low MW of peptides containing high hydrophilicity residues. However, the high solubility over a wide pH range is an advantage for the application in many foods (Gajanan *et al*., 2016).

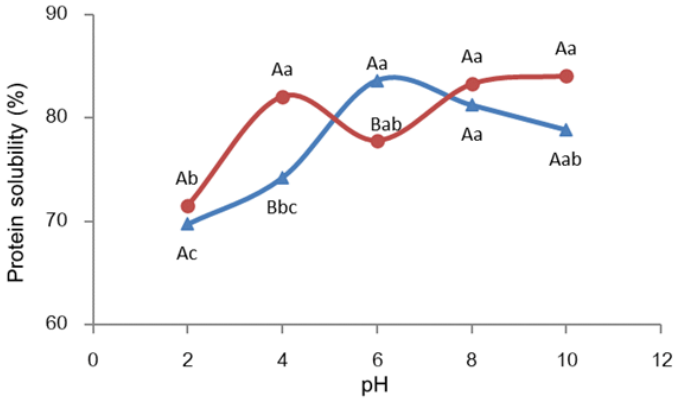

Figure 2. Protein solubility of the hydrolysates of bigeye snapper bone gelatin from the hot water extraction (HGH, ▲) and retort extraction (RGH, ●) at different pH values. Different capital letters in the same pH indicate the significant differences (P≤0.05) and different low-case letters in the same hydrolysate indicate the significant differences (P≤0.05).

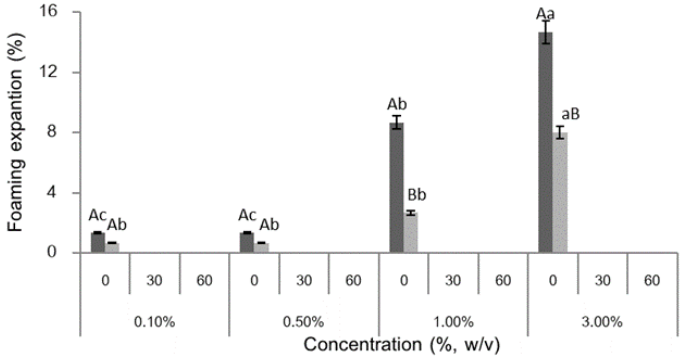

Figure 3. Foaming properties of the hydrolysates of bigeye snapper bone gelatin from the hot water extraction (HGH, ■) and retort extraction (RGH, ■). Different capital letters in the same time indicate the significant differences (P≤0.05) and different low-case letters among each hydrolysate indicate the significant differences (P≤0.05).

3.4.2.2 Foaming properties

The foaming properties of HGH and RGH are shown in Figure 3. The foam expansion (FE) and foam stability (FS) were evaluated with the various concentration (0.10, 0.50, 1.00, and 3.00%, w/v). The FE of the HGH and the RGH were observed in the range of 1.33-14.67% and 0.67-8.00%, respectively. FS of both hydrolysates were not observed at 30 and 60 mins, meaning those hydrolysates had low FS. The FE of HGH was higher than the RGH due to the higher DH and protein solubility as previously explained. This might be due to a higher solubility of gelatin hydrolysate had resulted in more incorporation between air and water resulting in higher foaming ability. Moreover, the foamability of both gelatin hydrolysates gradually increased when the concentration of both gelatin hydrolysates was increased. In the occurrence of FS, the molecule of protein should form continuous intermolecular polymers enwrapping air bubbles then the important factors for the production of stable foams are intermolecular cohesiveness and elasticity. Whereas, a smaller molecule of protein peptides does not have the strength to hold stable foam and results in decreasing of foam stability (Ktari *et al*., 2012).

3.4.2.3 Emulsifying properties

The mechanism of the emulsification process is the absorption of proteins to the surface of freshly formed oil droplets during homogenization and forming a protective membrane for the prevention of coalescence droplets (Ktari *et al*., 2012). The emulsifying properties of the HGH and the RGH are shown in Table 2. The emulsifying activity index (EAI) and emulsifying stability index (ESI) of both gelatin hydrolysates decreased when the concentration was increased from 0.1-3.0% (w/v). The EAI and ESI of the HGH were lower than the RGH (P≤0.05). This may affect by the DH value that the hydrolysate with high DH shows a lower emulsifying property as previously described by Gajanan *et al*. (2016). In addition, the low emulsion stability of gelatin hydrolysate may be due to the low MW of gelatin hydrolysate (Wangtueai *et al*., 2016). High MW peptides have greater amphiphilic than low MW peptides resulting in good emulsion properties (Klompong *et al*., 2007). As previously studied, carp flesh hydrolysate with an average peptide chain length lower than 20 residues presented the value of EAI and ESI range of 5.16-52.76 m^2/g and 14-85 min, respectively (Elavarasan *et al*., 2014).

