RESEARCH ON THE SUITABLE PARAMETERS FOR HYDROLYSIS REACTION OF RED MEAT OF STRIPED TUNA (SARDA ORIENTALIS) BY USING COMMERCIAL PROTAMEX

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Received: 15 August 2017; Accepted for publication: 11 October 2017

ABSTRACT

Red meat of Sarda orientalis is one of by-products of tuna processing industry, the source of Sarda orientalis red meat is abundant in Vietnam. Red meat of Sarda orientalis contained 27 % of protein and 0.53 % of lipid, thus, it was classified into lean fish meat and a perfect resource for protein hydrolysate production. The purpose of this research is to hydrolyze Sarda orientalis red meat by using protamex enzyme to obtain protein hydrolysates. The suitable parameters for hydrolysis reaction were reaction time of 4 h, ratio of Protamex enzyme to red meat of 0.4 % (based on the weight of red meat, w/w), and reaction temperature of 55 °C with the maximal value of degree of hydrolysis of 7.03 %. The highest degree of hydrolysis in this research can compare to previous researches.

Keywords: red meat of Sarda orientalis, protamex enzyme, hydrolysis reaction, protein hydrolysates, degree of hydrolysis.

1. INTRODUCTION

Tuna is one of fishery resources with high economic value and it is widely distributed in the sea of Vietnam. The export of Tuna reached at 400 million USD per year and the main fishery export of Vietnam in the sequence is shutchi catfish, shrimp and tuna (Vinatuna.org). Generally, tuna-processing industry develops by year and a large amount of by-products are created, including: head, bone, viscera, red meat, tail, etc. The by-products of tuna-processing industry account for 50 % of raw material. The solid by-products of tuna fillet processing are in the same pattern, and the percentage of red meat in the solid by-products of tuna fillet is 20% [1]. Around 100.000 tons of total weight of by-products of tuna processing industry is create
every year and the weight of red meat is 2,000 tons. The nutritional level of red meat is the highest in comparison to all of by-products of tuna processing industry. Though red meat is high in nutritional value, the utility of this source is not suitable. It is normally used for animal feed processing or sold at the wholesale market with a low price of 4,000 VND/kg to 5,000 VND/kg [2], which leads to the damage after harvest of tuna-processing industry. Biological treatment methods are attracted the interest of research community, especially, using enzymatic method to hydrolyze by-products to obtain protein because of special biological characters and high nutrition level of protein [3]. Using enzyme to hydrolyze protein is common in the fishery processing [4], especially, obtaining protein and fish oil from by-products of fishery processing. Furthermore, hydrolysis enzyme can be used in food industry to change functions and nutritional characters of protein by denaturation process [5]. The applications of hydrolyzed protein in food processing and animal feed processing have interested researchers in the world such as some studies from Kristinson and Rasco [6, 7, 8], Aristotelis [9], etc. Thus, the purpose of this research was determination of parameters for the hydrolysis reaction of red meat of Sarda orientalis tuna to obtain protein by using protamex enzyme (Novozyme, Denmark). The obtained protein will be used to produce nutrition powder to increase economic value of red meat of Sarda orientalis which is a by-product of tuna–processing industry.

2. MATERIALS AND METHODS

2.1. Materials and methods

2.1.1. Materials

Red meat of Sarda orientalis was purchased from Ha Long canned fishery Corporation, Da Nang city, Vietnam. Raw material was divided into block of 5 kg, preserved in styrofoam box at 0 - 4 °C before transporting to laboratory. Red meat of Sarda orientalis was preserved at -20 °C ± 2 °C in refrigerator at laboratory.

2.1.2. Protamex enzyme

Protamex enzyme was provided by STECH International Co., Ltd. Protamex enzyme is endopeptidase, which was synthesized by Bacillus bacteria. It is allowed to use in food based on regulation of FAO and WHO. The technical parameters of Protamex enzyme is 1.5 AU (Anson Units/g) of enzyme activity, 5.5 – 7.5 of the optimal pH, and 45 °C - 65 °C of the optimal temperature. The optimal preservation temperature of protamex enzyme is in the range of 0 °C - 5 °C [9].

