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RESEARCH ON PROTEIN HYDROLYSIS FROM SHRIMP WASTE USING COMMERCIAL PROTEASES

Tran Thi Linh*, Pham Thu Thuy

School of Biotechnology and Food Technology, Hanoi University of Science and Technology, 1 Dai Co Viet Road, Hanoi

*Email: *linhtt35@gmail.com*

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ABSTRACT

Protein hydrolysates were produced from shrimp waste mainly comprising head and shell by different commercial proteases (Alcalase, Protamex, Flavourzyme). Shrimp waste was mixed with water (ratio of 1:5 w/v) and was treated with protease individually or together, which were incorporated either simultaneously or sequentially. Temperature was kept at the optimum for each enzyme. pH was initially adjusted to the most favorable value for each enzyme. Result of study showed that in case of individial addition, the highest yield of protein recovery (YPR 48.88 %) was obtained with Alcalase (375 U/g material) at 50 °C, pH 8 for 4 hours. Besides, the maximal yield of amino acids recovery (2.91 %) was obtained with Flavourzyme (250 U/g material) at 50 °C, pH 7 for 4 hours. Specially, the sequential treatment with Alcalase and Flavourzyme improved significantly the hydrolysis of protein. The yield of protein recovery (YPR 49.13 %) and yield of amino acids recovery (YAR 6.91 %) were obtained by addition of Flavourzyme 6 hours after Alcalase, which is attributed to the combined nature of the endo- and exocatalytic action of Alcalase and Flavourzyme, respectively. This research shows the feasibility of hydrolyzing shrimp by-products using different commercial proteases.

Keywords: shrimp waste, enzyme hydrolysis, Alcalase, Flavourzyme, protein recovery.

1. INTRODUCTION

According to the Aquaculture Culture Asia-Pacific Magazine, global production of farmed shrimp increased from 3.4 million tonnes in 2013 to 3.6 million tonnes in 2014. Asian producers had the lion share at 3 million tonnes, whereas production in the Americas was estimated at 671 000 tonnes. In export trade, India was the lead supply source exporting 383 000 tonnes and Viet Nam was the second in 2015. However, shrimp processing industry produced considerable amount of waste depending on the species and processing method applied. With increasing competition on world markets there is a need to develop value-added products from the waste material to help maintain the economic viability of the industry as well as reduce environmental pollution [1]. The most important waste materials in shrimp processing industries are head and shell wastes, which comprise about 40 to 45 % of the whole shrimp weight. The shrimp waste is rich source of protein, fat, chitin and mineral. Recovery of protein fraction from the shrimp

waste by enzymatic hydrolysis has been widely studied [1, 2, 3] The enzymatic hydrolysis modifies physicochemical, functional and /or sensory properties of native protein without lost nutritional value [4]. These microbial enzyme preperations such as Alcalase, Neutrase, Protamex, Flavourzyme are used efficiently in the hydrolysis of shellfish proteins [5]. The proper combination of industrial Alcalase and Flavourzyme improved the degree of hydrolysis DH and free amino acid contents compared to individual one [6]. Desirable functional properties, high nutritive value and reduced bitterness shrimp waste hydrolysates were produced under controlled conditions [4]. It makes vast resources into useful food ingredients for direct human consumption [5]. The objective of this study was to optimize extraction procedure of protein from shrimp waste using commercialproteases i.e. high protein recovery yield and high amino acids recovery yield.

2. MATERIALS AND METHODS

2.1. Materials

Shrimp waste was from Haiphong Export Sea products processing Company - SPC (Haiphong, Viet Nam) and was stored at -20 °C until further used. Prior to the hydrolysis, the sample was divided in each part and was packed in PE bags overnight in refrigerator at 4 °C. The shrimp waste was defrosted, dried and grinded and was used as raw material (protein 35.81 %, W = 10.05 %) for experiment.

The commercial protease enzymes preparations Protamex 1.5 MG, Alcalase 2.4L and Flavourzyme 500 MG were obtained from Novozyme (Denmark). They were stored at 4 °C until further used.