3.4.4 Antioxidant activity of fish bone gelatin hydrolysate

Various researches reported that the fish gelatin hydrolysates compose the antioxidative peptides that play important roles in free radical scavenging (Lv *et al*., 2019). Fish gelatin hydrolysates have been used as a functional and bioactive component in healthy food production by increasing the antioxidant activity of products (Wangtueai *et al*., 2020). In this study, the antioxidant activity (DPPH and ABTS radical scavenging activity and FRAP assay) of Bigeye snapper bone gelatin hydrolysates were determined as shown in Table 3. The IC_{50} value on DPPH and ABTS radical scavenging activity were in the range of 0.002-0.007 g/mL and 0.002-0.07 mg/mL, respectively, while the FRAP value was in the range of 0.24-1.53 mmol $FeSO_4$/g. The previously reported IC_{50} values on DPPH radical scavenging activity of bigeye skin gelatin hydrolysate prepared using papain was 0.07 g/mL (Wangtueai *et al*., 2020), 14 mg/mL for blue sharkskin gelatin hydrolysate (Weng *et al*., 2014) and 1.98 mg/mL for thornback ray skin gelatin hydrolysate (Lassoued *et al*., 2015), 0.80-1.28 mg/mL for milkfish scale gelatin hydrolysate

Table 2. Emulsifying properties of the bigeye snapper bone gelatin hydrolysates

Bigeye snapper bone gelatin hydrolysates	Concentration (%, w/v)	Emulsifying activity index (m^2/g)	Emulsion stability index (min)
HGH	0.1	7.37±0.18[a]	33.5±5.80[a]
	0.5	4.04±0.02[b]	12.3±1.50[b]
	1	1.23±0.22[c]	7.18±2.34[bc]
	3	1.18±0.38[c]	5.33±1.96[c]
RGH	0.1	31.3±0.53[a]	76.8±7.48[a]
	0.5	5.71±0.07[b]	45.4±7.54[b]
	1	2.01±0.06[c]	27.9±3.19[c]
	3	0.41±0.01[d]	1.00±0.39[d]

Values are presented as mean±SD (n = 3). Values with different superscripts within the same column among each hydrolysate are significantly different (P≤0.05). HGH: gelatin hydrolysate of bigeye snapper bone gelatin from the hot water extraction, RGH: gelatin hydrolysate of bigeye snapper bone gelatin from the retort extraction.

(Huang *et al*., 2018). For ABTS radical scavenging activity, the IC_{50} values of bigeye skin gelatin hydrolysate produced using papain were about 0.001 g/mL (Wangtueai *et al.*, 2020), and 0.301, 0.235, and 0.186 mg/mL for carp roe protein hydrolysates produced using alcalase, pepsin, and trypsin, respectively (Chalamaiah *et al*., 2015). For the FRAP values, HGH and RGH in this study showed higher than bigeye snapper skin gelatin hydrolysate and catfish gelatin hydrolysates, which values were 0.08 mmol $FeSO_4$/g and the range of 6-8 μmol $FeSO_4$/g, respectively (Alemán *et al*., 2011; Wangtueai *et al*., 2020). In general, the peptides MW plays important role in the antioxidant activity of protein hydrolysate. The low MW peptides exhibited stronger antioxidative activity than the high MW peptides (Choonpicharn *et al*., 2015; Mongkonkamthorn *et al*., 2020). Regarding DH value, the HGH was higher than the RGH, resulting in higher antioxidant activity. The higher DH could result in low MW with a larger exposure of the active amino acids residues in peptide sequences that are capable of reacting with free radicals (He *et al*., 2019).

Table 3. Antioxidant properties of bigeye snapper bone gelatin hydrolysates

Antioxidant properties	HGH	RGH
DPPH (g/mL)	0.007±0.002[a]	0.002±0.0003[b]
ABTS (mg/mL)	0.070±0.031[a]	0.002±0.001[b]
FRAP (mmol $FeSO_4$/g)	0.244±0.023[a]	1.531±0.083[b]

Values are presented as mean±SD. Values with different superscripts within the same row are significantly different (P≤0.05). HGH: gelatin hydrolysate of bigeye snapper bone gelatin from the hot water extraction, RGH: gelatin hydrolysate of bigeye snapper bone gelatin from the retort extraction, DPPH: the IC50 value of DPPH radical scavenging activity, ABTS: the IC_{50} value of ABTS radical scavenging activity, FRAP: FRAP assay activity.

