

EXPLORATION OF THE FACTORS AFFECTING THE SOLUBLE PROTEIN EXTRACTION FROM CULTURED SNAKEHEAD FISH (*Channa striata*) MUSCLE

Vo Hoang Ngan^{1,*}, Tran Thanh Truc², Nguyen Van Muoi²

¹*Department of Science and Technology of Vinh Long province, 111 Nguyen Hue street, Ward 2, Vinh Long City, Vinh Long Province*

²*Can Tho University, Campus 2, 3/2 Street, Ninh Kieu District, Can Tho City*

*Email: nganp1115002@gstudent.ctu.edu.vn

Received: 15 August 2017; Accepted for publication: 10 October 2017

ABSTRACT

This study was conducted to identify the factors affecting the soluble protein extraction from cultured snakehead fish muscle, a protein having bioavailability and nutritional value. The study investigated the ratio of fish muscle to water (from 1:1 to 1:5 w/v) and salt (NaCl) concentration (from 0.05 M to 0.25 M) that are suitable for extracting soluble protein effectively. Research has applied a response surface methodology using a central composite design to optimize the effect of temperature and time extraction on soluble protein extraction efficiency. In addition, the study investigated the effect of pH of salt solution (pH 2 to pH 12) on the efficiency of soluble protein extraction from cultured snakehead fish muscle. As a result, the use of 0.15 M NaCl solution with 1:2 ratio of fish muscle to water gave high soluble protein extraction efficiency. The optimum extraction temperature and time were determined as 34.8 °C and 15.2 min respectively, and the appropriate pH value was also determined as pH 10. At the optimum extraction conditions, soluble protein recovery calculated by the amount of soluble protein extraction dividing total protein of fish muscle was 14.39 ± 0.18 %.

Keywords: extraction temperature, extraction time, NaCl, pH, snakehead fish, soluble protein.

1. INTRODUCTION

Fish is a very important source of protein for human nutrition. Among protein sources, fish is believed to provide up to 17 % of the global protein requirement [1]. Snakehead fish has long been known to be the source of protein needed for human consumption, especially for people with poor health. The consumption of snakehead fish protein can help support wound healing, analgesic and additional energy during the recovery process. These values are due to the adequate supply of fish protein, the balance of essential and non-essential amino acids [2]. Recent research by Muhammad (2014) [3] has shown that it is possible to extract soluble protein, mainly albumin, from snakehead fish to produce fish protein concentrate with high protein (20.80 %) and low lipid content (1.78 %). This brings great promise in extracting soluble

protein from cultured snakehead fish for processing of rich nutrient foods with high protein content. In addition, the soluble protein of snakehead fish is also used in medicine - an effective source of functional food to provide protein for patients and has a positive effect in preventing obesity [4]. On the other hand, in the manufacturing industry of surimi, the soluble proteins needed to be removed to increase gel formation, improve the stability and functional properties of proteins in the product [5]. All of this showed that soluble protein in cultured snakehead fish was an indispensable source of raw materials contributing to the development of Vietnam food and pharmaceutical industry in the future. Therefore, the study was conducted to determine the factors that affect the efficiency of soluble protein extraction from cultured snakehead fish.

2. MATERIALS AND METHODS

2.1. Materials and equipments

The research was conducted at the Laboratory of Food Technology, Faculty of Agriculture and Applied Biology, Can Tho University. Major equipment and chemicals used in the study include Visible Spectrophotometer 722 (China), Cold centrifuge (HermLe Labortechnik GmbH Siemensstr., Type: Z232K, Germany), Reagent Folin Ciocalteu (Merck, Germany) and Bovine serum albumin (India).

Cultured snakehead fish (from 400÷700 g) was purchased directly at the farming area in Tam Binh district, Vinh Long province. After collection, the fish was transported live (in a bucket of water) to the laboratory, which took about 1 hour. At the laboratory, live fish was kept stable in the water tank for at least one hour before further processing.

2.2. Extraction process

Snakehead fishes were weighed prior to preliminary processing. Fish was fainted, cut and discharge blood in the water tank (5 minutes to ensure complete separation of blood). Fish after exhaustion of blood was removed fins, skin, viscera, head and wash in 0.5 % NaCl solution. After preliminary processing, fillet was taken to recover fish meat and washed with water at low temperature (5÷10 °C). Cut fish fillets (2 × 2 cm) were put into PE bags (0.2 kg/bag) for freezing at -18 ± 2 °C. Fish meat was stored frozen at least 24 hours before the study.