2.2. Methods

2.2.1. Determination of optimal parameters for hydrolysis reaction of red meat of Sarda orientalis by using protamex enzyme

Red meat of Sarda orientalis was thawed at room temperature to reach temperature of center point of block of -1 °C - 0 °C. Sarda orientalis red meat was milled into particle size of 3 mm – 3.5 mm, thoroughly mixed, and divided into blocks of 100 g for further experiments. Enzyme activity of Protamex enzyme was regularly verified and results showed that enzyme activity of protamex enzyme did not decrease during preservation time and this research was
carried out in a short time, thus, percentage was used as a unit of ratio of protamex enzyme to red meat of *Sarda orientalis*. Hydrolysis reactions were carried out at natural pH of red meat because of the optimal pH of protamex enzyme in the range of 5.5 to 7.5, and the random of ratio of distilled water to red meat of *Sarda orientalis* was selected at 1:1.

- Optimal reaction time determination: Hydrolysis reactions were carried out with reaction time in the range of 0 h to 5 h (1 h of increments), reaction temperature of 45 °C ± 2 °C, ratio of protamex enzyme to *Sarda orientalis* red meat of 0.3 % (based on the weight of red meat), natural pH of red meat, and ratio of red meat to distilled water of 1:1.

- Optimal ratio of protamex enzyme to red meat of *Sarda orientalis* determination: Conditions for hydrolysis reaction was set at optimal reaction time, ratio of protamex enzyme to *Sarda orientalis* red meat in the range of 0.1 % to 0.7 % (0.1 % of increments), reaction temperature of 45 ± 2 °C, and natural pH of red meat.

- Optimal reaction temperature determination: Hydrolysis reactions were performed at optimal reaction time, optimal ratio of protamex enzyme to red meat of *Sarda orientalis*, reaction temperature in the range of 35 °C to 60 °C (5 °C of increments), and natural pH of red meat.

Protamex enzyme was inactive at 95 °C in 15 min for all of hydrolysis reactions. The yield of hydrolysis reaction was determined via two factors: nitrogen amino acids content (%), based on the weight of red meat of *Sarda orientalis* and degree of hydrolysis (DH). Degree of hydrolysis was calculated by equation (1):

$$ DH = \frac{N_{AA} - N_{AA0}}{N_{OA} - N_{AA0}} \times 100\% $$

where, $N_{AA}$ is nitrogen amino acids content (%), based on the weight of red meat of *Sarda orientalis*, $N_{AA0}$ is nitrogen amino acids percentage in *Sarda orientalis* red meat (%), and $N_{OA}$ is total nitrogen in red meat (%). Nitrogen amino acids content and degree of hydrolysis was selected to remark hydrolysis reaction because of high exact degree and low labor consume. The target for optimal parameters of hydrolysis reaction of *Sarda orientalis* red meat was the lowest of nitrogen amino acids content and the highest degree of hydrolysis.

2.2.2. Analysis methods

Proximate compositions of red meat, including protein content, lipid content, moisture content, and ash content, was analyzed by NMKL No.6, 4th 2003 method; NMKL No. 31, 1989 method; TCVN 3706-1190 method; and NMKL No.173, 2nd Ed., 2005 method; respectively. Nitrogen amino acids content and total nitrogen was determined by TCVN 3708-1990 method and NMKL No.6, 4th Ed., 2003 method.

2.3. Statistical analysis

All of experiment were repeated three time, three samples was performed for one time. Microsoft Office Excel software was used for solving collected data (significant mean of statistic with P value > 0.05).

