

IMPROVING THE PHYSICOCHEMICAL PROPERTIES OF SNAKEHEAD FISH (*Channa striata*) SAUSAGE BY PROTEASE FROM ITS VISCERA

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ABSTRACT

The study was conducted to investigate the possibility of enhancing the quality of cultured snakehead fish (*Channa striata*) protein, especially used in the processing of sausage products, by treatment with protease from its viscera. The change in physicochemical properties (expressed by water holding capacity, emulsifying capacity of protein, texture characteristics and whiteness index) of snakehead fish paste related to various protease activity (0, 3, 6, 9, 12, 15 U/L) and washing time (20, 30 and 40 min) were determined. In addition, protease treatment conditions were fixed at ambient temperature (30 ± 2 °C) and the ratio of minced fish and protease solution was 1:1(w/v). The results showed that physicochemical properties, gel quality and sensory properties of sausage significantly improved from soaked minced fish in protease solution at 9 U/L for 30 minutes.

Keywords: physicochemical properties, protease treatment, sausage, snakehead fish.

1. INTRODUCTION

There are many species from *Channa* family (snakehead fish), *Channa striata* has been considered the most widely introduced species of those, is one of the freshwater fish species [1]. *C. striata* was considered as a potential food containing completed and balanced essential and non-essential amino acids [1]. In Vietnam, snakehead fish, called as “ca loc”, was newly commercialized with a large area of land for cultivation. The main advantages of snakehead fish are less disease, high yield and high price which take *C. Striata* into account as a source of income for farmers in Mekong Delta [2]. The strong development of snakehead fish cultivation has resulted in surplus raw materials so the demand for fish products is becoming more urgent.

The uses of enzymes as biological catalysts are well-known. A larger number of enzymes extracted through by-products of seafood processing can improve manufacturing efficiency as well as reducing environmental pollution [3]. Many studies have suggested the use of protease in improving the quality of emulsion products. The addition of Flavourzyme can effectively accelerate the proteolysis of native proteins and improve sensory properties and storage stability of Chinese sausage [4]. Actinidin (a sulfhydryl protease from kiwi fruit) was used to improve the

quality attributes of an emulsion product made from this protease-treated beef [5]. Although various uses of protease on emulsion product were reported, little information is available on the application of protease to improve physicochemical properties of snakehead fish protein and in manufacturing snakehead fish sausage. Therefore, the objective of this study was to investigate the effect of protease extracted from snakehead fish viscera on physicochemical properties of cultured snakehead fish protein and quality of sausage product made by this protein.

2. MATERIALS AND METHODS

2.1. Materials

Alive snakehead fishes, weighted 400÷700 g, were purchased from Vinh Long, Vietnam. Fishes were allowed to rest for 1 hour before slaughtering and filleting. The fillets were cut into pieces, divided into PE package and kept frozen at -18 ± 2 °C until use.

Snakehead fish viscera were collected during slaughter operation, stored in PE package, 50 g/sample. The activity of protease in the sample could be maintained by quick-freezing and frozen storage under -18 ± 2 °C for 5 weeks without a decrease in specific activity.

The chemicals used in this work were of analytical grade. Other ingredients in sausage recipe were purchased from local market.

2.2. Protease extraction, partial purification and protease activity

Crude protease extract and partial purification: Crude protease extract and partial purification was carried out as described by Vuong *et al.* (2011) with some modification [3]. 50 g of frozen snakehead fish viscera was homogenized in 100 mL of pre-cooling 0.1 M sodium phosphate buffer pH 8.0 by blender jar for 3 min at 3000 rpm, which maintained the temperature < 5 °C. The homogenate was then heated to 45 °C and stirred for 30 minutes to extract protease. The extract was cleared by centrifuging at 6000 rpm, 4 °C for 20 minutes (Rotanta 46R, German). Ammonium sulfate was added in crude protease extract to 50% saturation. The solution was allowed to stand in cold (4 °C) for 30 minutes. The precipitate was removed by centrifugation at 6.000 rpm, 4 °C for 20 minutes (Rotanta 46R, German). The collected solution was treated similarly with ammonium sulfate to 60% saturation. This precipitate is partial purified protease and was collected by centrifuging at 6.000 rpm, 4 °C for 20 minutes. Re-solubilize protease with 0.1 M sodium phosphate buffer pH 8.0 (just enough for complete solution) and dialyze overnight against water through cellophane membrane at 4 °C before use. Although protease was usually used within 1 day, it can be kept stored at -18 °C for up to 1 month without a decrease in specific activity.