Frozen fish meat was cut evenly (< 5 mm) prior to the extraction of the water soluble protein in by a heated magnetic stirring according to method of Muhammad et al. (2014) [3]. Protein extraction was performed in 2000 mL glass beaker, each weighing 200 ± 0.1 g. The ratio of minced fish to water, addition of salt, temperature, time and pH of salt solution were investigated. After extraction, the mixture was centrifugated at 4 °C for 20 minutes with 12,000 g centrifugal force [6]. The sample after centrifugation was filtered through filter paper to collect fluid (water soluble protein). In order to investigate the influence of extraction conditions on soluble protein extraction efficiency, 4 experiments were prepared:

Experiment 1: Extracting soluble protein from cultured snakehead fish muscle with a ratio of fish muscle and water from 1:1, 1:2 to 1:5 (w/v). Extraction of soluble protein was performed at room temperature for 40 minutes.

Experiment 2: Extraction was performed by varying concentration of the salt (from 0.05 M to 0.25 M), control with no added salt. Other conditions were fixed as the results of experiment 1.

Experiment 3: Response surface methodology with a central composite design was used to determine the influence of two independent variables (temperature, time) and the optimum conditions of protein extraction. Other conditions were fixed as the results of experiment 2.

Experiment 4: Perform soluble protein extraction when pH of salt solution is changed from pH 2, pH 3 to pH 12, and control sample with no adjusted pH. Other conditions were fixed as the results of experiment 3.

2.3. Proximate analysis

Total protein content was analyzed using Vietnam standard TCVN 3705-90. Soluble protein concentration was determined by the method of Lowry et al. (1951) [7] using bovine serum albumin as a standard protein. Absorbance was measured at 750 nm. Extraction efficiency of soluble protein was expressed as a percentage of total protein in fish muscle.

2.4. Data analysis

Using Statgraphics Centurion 16.1 program, data were analyzed for the degree of variation and significance of difference based on the analysis of variance (ANOVA) to determine if significant differences ($p \leq 0.05$) existed between treatments. Optimization of Extraction Parameters was conducted by Using Response Surface Methodology with central composite design.

3. RESULTS AND DISCUSSION

3.1. Effect of the ratio of fish muscle to water on soluble protein extraction from cultured snakehead fish

The efficiency of soluble protein extraction depends on the proportion of fish muscle and water. If the amount of water is too low, the dissolution and contact of the extracted components with the water is very limited, the diffusion process is very low. This has made the extraction efficiency poorer than other ratios.

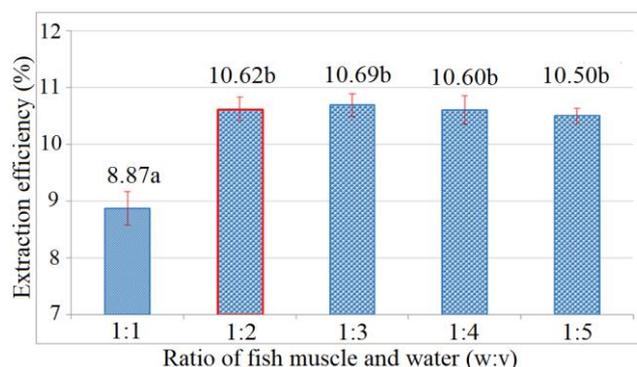


Figure 1. Effect of the ratio fish muscle to water on soluble protein extraction efficiency.

The result from Figure 1 showed that the lowest recovery of soluble protein was by extraction with the ratio of 1:1 (8.87 %), whereas the highest was by the ratio of 1:3 (10.69 %).

In addition, there was no difference in extraction performance when the ratio increased from 1:2 to 1:5. According to Muhammad et al. (2014) [3], the ratio of raw materials and water used in extracting albumin from snakehead fish was 1:1. Increasing the ratio of raw materials and water does not mean increasing the protein content of the extract, this will result in the phenomenon of balance and slow diffusion process despite increased water use. Meanwhile, the study by Lopez-Enriquez et al. (2015) [6] was found the ratio of mince and water was 1:3 (w/v) for the extraction of protein from squid (*Dosidicus gigas*) muscle. From the above result, a fish muscle: water ratio of 1:2 (w/v) was chosen and used for the following experiments to determine the factors affecting the soluble protein extraction from snakehead fish.