3. RESULTS AND DISCUSSION

3.1. Analysis of proximate compositions of *Sarda orientalis* red meat

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Red meat of *Sarda orientalis* contents the highest percentage of protein (27 %). Protein content in red meat of *Sarda orientalis* is higher than other fish meat: 19.25 % of protein content in meat of *Sardinella* [9], 17 – 21 % of protein content in meat of *Squid* [10, 11]. The second composition found in red meat was others with 3 %, followed by ash with 2 %. The lowest percentage belonged to lipid content with 0.53 %, thus, *Sarda orientalis* red meat is classified into lean fish meat and red meat of *Sarda orientalis* was a perfect material for protein hydrolyzed production.

![Figure 1. Proximate compositions of red meat of *Sarda orientalis*.](image1)

### 3.2. Determination of optimal parameters for hydrolysis reaction of red meat of *Sarda orientalis* by using Protamex enzyme

#### 3.2.1. Optimal reaction time determination

The effect of reaction time on degree of hydrolysis was carried out in order to determine optimal reaction time when protein (polymer) of red meat of *Sarda orientalis* was hydrolyzed by protamex enzyme to release peptide and free amino acids (monomer). The effect of reaction time on nitrogen amino acids content and degree of hydrolysis was shown in Figure 2.

![Figure 2. The effect of reaction time on nitrogen amino acid content (a) and degree of hydrolysis (b).](image2)

Nitrogen amino acids content and degree of hydrolysis increased with increasing reaction time. They dramatically increased at 1 h of reaction time and their increase was slow afterward. Nitrogen amino acids content and degree of hydrolysis increased from 0.343 % to 0.575 % and 4.38 % to 5.75 % corresponding to an increase of reaction time from 1 h to 4 h, respectively.
Significant difference of degree of hydrolysis does not have between hydrolysis reaction with 1 h of reaction time and 2 h of reaction time. Results of previous researches had indicated that degree of hydrolysis increased with increasing reaction time [12, 13, 14, 15, 16, 17]. Optimal reaction time was determined to obtain desirable product (protein) by breaking linkages of substrate and avoiding creating low degree of hydrolysis products: NH₃, H₂S, indol, scaptol ... which were negative effect on final products. Amino acids were converted into secondary products of hydrolysis reaction (NH₃, H₂S, indol, scaptol …) by rotten microorganism [9]. Based on experimental results, optimal reaction time was selected at 4 h to increase economic value of production with large scale.

3.2.2. Optimal ratio of protamex enzyme to Sarda orientalis red meat determination

The effect of ratio of protamex enzyme to red meat of Sarda orientalis on nitrogen amino acids content and degree of hydrolysis was shown in Figure 3. Nitrogen amino acids content increased from 0.376 % to 0.628 % when ratio of protamex enzyme to Sarda orientalis red meat increased from 0.1 % to 0.4 % (Fig. 3 a). The degree of hydrolysis was in the same pattern, increasing from 0.66 % to 7.03 %.

However, nitrogen amino acids content and degree hydrolysis significantly decreased with increasing ratio of protamex enzyme to Sarda orientalis red meat from 0.5 % to 0.7 %. Previous researches showed that nitrogen dissolution increased with an increase of ratio of enzyme to substrate, however, obtained nitrogen concentration decreased with increasing ratio of enzyme to substrate because degree of break linkage of peptide increased when enzyme concentration increase [15, 18, 9].

Degree of hydrolysis increased when ratio of enzyme to substrate increased because enzyme catalyzed linkage break to release low degree products of hydrolysis reaction [19]. Ratio of protamex enzyme to Sarda orientalis red meat of 0.4 % had the perfect of nitrogen amino acids content and degree of hydrolysis; thus, it was selected for optimal reaction temperature determination.

3.2.3. Optimal reaction temperature determination

According to Nguyen Duc Luong, temperature has significant affected to velocity of
reaction catalyzed by enzyme and temperature can be used as an important parameter to control the action of enzyme and velocity of reaction in food preservation and food processing [20]. Reaction temperature has significantly affected to degree of hydrolysis in hydrolysis reaction of Sarda orientalis red meat by using Protamex enzyme.