Protease activity: The activity of the proteolytic enzymes was estimated by the method of Anson with modification [6]. The enzymatic reaction mixture contained 5 mL of 1 % casein as a substrate (pre-prepared in Sorensen buffer pH 7.6) and 1 mL of diluted enzyme for 30 min at 30 °C. The reaction was terminated by the addition of 5 mL of 10 % (w/v) trichloroacetic acid (TCA). Blank samples were prepared by 5 mL of casein, 5 mL of TCA, and 1 mL of enzyme added after 30 min. To 1 mL of filtrate, 2 mL of 0.5 M NaOH and 0.6 mL of three-fold diluted Folin - Ciocalteu reagent were added. The color developed after 10 min of incubation at ambient temperature was measured at 660 nm (Visible Spectrophotometer 722, China) [7]. The slope of the standard curve determined with tyrosine was used in the calculation of protease

activity. One unit of protease activity (U) was defined as the amount of enzyme required to produce 1 μmol of tyrosine/mL/min at 30 °C [7].

2.3. Protease treatment and sausage process procedure

Protease treatment: Frozen snakehead fish fillets were ground through a meat grinder with a 1/16-inch plate and unfrozen using a water bath (Memmert, WB 10, German) at a mince:water ratio of 1:1 (w/v), 30 ± 2 °C. Various amount of protease (3, 6, 9, 12, 15 U/L, calculated on the water volume) were added and stirred for 20, 30 and 40 minutes. Dewatered minced meat was subjected to chilled washing (4 °C) twice with 0.5 % NaCl and 0.3 % NaCl, at a ratio of 1:3, by the following steps: stirring for 4 minutes, keeping still for 15 minutes, and then dewatering [8]. The sample with no protease treatment and only washed with NaCl solution was taken as a control (0 U/L). For standardizing, the moisture content of the mince was then measured and ice was added to moisture content of 83 %. The mixture was grounded for 1 minute to obtain snakehead fish paste.

Sausage process procedure: Snakehead fish emulsion was prepared according to the following recipe: Snakehead fish paste (700 g); NaCl (15 g) and PDP (4g); Sucrose (15 g) and Sorbitol (15 g); Dried garlic (5 g), Pepper (5 g) and MSG (3 g); Pork fat (300 g); Modified starch (40 g). The emulsion was obtained by mixing those ingredients in 6 groups sequentially with meat blender, 20 s for 1 group, and finishing by mixing for 60 s [9]. The mixture was kept at the temperature below 4 °C during mixing. Treated snakehead fish pastes with good quality were chosen as sausage ingredient. Two control samples were taken, one was the sausage processed with snakehead fish fillet (control 1, no treatment), and the other was the sausage processed with snakehead fish paste only washed with NaCl solution with no protease treatment (control 2, 0 U/L). The emulsion was stuffed into PVDC casings with a diameter of 30–32 mm, approximate 15 cm length then steamed at $75 \div 80$ °C for 135 minutes [9]. Immediately after steamed, the sausages were pre-cooled with ice water and kept at 4 °C for further use.

2.4. Physical and chemical characterization

Moisture content: Moisture content was determined by rapid microwave drying method (AOAC Official Method 985.14).