3.2. The role of NaCl in soluble protein extraction

The results of Figure 2 show that the use of NaCl solution at different concentrations has a significant change in soluble protein extraction efficiency. When extracted with distilled water, the soluble protein extraction yield was 9.78 % and when supplemented with 0.05 M NaCl, the soluble protein extraction yield increased to 10.87 %. Within certain limit, when the concentration of salt solution increases, the soluble protein extraction performance increases, particularly when NaCl levels increase to 0.1 M and 0.15 M, the amount of dissolved protein increases accordingly 11.17 % and 11.70 %. The soluble protein extraction performance was highest efficiency for 0.15 M NaCl salts, but as the salt concentration more increased, the soluble protein extraction efficiency decreased (10.39 % at 0.25 M NaCl). The solubility of proteins increases at low ionic concentrations because the protein is surrounded by salt ions and reduces the electrical energy of the proteins together "salting-in", thereby increasing the solubilization efficiency of the protein outside the medium of salt solution [8]. In contrast, increasing the salt concentration in the solution will reduce the solubility of the protein due to reduced solvate. This phenomenon is called "salting-out" [9].

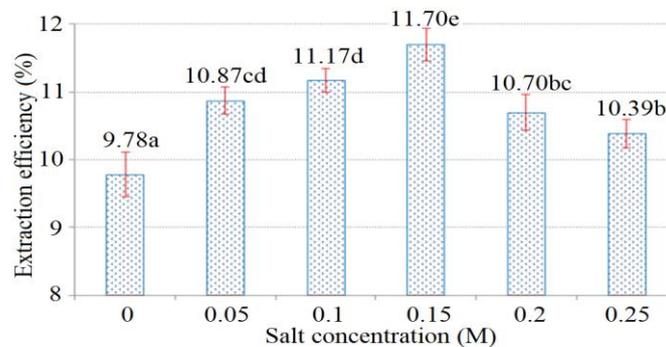


Figure 2. Effect of salt concentration on soluble protein extraction efficiency.

As a result, the concentration of salt solution has a great influence on the efficiency of soluble protein extraction from snakehead fish. To select a concentration of salt to increase the efficiency of extracting soluble protein from fish snake species, 0.15 M NaCl was the most suitable. This result was also consistent with Chang (2010) [10].

3.3. Optimizing the effect of temperature and time of soluble protein extraction

In external surveys, the effect of each factor (temperature, time) on the soluble protein extraction efficiency was determined by the classical method (single-factor design) (unpublished

data). It pointed out that temperature and time very strong impacted on soluble protein extraction. However, the values depended on the “one-variable-at-a-time” approach couldn’t explain the mutual interactions between the independent variables. Thus, in this study, the interactive effects of these two factors selected as key parameters were investigated for further optimization to maximize the soluble protein extraction by response surface methodology approach basing on single-factor design optimization results.

Response surface methodology with central composite design was applied to optimize the variables of the key factors (temperature and time extraction) and the effect of their interactions on soluble protein extraction with the purpose of obtaining the highest soluble protein yield during the extraction process. Based on the central composite design analysis, the experimental design and results are displayed in Table 2. The response of the center point (temperature = 30 °C, time = 20 minutes). The regression equation obtained after the analysis of variance gave the level of response as a function of two independent variables. The final model was obtained by multiple regression analysis of the experimental data and expressed by the following equation:

$$Y = 0.6 + 0.59X_1 + 0.35X_2 - 0.008X_1^2 - 0.005X_1X_2 - 0.006X_2^2.$$

where, Y is the predicted soluble protein extraction efficiency potential (mg soluble protein/100 mg total protein of fish muscle); X₁ and X₂ are the coded values of temperature and time extraction, respectively.

Table 2. ANOVA for the soluble protein extraction potential.

Factors	Sum of Squares	Degree of freedom	Mean Square	F-value	P-value
X ₁ : Temperature, °C	4.57027	1	4.57027	261.37	0.000
X ₂ : Time, minute	2.60681	1	2.60681	149.08	0.000
X ₁ X ₁	4.51001	1	4.51001	257.92	0.000
X ₁ X ₂	2.56688	1	2.56688	146.80	0.000
X ₂ X ₂	3.15005	1	3.15005	180.15	0.000
Blocks	0.04437	2	0.02219	1.27	0.327
Lack-of-fit	0.63511	19	0.03343	1.91	0.160
Pure error	0.15738	9	0.01749		
Total (corr.)	22.02530	35			
R-squared = 96.40 percent; R-squared (adjusted for d.f.) = 95.80 percent					