4. Conclusion

In the present study, two methods were used to extract the gelatin from the bigeye snapper bone. Gelatin extracted with the hot water extraction method showed good gel strength and viscoelastic properties but a lower yield than the retort extraction. The bigeye snapper bone gelatin could be used to produce the gelatin hydrolysates with high antioxidant activity. Based on the functional properties and antioxidant capacities, both the HGH and the RGH have the potential to be used as functional ingredients in nutraceuticals and foods.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

This work was supported by Chiang Mai University, Thailand.

References

Ahn, C.B., Kim, J.G. and Je, J.Y. (2014). Purification and antioxidant properties of octapeptide from salmon by-product protein hydrolysate by gastrointestinal digestion. *Food Chemistry*, 147, 78-83. https://doi.org/10.1016/j.foodchem.2013.09.136

Akagündüz, Y., Mosquera, M., Giménez, B., Alemán, A., Montero, P. and Gómez-Guillén, M.C. (2014). Sea bream bones and scales as a source of gelatin and ACE inhibitory peptides. *LWT-Food Science and Technology*, 55(2), 579-585. https://doi.org/10.1016/j.lwt.2013.10.026

Alemán, A., Giménez, B., Ǵomez-Guillén, M.C. and Montero, P. (2011). Enzymatic hydrolysis of fish gelatin under high pressure treatment. *International Journal of Food Science and Technology*, 46(6), 1129-1136. https://doi.org/10.1111/j.1365-2621.2011.02590.x

AOAC. (2000). Official Methods of Analysis', Proximate Analysis. 17th ed. Gaithersburg, USA: AOAC.

Benjakul, S. and Morrissey, M. (1997). Protein hydrolysate from Pacific whiting solid wastes. *Journal of Agricultural and Food Chemistry*, 45(11), 3423-340. https://doi.org/10.1021/jf970294g

Benjakul, S., Oungbho, K., Visessanguan, W. and Thiansilakul, Y. (2009). Characteristics of gelatin from the skins of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*. *Food Chemistry*, 116(2), 445-451. https://doi.org/10.1016/j.foodchem.2009.02.063

Chalamaiah, M., Jyothirmayi, T., Diwan, P.V. and Kumar, B.D. (2015). Antioxidant activity and functional properties of enzymatic protein hydrolysates from common carp (*Cyprinus carpio*) roe (egg). *Journal of Food Science and Technology*, 52(9), 5817-5825. https://doi.org/10.1007/s13197-015-1714-6

Choonpicharn, S., Jaturasitha, S., Rakariyatham, N., Suree, N. and Niamsup, H. (2015). Antioxidant and antihypertensive activity of gelatin hydrolysate from Nile tilapia skin. *Journal of Food Science and Technology*, 52(5), 3134-3139. https://doi.org/10.1007/s13197-014-1581-6

Dhanabalan, V., Xavier, M., Murthy, L.N., Asha, K.K., Balange, A.K. and Nayak, B.B. (2020). Evaluation of physicochemical and functional properties of spray-dried protein hydrolysate from non-penaeid shrimp (*Acetes indicus*). *Journal of Science Food Agricultural*, 100(1), 50–58. https://doi.org/10.1002/jsfa.9992

Elavarasan, K., Kumar, V.N. and Shamasundar, B.A. (2014). Antioxidant and functional properties of fish protein hydrolysates from fresh water carp (*Catla catla*) as influenced by the nature of enzyme. *Journal of Food Processing and Preservation*, 38(3), 1207–1214. https://doi.org/10.1111/jfpp.12081

Gajanan, P.G., Elavarasan, K. and Shamasundar, B.A. (2016). Bioactive and functional properties of protein hydrolysates from fish frame processing waste using plant proteases. *Environmental Science and Pollution Research*, 23(24), 24901-24911. https://doi.org/10.1007/s11356-016-7618-9

Giménez, B., Alemán, A., Montero, P. and Gómez-Guillén, M.C. (2009). Antioxidant and functional properties of gelatin hydrolysates obtained from skin of sole and squid. *Food Chemistry*, 114(3), 976-983. https://doi.org/10.1016/j.foodchem.2008.10.050

Haug, I.J., Draget, K.I. and Smidsrød, O. (2004). Physical behaviour of fish gelatin-κ-carrageenan mixtures. *Carbohydrate Polymers*, 56(1), 11-19. https://doi.org/10.1016/j.carbpol.2003.10.014