Water holding capacity (WHC) evaluation: The WHC of fish samples were determined using the filter paper press method (FPPM) [10]. One sample of about 0.3 g was weighted on a film and put on a filter. The filter has a known property concerning the absorption of water. The filter was put inside two glass tops which stressed with a weight of 1 kg for 10 min. After that, the inside and outside line of the probe were marked with a pencil. The inside and outside areas were measured with a planimeter. The water holding capacity was calculated as follows:

$$\text{WHC (\%)} = \text{Total water (in product)} - \text{Loose water}$$

$$\text{With: Loose water} = (b - a) \times 0.0064/m \times 100 (\%)$$

$$b: \text{area of outside, cm}^2 \quad a: \text{area of inside, cm}^2 \quad m: \text{mass of sample (g)}$$

$$0.0064: 1 \text{ cm}^2 \text{ area of filter paper} = 0.0064 \text{ g water (g/cm}^2\text{)}$$

Emulsifying capacity (EC): EC was determined by using a model system described by Ockerman (1985) with modification [11]. To measure EC, 25 g of meat was ground with 100 mL cold NaCl 1M ($0 \div 5$ °C) at 15,000 rpm for 2 min to obtain the slurry. The emulsions were prepared with 2.5 g of slurry and 7.5 mL of cold 1M NaCl, transferred to a blender jar and

homogenized for 10 s at low speed (8,000 rpm) with 20 mL of soybean oil added at first. Oil was added at 0.4 mL/s by burette (volume 100 mL, accuracy 0.1 mL) until the breaking point of the emulsions. The total amount of emulsified oil was measured and calculated by considering the first 20 mL oil added. EC was defined as mL of oil emulsified per gram of protein (Kjeldahl).

Emulsion stability (ES): A newly sausage emulsion was formed as described above in sausage process procedure. Then, 10 g emulsion was weighted into cellulose nitrate test tubes with a cap and transferred to a water bath at 80 °C until the internal temperature reached to 72 °C (15 min). The test tubes were centrifuged for 15 min at 9000 rpm and were drained into a volume tricylinder for 12 h to collect the unbound oil and water. ES was calculated as a percentage (%) from the amount of separated oil plus separated water released [11].

Texture characteristics: For gel preparation, 1.23 % NaCl was comminuted into snakehead fish paste at 5 °C. The paste was then stuffed into petri dishes (70 × 15 mm), set at 60 °C for 30 min and refrigerated overnight. After gel was equilibrated to room temperature, the gel was cut into 22 x 15 mm cylinder pieces [12]. The similar cylinder size was also applied to snakehead fish sausage. The texture of samples was objectively measured using a Rheotex (Japan) with following compression test parameters: load cell = 25 kg, probe = P5 cylinder (5 mm diameter) with test speed = 1 mm/s and distance = 10 mm. Gel strength is defined as the multiplication of breaking strength (g_f) (the peak force of the first compression) by deformation (mm) [13].

Whiteness Index (WI): ACIE Laboratory color scale was used to measure the degree of lightness (L^*), and yellowness or blueness ($\pm b^*$) of gels (Colorimeter NH300 D65, China). Whiteness was calculated as whiteness index, $WI = L^* - 3b^*$ [14].

Adhesion: The adhesion of the sausage was assessed by the weight (g) of sausage (Ohaus, AR-240, accuracy 0.01 g, Japan) per casing area (mm^2) (Mitoot, Y308, accuracy 0.01 mm, China) after peeled.

Sensory evaluation: Organoleptic characteristics of the fish balls were evaluated based on color, aroma, taste, smoothness and texture using 5-point scale and overall acceptability using 9-point hedonic scale by seven trained panelists.

Statistical analysis: All data were statistically analyzed, the analysis of variance and Duncan's Multiple Range Test was applied to assess the difference between means, processed by Statgraphics Centurion 16.2 (Copyright (C) PP, USA) ($p \leq 0.05$) and Excel 2016 programs.

3. RESULTS AND DISCUSSION

3.1. Composition of snakehead fish muscle

The composition of raw material was showed in Table 1. The percent of protein and ash were high while the percent of lipid in snakehead fish fillet were low. Subsequently, this is a good source for protein and mineral intake. The high WHC and EC values indicated that high quality emulsion product can be produced from snakehead fish fillet. Moreover, the fillet yield was pretty high at 47.25 % whilst pH value around 7 (6.71) indicated freshness condition of raw material. It could be concluded that Vinh Long's snakehead fish was a suitable ingredient for food processing, especially in emulsion products.

Table 1. Physicochemical properties of snakehead fish muscle.