To validate the statistical results and the model equation, an analysis of variance (ANOVA) was conducted and the results are shown in Table 2. The high F-values and the values of P less than 0.05 implied significant model fit. The "Lack of Fit F-value" of 1.91 implies the Lack of Fit is not significant relative to the pure error. There is a 16 % chance that a "Lack of Fit F-value" this large could occur due to noise. Thus, the lack of fit is insignificant. It meant the model was good. The high value of regression coefficient (R-squared = 96.40 %) and the adjusted R-squared of 95.80 %. These suggested that the regression model was an accurate representation of the experimental data. These findings indicated that the model equation obtained was statistical

for predicting the effects of temperature and time on soluble protein extraction potential. It can also be seen from Table 2 that the linear and quadratic effects of temperature and time, as well as the interactive effect between temperature and time extraction were highly significant ($P < 0.05$). The optimum level of each variable and the effect of their interactions on the extraction were studied by plotting three dimensional response surfaces (Figure 3).

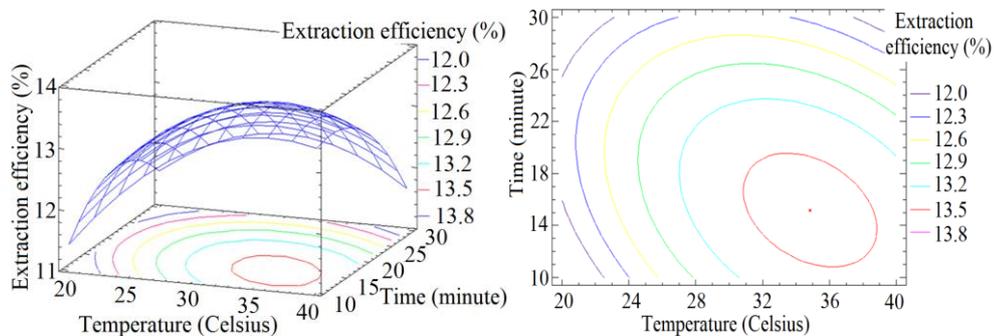


Figure 3. Three-dimensional response plots showing interaction effects of temperature and time on the soluble protein extraction efficiency.

The three-dimensional curves of the calculated responses show the interactions between temperature and time. To further validate optimal values, the optimal values of the variables affected the yield of the hydrogen production given by the software which calculated the equation giving the following results: $X_1 = 34.80$; $X_2 = 15.20$. Therefore, the optimal values of the variables combination were the following: temperature extraction was 34.80 °C, time extraction was 15.20 minutes. According to the results of the statistically designed experiments, the soluble protein extraction was performed under this optimal condition. The maximum predicted value of soluble protein extraction efficiency was 13.61 %, more than previous optimization result by “one-variable-at-a-time” method (12.80 %) (unpublished data). Therefore, the response surface optimization could be successfully used to evaluate the soluble protein extraction efficiency and to achieve higher yield of soluble protein in this study.

3.4. Effect of salt solution pH on the soluble protein extraction

In protein extraction, pH adjustment is also an effective solution for the solubilization of proteins in the material into salt solution, thereby enhancing the protein extraction efficiency. In the study, the pH survey was conducted at pH 2 to pH 12. The results shown in Figure 4 show that protein extraction efficiency was high at pH 3 and pH 10, the extraction efficiency at these pH values was 14.48 and 14.39, respectively. However, if the pH value of the extraction continues to decrease or increase, the soluble protein extraction efficiency will be decreased.

Park (2008) [11] studied protein extraction from sardines in the Pacific Ocean indicating that protein extraction was highly effective at pH 3÷11. However, if protein extraction at lower or higher pH results in protein denaturation and reduced yields of soluble protein. Consequently, the solubility of soluble protein at pH 2 (10.7 %) and pH 12 (10.85 %) was very low. It decreased nearly 4 % compared to the extraction efficiency at pH 3 and pH 10. The results also showed that the soluble protein extraction efficiency was significantly reduced at pH 5 and pH 6, respectively, only about 12.83 % and 13.08 % of the total protein content of fish muscle. This could be explained as the approximate value of the protein's isoelectric point (pI) in snakehead

meat [12] and at this value the total charge of the groups would be zero. Kim et al. (2005) [13] also showed that the solubility of muscle protein from *Sebastes flavidus* was highest at pH 10, while the study also showed that muscle protein was more stable at neutral and alkaline conditions. The efficiency of soluble protein extracts from cultured snakehead fish muscle was 14.39 % of total protein content, not significantly different from that of Lopez-Enriquez et al. (2015) [6] on squid muscle (14.02 %). This result is lower than that of Muhammad et al. (2014) [3] on snakehead fish muscle (albumin content of 15.32 %). However, research results have initially confirmed the ability to obtain soluble protein from snakehead fish in the Mekong Delta.