![Figure 4. The effect of reaction temperature on nitrogen amino acids content and degree of hydrolysis.](image)

The remarkable increase of degree of hydrolysis was from 3.68 % to 4.17 % when reaction temperature increased from 35 °C to 40 °C. Degree of hydrolysis decreased with increasing reaction temperature from 40 °C to 45 °C. Afterward, degree of hydrolysis gradually increased with reaction temperature in the range of 45 °C to 55 °C and reached the highest value of 4.79 at 55 °C. Nitrogen amino acids content and degree of hydrolysis started decreasing when reaction temperature was higher than 55 °C. Nitrogen amino acids content and degree of hydrolysis in hydrolysis of reaction of red meat of Sarda orientalis was in the same pattern of hydrolysis of reaction of Atlantic salmon meat and Sardinella meat. Velocity of hydrolysis reaction increased with an increase of reaction temperature because enzyme molecules have high kinetic energy at high temperature, leading to increase the interaction between enzyme and substrate. Thus, velocity of hydrolysis reaction increased and reached maximal value at the optimal reaction temperature of protamex enzyme in the range of 50 - 55 °C. However, reaction temperature increased higher than 50 °C, leading to decrease activity of Protamex enzyme and denature Protamex enzyme [9, 21]. Based on obtained results, the optimal reaction temperature was 55 °C and action of enzyme at this temperature was perfect.

4. CONCLUSIONS

In conclusion, red meat of Sarda orientalis was rich in protein and it was classified into lean fish meat. Specially, protein content of Sarda orientalis red meat was 27 % and minor lipid (0.53 %) was found in red meat of Sarda orientalis. Thus, Sarda orientalis red meat was a perfect resource to produce protein hydrolysates. The optimal parameters for hydrolysis reaction of Sarda orientalis red meat by protamex enzyme to obtain protein hydrolyzed solution was 4 h of reaction time, ratio of protamex enzyme to Sarda orientalis red meat of 0.4 % (based on the weight of Sarda orientalis red meat), and reaction temperature of 55 °C. Furthermore, optimization of hydrolysis reaction of Sarda orientalis red meat by protamex enzyme to obtain protein hydrolysates should be carry out to determine the optimal conditions and characters of protein hydrolysates need to be determined.
REFERENCES


**TÓM TÁT**

NGHIÊN CƯ Ý ĐIỀU KIỆN THÍCH HỢP THỦY PHÂN THỊT ĐỎ CÁ NGỪ SỌC DƢA (SARDA ORIENTALIS) BẰNG ENZYME PROTAMEX THƯƠNG MẠI

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Thịt đỏ cá ngừ sok dura (*Sarda orientalis*) là nguồn phụ phẩm của ngành công nghiệp chế biến cá ngừ với trữ lượng lớn ở Việt Nam. Thịt đỏ cá ngừ sok dura có hàm lượng protein chiếm 27,0 % và hàm lượng lipit chiếm 0,53% trong lượng nguyên liệu nên được coi là nguồn nguyên liệu thích hợp để sản xuất dịch đạm thủy phân. Mục đích của nghiên cứu này là thủy phân thịt đỏ cá ngừ sok dura bằng phương pháp sử dụng enzyme protamex để sản xuất dịch đạm thủy phân. Các thông số tối ưu của phân ứng thủy phân xác định được là thời gian phân ứng 4 giờ, nồng độ enzyme protamex 0,4 % (tính trên khối lượng thịt đỏ) và nhiệt độ môi trường phân ứng là 55 °C, ở điều kiện tối ưu mức độ thủy phân đạt 7,03 %. Mức độ thủy phân cao nhất trong nghiên cứu này xác định được tương đương với các nghiên cứu trước đây của Batista và Trần Thị Bích Thúy.

*Từ khóa:* thịt đỏ cá ngừ sok dura (*Sarda orientalis*), enzyme protamex, phân ứng thủy phân, dịch đạm thủy phân.