Parameter	Value	Parameter	Value
Moisture (%)	77.71 ± 0.6	pH	6.71 ± 0.21
Protein (%)	19.23 ± 0.48	WHC (%)	68.11 ± 1.49
Lipid (%)	2.56 ± 0.26	EC (mL oil/g protein)	625 ± 21
Ash (%)	0.5 ± 0.03	Fillet yield (%)	47.25 ± 1.4

3.2. Effect of protease treatment on physicochemical properties of snakehead fish paste

Various protease treatment conditions were applied on snakehead fish mince. That effected on the quality of snakehead fish paste and was showed in Figure 1. From the result, treatment conditions at 6 U/L, 9 U/L and 12 U/L of protease activity, for 30 minutes resulted in the pastes with the highest WHC and WI whilst the paste treated at 6 U/L for 30 min achieved the highest gel strength and treated at 12 U/L for 30 min achieved the highest EC.

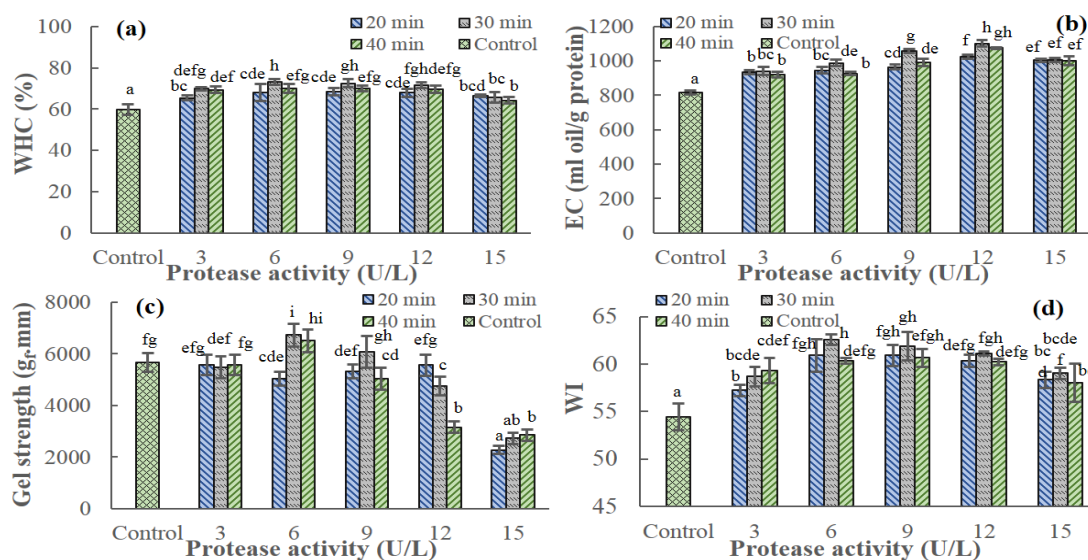


Figure 1. Effect of different protease treatment conditions on water holding capacity (a), emulsifying capacity (b), gel strength (c) and whiteness index (d) of snakehead fish paste. (Control: no protease treatment, washing with salt solution only).

Proteolytic enzymes were known that can increase the quality of meat and meat product when properly used. The appropriate breakdown of myofibril protein can have helped to inducing crosslinking of the protein molecules which ultimately improves their physiochemical and functional properties. In beef tenderization, the increase in solubility of proteins was observed when ficin and actinidin was used at appropriate concentrations, which resulted in an increase in the water holding capacity and texture score. The decreased intensity of the band corresponding to myosin heavy chain (MW = 200 kDa) by SDS-PAGE results, indicating degradation of this protein [5]. Appropriate using of protease from snakehead fish viscera may have the effect to induce the crosslinking between myofibrillar components, thus can improve