2.2. Methods

2.2.1. Enzymatic hydrolysis

The shrimp wastes were mixed with water.The pH of the mixture was adjusted to pHas indicated in text by adding HCl 2N. All reactions were performed in 100 ml glass vessels attemperature as indicated in text. After hydrolysis, the reaction was stopped by heating upto 90 °C for 5 - 10 min. The hydrolysates were centrifuged at 6000 rpm for 20 min to collect the soluble fraction in supernatant. The soluble fraction was used for protein and aminoacid quantification.

2.2.2. Calculation of protein recovery yield (YPR) and amino acids recovery yield (YAR)

The total protein content in soluble fractions was determined using Biuret method using BSA as standard protein. The amino acid content in soluble fraction was determined by Ninhydrin method.

YPR(%) = [total protein in soluble fraction (g)/ total protein of shrimp waste (g)] × 100

YAR (%) = [total amino acids in soluble fraction (g)/ total protein of shrimp waste (g)] × 100.

3. RESULTS AND DISCUSSION

3.1. Effect of pH on protein hydrolysis

The protein hydrolysis was carried out as described in 2.2.1 at pH range for each enzyme as indicated by manufacture (Alcalase pH 7-8.5; Protamex and Flavourzyme pH 6 - 7.5) at 50 °C at enzyme dose 125 U/g raw material, ratio shrimp waste/water 1/5. The results from Figure 1 indicated that the optimum pH for protein recovery and aminoacid recovery of Alcalase was 8 and of Protamex and Flavourzyme was 7. Optimum pH of Alcalase for protein recovery was found 8 ÷ 8,5 by Mukhin and Novikov [7]; 8 by Nchienzia [6]; 8.25 by Dey and Dora [8]. Optimum pH of Flavourzyme was reported by Nchienzia [6], Satya [8].

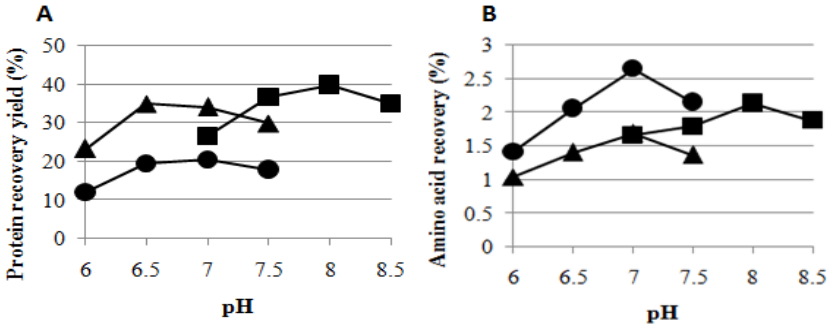

Figure 1. Effect of pH on protein hydrolysis. Alcalase (■); Protamex (▲) and Flavouzyme (●).

3.2.Effect of temperature on protein hydrolysis

The protein hydrolysis was carried out as described in 2.2.1 at temperature range for each enzyme as indicated by manufacture (Alcalase, Protamex and Flavourzyme 35 – 50 °C) at pH 8 for Alcalase and pH 7 for Protamex and Flavourzyme at enzyme dose 125 U/g raw material, ratio shrimp waste/water 1/5. The results from Figure 2 indicated that the optimum temperature for protein recovery and aminoacid recovery of Alcalase, Protamex and Flavourzyme was 50 °C. Optimumtemperatureof Alcalase for protein recovery and of Flavourzyme for amino acid yield was 50 °C according to Nchienzia [9].

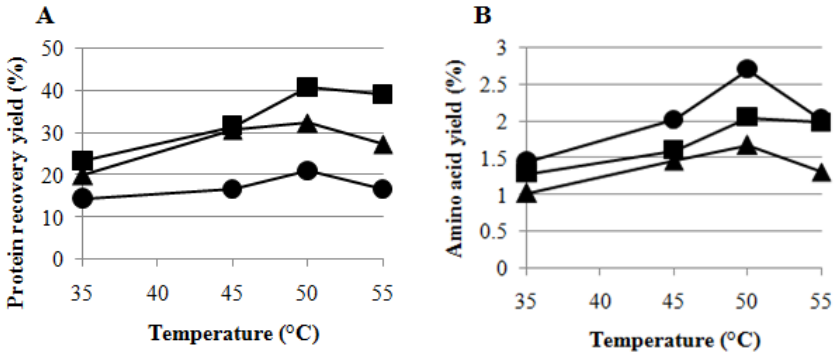

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Figure 2. Effect of temperature on protein hydrolysis. Alcalase (■), Protamex (▲) and Flavouzyme (●).