He, S., Zhang, Y., Sun, H., Du, M., Qiu, J., Tang, M., Sun, X. and Zhu, B. (2019). Antioxidative peptides from proteolytic hydrolysates of false abalone (*Volutharpa ampullaceal perryi*): Characterization, identification, and molecular docking. *Marine Drugs*, 17(2), 116. https://doi.org/10.3390/md17020116

Huang, C.Y., Tsai, Y.H., Hong, Y.H., Hsieh, S.L. and Huang, R.H. (2018). Characterization and antioxidant and angiotensin I-converting enzyme (ACE)-inhibitory activities of gelatin hydrolysates prepared from extrusion-pretreated milkfish (*Chanos chanos*) scale. *Marine Drugs*, 16(10), 346. https://doi.org/10.3390/md16100346

Huang, T., Zhao, H., Fang, Y., Lu, J., Yang, W., Qiao, Z., Lou, Q., Xu, D. and Zhang, J. (2019). Comparison of gelling properties and flow behaviours of microbial transglutaminase (MTGase) and pectin modified fish gelatin. *Journal of Texture Studies*, 50(5), 400–409. https://doi.org/10.1111/jtxs.12405

Islam, Md.R., Yuhi, T., Meng, D., Yoshioka, T., Ogata, Y., Ura, K. and Takagi, Y. (2021). Purity and properties of gelatins extracted from the head tissue of the hybrid kalamtra sturgeon. *LWT-Food Science and Technology*, 142, 110944. https://doi.org/10.1016/j.lwt.2021.110944

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. (2006). Skin gelatin from bigeye snapper and brownstripe red snapper: Chemical compositions and effect of microbial transglutaminase on gel properties. *Food Hydrocolloids*, 20(8), 1216-1222. https://doi.org/10.1016/j.foodhyd.2006.01.006

Kaewrung, P., Benjakul, S., Prodpran, T. and Nalinanon, S. (2013). Physicochemical and functional properties of gelatin from the skin of unicorn leatherjacket (*Aluterus monoceros*) as affected by extraction conditions. *Food Bioscience*, 2, 1-9. https://doi.org/10.1016/j.fbio.2013.03.002

Karim, A. and Bhat, R. (2009). Fish gelatin: Properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocolloids*, 23(3), 563-576. https://doi.org/10.1016/j.foodhyd.2008.07.002

Koli, J.M., Basu, S., Nayak, B.B., Patange, S.B., Pagarkar, A.U. and Gudipati, V. (2012). Functional characteristics of gelatin extracted from skin and bone of tiger-toothed croaker (*Otolithes ruber*) and pink perch (*Nemipterus japonicus*). *Food and Bioproducts Processing*, 90(3), 555–562. https://doi.org/10.1016/j.fbp.2011.08.001

Klompong, V., Benjakul, S., Kantachote, D. and Shahidi, F. (2007). Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (*Selaroides leptolepis*) as influenced by the degree of hydrolysis and enzyme type. *Food Chemistry*, 102(4), 1317–1327. https://doi.org/10.1016/j.foodchem.2006.07.016

Ktari, N., Jridi, M., Bkhairia, I., Sayari, N., Salah, R.B. and Nasri, M. (2012). Functionalities and antioxidant properties of protein hydrolysates from muscle of zebra blenny (*Salaria basilisca*) obtained with different crude protease extracts. *Food Research International*, 49(2), 747-756. https://doi.org/10.1016/j.foodres.2012.09.024

Lassoued, I., Mora, L., Nasri, R., Jridi, M., Toldŕa, F., Aristoy, M.C., Barkia, A. and Nasri, M. (2015). Characterization and comparative assessment of antioxidant and ACE inhibitory activities of thornback ray gelatin hydrolysates. *Journal of Functional Foods*, 13, 225-238. https://doi.org/10.1016/j.jff.2014.12.042

Liu, H.Y., Han, J. and Guo, S.D. (2009). Characteristics of the gelatin extracted from channel catfish (*Ictalurus punctatus*) head bones. *LWT-Food Science and Technology*, 42(2), 540-544. https://doi.org/10.1016/j.lwt.2008.07.013

Liu, H.Y., Li, D. and Guo, S.D. (2008). Extraction and properties of gelatin from channel catfish (*Ietalurus punetaus*) skin. *LWT-Food Science Technology*, 41 (3), 414-419. https://doi.org/10.1016/j.lwt.2007.03.027