WHC and gel strength of snakehead fish paste. The overuse of protease may cause the breakdown of the crosslinking, and ended up in the decreasing of WHC and gel strength. In addition, the appropriate breakdown of myofibril protein (the insoluble components) can also help to extract more heme pigments and other impurities in the washing process, thus increase the whiteness index [5]. Ramezani *et al.* (2003, cited by Aminlari *et al.*, 2009) [5] demonstrated that, the proteolysis of myofibrillar can increase the viscosity of beef emulsions. These proteins may associate along their lengths giving rise to a vast number of total molecular weight permutations. The aggregation of these molecules then increases the viscosity of the emulsion and improves the texture of the final product. The EC of protein related to the ability of this protein to form and maintain a stable sausage emulsion. The EC and WHC are two factors that affecting the stability of the emulsion, thus affecting the texture of the final product [11]. Protease treatment condition at 6 U/L, 9 U/L and 12 U/L for 30 minutes could improve physicochemical properties of snakehead fish paste and had been chosen as ingredients for snakehead fish sausage processing.

3.3. Influence of protease treatment condition on sausage quality

Several tests were applied to assess the physicochemical characteristics and the quality of sausage products. The results were showed in Table 2 and Figure 2. All snakehead fish emulsions and sausage were obtained with high quality. The high level of WHC and ES in all samples indicated the stable emulsions, especially in control 2 and all protease treatment samples (above 95 % for WHC and 95 % for ES). All the treatment samples had the whiteness higher than the controls. The whiteness was the highest from pastes treated with 6 U/L and 9U/L of protease for 30 minutes (60.84 and 61.19). Regarding to packaging of sausage, the adhesion was small and did not meaningfully effect on production efficiency whereas it could steadily affect to the appearance of the sausage skin. The lowest adhesion was observed in sausage processed with paste treatment with 6 U/L of protease for 30 minutes (3.66 mg/cm²) whereas paste treatment with 12 U/L of protease resulted in the highest adhesion (20.54 mg/cm²). The different in the emulsion structure may be the cause for the different in WI and adhesion [15].

Texture characteristic was a wide range of diverse value in all samples. Particularly, sausage processed with paste treated at 12 U/L of protease reach the highest gel strength maybe due to the highest EC as explained above. However, sausage processed with paste treated at 12 U/L of protease for 30 minutes had texture score lower than with paste treated at 6 U/L and 9U/L of protease for minutes although it had the highest gel strength. This result was also observed in Chinese sausage [4], which “too hard” or “too soft” sausage was not accepted among the panelists. On the other hand, sausage processed with paste treated at 9 U/L of protease had the best texture with moderate gel strength and the highest color score due the highest WI. The smoothness of the skin was lower than sausage processed with paste treated at 6 U/L (due to the adhesion on the casing) but sausage processed with paste treated at 9 U/L was widely accepted among the panelists (8.71 score for overall acceptability). Therefore, protease treatment with 9 U/L in 30 minutes was employed in this study.

Table 2. Physicochemical properties of snakehead fish emulsion and sausage.

Parameter	Control 1	Control 2	6 U/L - 30 min	9 U/L - 30 min	12 U/L - 30 min
Snakehead fish emulsion					
Moisture* (%)	55.28 ± 0.28	55.18 ± 0.97	54.75 ± 0.10	55.35 ± 0.19	55.11 ± 0.51
WHC (%)	92.90 ^a ± 1.30	95.04 ^{ab} ± 2.68	96.72 ^b ± 0.40	97.00 ^b ± 0.73	96.02 ^b ± 0.15
WI	42.40 ^a ± 0.52	57.28 ^b ± 0.66	60.84 ^d ± 0.13	61.19 ^d ± 0.19	59.16 ^c ± 0.16
ES (%)	97.32 ^a ± 0.28	99.23 ^b ± 0.21	99.40 ^b ± 0.20	99.02 ^b ± 0.89	99.32 ^b ± 0.20
Snakehead fish sausage					
Moisture* (%)	56.97 ± 0.19	57.34 ± 0.25	55.84 ± 0.42	56.60 ± 0.75	57.36 ± 0.69
WHC (%)	91.79 ^a ± 0.57	95.53 ^b ± 0.46	96.43 ^{bc} ± 0.49	96.91 ^c ± 1.15	96.98 ^c ± 0.15
WI	45.07 ^a ± 0.75	54.13 ^b ± 1.07	57.78 ^d ± 0.44	59.15 ^d ± 0.47	55.59 ^c ± 0.95
Gel strength (gf.mm)	2785 ^a ± 201	4264 ^b ± 121	4528 ^c ± 102	4748 ^d ± 139	5825 ^e ± 122
Adhesion (mg/cm ²)	14.77 ^d ± 0.73	9.97 ^c ± 0.85	3.66 ^a ± 0.67	6.23 ^b ± 0.38	20.54 ^e ± 1.21