3.3. Effect of ratio of shrimp waste and water on proteinhydrolysis

Different ratio of shrimp waste/ water (w/v) 1:4; 1:5; 1:6 was used for hydrolysis. Alcalase, Flavourzyme and Protamex with the enzyme dose125 U/g of raw material was added.

Hydrolysis was performed at 50 °C, pH 8 for Alcalase and pH 7 for Protamex and Flavourzyme. The results presented on Figure 3 showed that the higher ratio of resulted on higher protein recovery yield for all three investigated enzymes. Alcalase gave highest protein recovery yield whereas Flavourzyme gave the lowest one. The obtained protein recovery yield with ratio 1:6 for Alcalase 40.66 %; Protamex 33.42 % and Flavourzyme 23.92 % (Figure 3). Howerver the protein recovery yield was only slightly reduced at ratio 1:5. Therefore for higher protein concentration in hydrolysate, ratio 1/5 was chosen. Herpandi *et al.* (2012) demonstrated also that Alcalase gave the higest yields of protein recovery from Skipjack Tuna among all proteases followed by Protamex, Flavourzyme and Neutrase with ratio of shrimp waste/ water (w/v) 1:5 [5]. In contrast, Nchienzia found similar protein recovery yield by Protamex and Alcalase. The different of poultry meat and shrimp meat might explain this phenomenom [6]. From all date (Figure 1, 2, 3), Alcalse and Flavourzyme were chosen for further study.

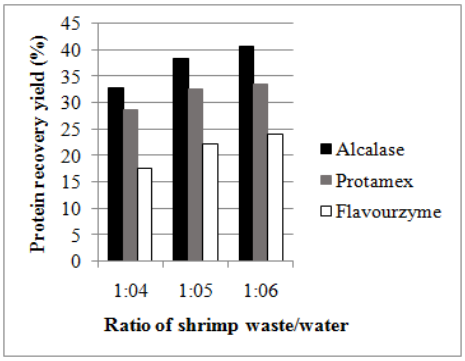

Figure 3. Effect of ratio of shrimp waste / water on protein hydrolysis.

3.4. Effect of protease dose on protein hydrolysis

The hydrolysis of shrimp waste was carried for 4h with different enzyme doseof Alcalase and Flavourzyme, which varied from 125 to 500 U/g raw material.

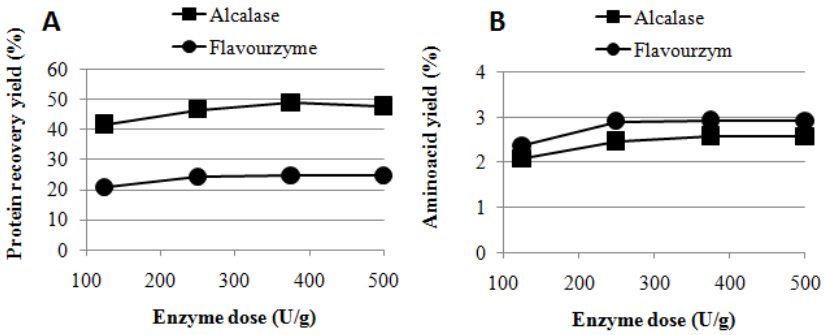

Figure 4. Influence of enzymedose on protein hydrolysis.

The results in Figure 4demonstrated an increase of protein recovery yield with the increament of protease dose.At all enzyme concentration studied, Alcalase always gave higher protein recovery yield than Flavourzyme. This result was in accordance with previous for the enzymatic hydrolysis of Skipjack Tuna [5] and shrimp waste [8]. Alcalase with 375 U/g gave the

highest protein recovery yield 48.88 %, which was 1.98 timeshigher to that of Flavourzyme (24.61 %). However, Flavourzyme at the same enzyme dose always gave higher aminoacid yield, e.g. at 250 U/g gave amino acidsyield 2.91 % which was higher than Alcalase 2.47 %. The results were in agreement with prevous studies and with properties of protease since Alcalase is endoprotease whereas Flavourzyme is a mixture of exo- and endoprotease. It should be mentioned that though amino acid yield of protein hydrolysates by Flavourzyme was not much higher than Alcalase, the ratio of amino acid to total protein in soluble fraction by Flavourzyme and Alcalse were 11.59 % and 5.57 %. Therefore the hydrolysates by Flavourzyme possessed much better sensory properties.