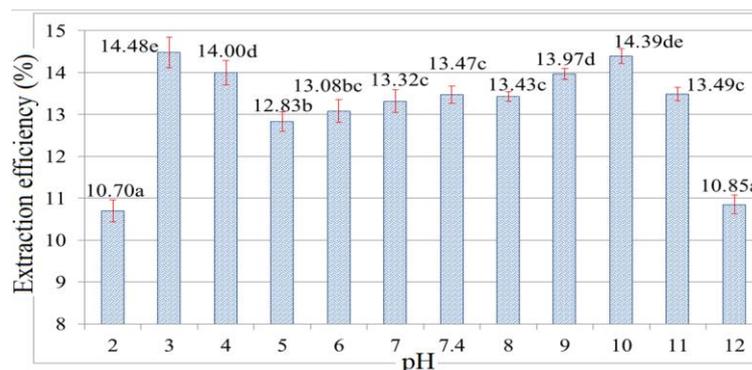


Figure 4. Effect of pH on soluble protein extraction efficiency.

4. CONCLUSIONS

The results of this study have shown the feasibility of soluble protein extraction from cultured snakehead fish muscle towards processing fish protein concentration. The basic parameters of soluble protein extraction from cultured snakehead fish include fish muscle and salt solution ratio of 1:2 (w/v), suitable salt concentration of 0.15 M and pH of salt solution is 10. The response surface methodology helped to determine the optimum temperature and time extraction are 34.80 °C and 15.20 minutes, respectively. Under the appropriate extraction conditions, the efficiency of soluble protein extracted from snakehead fish muscle was 14.39%.

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TÓM TẮT

CÁC YẾU TỐ ẢNH HƯỞNG ĐẾN HIỆU QUẢ TRÍCH LI PROTEIN HÒA TAN TỪ THỊT CÁ LÓC NUÔI (*Channa striata*)

Võ Hoàng Ngân^{1,*}, Trần Thanh Trúc², Nguyễn Văn Mười²

¹Nghiên cứu sinh ngành Công nghệ thực phẩm Khóa 2015, Trường Đại học Cần Thơ, Chuyên viên Sở Khoa học và Công nghệ tỉnh Vĩnh Long, số 111, đường Nguyễn Huệ, P.2, TP. Vĩnh Long, tỉnh Vĩnh Long

²Trường Đại học Cần Thơ, Khu 2, đường 3/2, P. Xuân Khánh, Q. Ninh Kiều, TP. Cần Thơ

*Email: nganp1115002@gstudent.ctu.edu.vn

Mục tiêu của nghiên cứu là xác định các yếu tố có ảnh hưởng đến quá trình trích li protein hòa tan từ thịt cá lóc nuôi - một loại protein có giá trị sinh học và dinh dưỡng cao. Nghiên cứu đã tiến hành khảo sát tỉ lệ nguyên liệu và nước (từ 1:1 đến 1:5, w/v) cũng như nồng độ muối NaCl (từ 0,05 M đến 0,25 M) thích hợp giúp quá trình trích li protein hòa tan đạt hiệu quả cao. Phương pháp bề mặt đáp ứng với mô hình có cấu trúc tâm được sử dụng để tối ưu hóa ảnh hưởng của nhiệt độ và thời gian trích li protein hòa tan. Ngoài ra, tác động của pH dung môi

trích li (từ pH 2 đến pH 12) đến hiệu quả quá trình trích li protein hòa tan từ thịt cá lóc nuôi cũng được khảo sát. Kết quả nghiên cứu cho thấy, sử dụng muối NaCl 0,15 M với tỉ lệ nguyên liệu thịt cá lóc với dung môi là 1:2 cho hiệu quả trích li protein hòa tan cao. Điều kiện tối ưu để trích li thu hồi protein hòa tan đạt được ở nhiệt độ 34,8 °C và thời gian 15,2 phút, đồng thời giá trị pH trích li thích hợp cũng được xác định là pH 10. Tại các điều kiện trích li tối ưu, hiệu suất thu hồi protein hòa tan so với protein tổng số của thịt cá là $14,39 \pm 0,18 \%$.

Từ khóa: cá lóc, muối NaCl, nhiệt độ trích li, pH, protein hòa tan, thời gian trích li.