Lv, L.C., Huang, Q.Y., Ding, W., Xiao, X.H., Zhang, H.Y. and Xiong, L.X. (2019). Fish gelatin: The novel potential applications. *Journal of Functional Foods*, 63, 103581. https://doi.org/10.1016/j.jff.2019.103581

Mongkonkamthorn, N., Malila, Y., Yarnpakdee, S., Makkhun, S., Regenstein, J.M. and Wangtueai, S. (2020). Production of protein hydrolysate containing antioxidant and angiotensin -I-converting enzyme (ACE) inhibitory activities from tuna (*Katsuwonus pelamis*) blood. *Processes*, 8(11), 1518. https://doi.org/10.3390/md17050251

Muyonga, J., Cole, C. and Duodu, K. (2004). Extraction and physico-chemical characterisation of Nile perch (*Lates niloticus*) skin and bone gelatin. *Food Hydrocolloids*, 18(4), 581-592. https://doi.org/10.1016/j.foodhyd.2003.08.009

Naqash, S.Y. and Nazeer, R.A. (2013). Antioxidant and functional properties of protein hydrolysates from pink perch (*Nemipterus japonicus*) muscle. *Journal of Food Science and Technology*, 50(5), 972–978. https://doi.org/10.1007/s13197-011-0416-y

Sai-Ut, S., Benjakul, S., Sumpavapol, P. and Kishimura, H. (2015). Antioxidant activity of gelatin hydrolysate produced from fish skin gelatin using extracellular protease from *Bacillus amyloliquefaciens* H11. *Journal of Food Processing and Preservation*, 39(4), 394-403. https://doi.org/10.1111/jfpp.12244

Shakila, R.J., Jeevithan, E., Varatharajakumar, A., Jeyasekaran, G. and Sukumar, D. (2012). Functional characterization of gelatin extracted from bones of red snapper and grouper in comparison with mammalian gelatin. *LWT-Food Science and Technology*, 48(1), 30-36. https://doi.org/10.1016/j.lwt.2012.03.007

Wangtueai, S. and Noomhorm, A. (2009). Processing optimization and characterization of gelatin from lizardfish (*Saurida spp*.) scales. *LWT-Food Science and Technology*, 42(4), 825-834. https://doi.org/10.1016/j.lwt.2008.11.014

Wangtueai, S., Noomhorm, A. and Regenstein, J.M. (2010). Effect of microbial transglutaminase on gel properties and film characteristics of gelatin from lizardfish (*Saurida spp*.) scales. *Journal of Food Science*, 75(9), c731-c739. https://doi.org/10.1111/j.1750-3841.2010.01835.x

Wangtueai, S., Noomhorm, A., Regenstein, J.M. and Anal, A.K. (2013). Value-added bioprocessing of fish waste to gelatin. In Boran, G. (Ed.). Gelatin: Production, Applications and Health Implications, p. 185-211. New York, USA: Nova Science Publishers, Inc.

Wangtueai, S., Phimolsiripol, Y., Vichasilp, C., Regenstein, J.M. and Schoenlechner, R. (2020). Optimization of gluten-free functional noodles formulation enriched with fish gelatin hydrolysates. *LWT-Food Science and Technology*, 133, 109977. https://doi.org/10.1016/j.lwt.2020.109977

Wangtueai, S., Siebenhandl-Ehn, S. and Haltrich, D. (2016). Optimization of the preparation of gelatin hydrolysates with antioxidative activity from lizardfish (*Saurida spp*.) scales gelatin. *Chiang Mai Journal of Science*, 43(1), 1122-1133

Weng, W., Tang, L., Wang, B., Chen, J., Su, W., Osako, K. and Tanaka, M. (2014). Antioxidant properties of fractions isolated from blue shark (*Prionace glauca*) skin gelatin hydrolysates. *Journal of Functional Foods*, 11, 342-351. https://doi.org/10.1016/j.jff.2014.10.021

Yang, J.I., Liang, W.S., Chow, C.J. and Siebert, K.J. (2009). Process for the production of tilapia retorted skin gelatin hydrolysates with optimized antioxidative properties. *Process Biochemistry*, 44 (10), 1152-1157. https://doi.org/10.1016/j.procbio.2009.06.013

Zhou, P., Mulvaney, S J. and Regenstein, J.M. (2006). Properties of Alaska pollock skin gelatin: A comparison with tilapia and pork skin gelatins. *Journal of Food Science*, 71(6), 313-321. https://doi.org/10.1111/j.1750-3841.2006.00065.x