Control 1: no treatment, fillet only; Control 2: no protease treatment, only washing with NaCl solution

* No significantly different

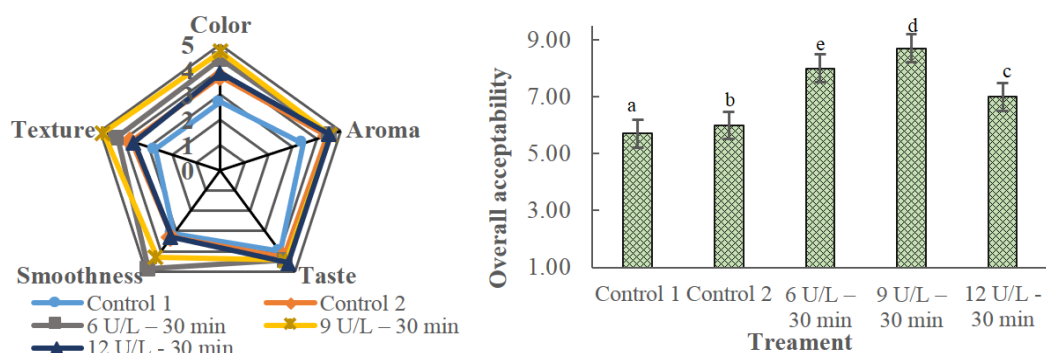


Figure 2. Sensory evaluation of sausages processed with protease-treated snakehead fish pastes. (Control 1: no treatment, fillet only; Control 2: no protease treatment, only washing with NaCl solution)

4. CONCLUSIONS

In this study, the changes in physicochemical properties of snakehead fish protein by viscera protease activity and washing time were determined. Under treatment conditions with 6 U/L, 9 U/L and 12 U/L of protease activity in 30 minutes, the WHC and WI of minced fish was significantly improved. However, the highest gel strength was obtained by treated with 6 U/L of protease activity, 30 min and using 12 U/L of protease increased EC value of product. Among this, the gel quality and sensory properties of sausage product were improved from soaked minced fish in protease solution at 9 U/L for 30 minutes. At the chosen treatment condition, the sensory score of sausage product reached 8.71 points for overall acceptability.

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TÓM TẮT

CẢI THIỆN TÍNH CHẤT HÓA LÝ CỦA XÚC XÍCH CÁ LÓC (*Channa striata*) BẰNG CHẾ PHẨM PROTEASE NỘI TẠNG

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Nghiên cứu được tiến hành nhằm khảo sát khả năng nâng cao chất lượng protein từ thịt cá lóc nuôi (*Channa striata*), đặc biệt ứng dụng trong chế biến sản phẩm sausage, thông qua xử lý bằng chế phẩm protease nội tạng. Sự thay đổi về đặc tính hóa lý (thể hiện qua khả năng giữ nước, khả năng nhũ hóa của protein, đặc tính cấu trúc và độ trắng) của thịt cá lóc ở dạng paste theo hoạt độ protease sử dụng (thay đổi từ 0, 3, 6, 9, 12 U/L) và thời gian xử lý (20, 30, 40 phút) đã được xác định với điều kiện xử lý được cố định theo tỷ lệ nguyên liệu và dung dịch protease là 1:1 (w/v) ở nhiệt độ phòng (30 ± 2 °C). Kết quả nghiên cứu cho thấy rằng đặc tính hóa lý, chất lượng gel và giá trị cảm quan của xúc xích được cải thiện đáng kể khi sử dụng sử dụng thịt cá lóc nghiền đã qua xử lý protease ở hoạt độ 9 U/L trong 30 phút.

Từ khóa: cá lóc, tính chất hóa lý, xúc xích, xử lý protease.