3.5. Kinetic of protein hydrolysis

Hydrolysis of shrimp waste with Alcalase and Flavourzyme were caried out at initial enzyme dose 375 U/g weigh of raw material. The protein hydrolytic curves obtained are given in Figure 5. The plot illustrated that the enzymatic hydrolysis was characterized by an initial rapid increase in the protein recovery yield during 4 hrs, which is an indication of a large number of peptide bonds being cleaved. The rate of the enzymatic hydrolysis subsequently decreased until the end of experiment. After 8 hrs of hydrolysis, Alcalase gave the yield of protein recovery 47.64 %, which was 1.67 times higher than Flavourzyme (28.46 %).The protein recovery yield by Alcalase in this study was similar to previous study by Mizani et al., who achieved protein recovery yield of 47.1 % [3] but lower than that of study by Satya (59 – 60 %) [8].

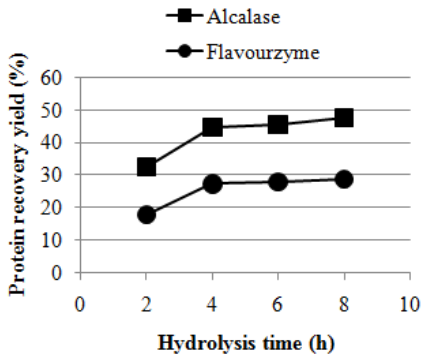

Figure 5. Kinetic of protein hydrolysis of shrimp waste by proteases.

3.6. Protein hydrolysis of shrimp waste by combination of Alcalase and Flavourzyme

In oder to improve the protein hydrolysis, the combination of Alcalase and Flavourzyme were used. From above results, it was revealed that for the combination of two these protease, temperature 50°C was optimum. The pH for both enzyme should be in range 7-8. In our unpublished study, protein recovery yield was highest at pH 7.5 and slightly lower (about 1 %) at pH 7 and 8. For maximum activity of Flavouzyme, protein hydrolysis was performed at pH 7. The ratio of shrimp waste/water 1:5 and Alcalase dose 375 U/g raw material were kept. Flavourzyme at 250 U/g raw material was added simultaneously or sequentiallyafter 4 hrs or 6 hrs. Hydrolysis was prolonged for 12 hrs and protein recovery and amino acidrecovery was determined (Figure 6).

The simultaneous use of Alcalase and Flavourzyme produced a lower protein recovery yield and lower amino acid yield than when they were added sequentially (Figure 6). The protein recovery was even lower than compared to Alcalase alone (42.75 compared with 46.65 %, Fig. 4A). However sequential adding Flavourzyme at latter stage i.e at 4 or 6 hours after Alcalasegave higherprotein recovery yield than using Alcalase alone (49.13 - 48.55 % compared with 46.65 %), though was similar to that of Alcalase alone at pH optimum 8 for Alcalase (48.88 %, Figure 5).The combination of these proteases produced a significant increase in amino acid yield in comparison to Flavourzyme alone even in simultaneous mode (5.73 % compared with 3.07 %) indicating the role of Flavouzyme in production of amino acid. Specially when Flavourzyme was added at 6 hours after Alcalase, the amino acid yield was highest 6.91 %. The ratio of amino acid to protein was 14.24 %, 3-fold higher than Alcalase alone 5.27 %, which could improve the sensory properties of hydrolysate. Starting the hydrolysis withAlcalase, which degraded proteininside the protein chain led to increasing the number of N-terminal sites available for the exopeptidase action of Flavourzyme. Nchienzia *et al* [7] found that combination of Alcalase and Flavourzyme for hydrolysis of poultry meal gave a higher protein recovery yield (58.1 % compared to 46.77 %) and a higher free amino acids than using Alcalase andFlavourzyme alone.Differing from Nchienzia, who used pH 8 for Alcalase and pH 7 for Flavourzyme, in our study pH 7 was used for both enzyme thus facilitate the protein hydrolysis process.

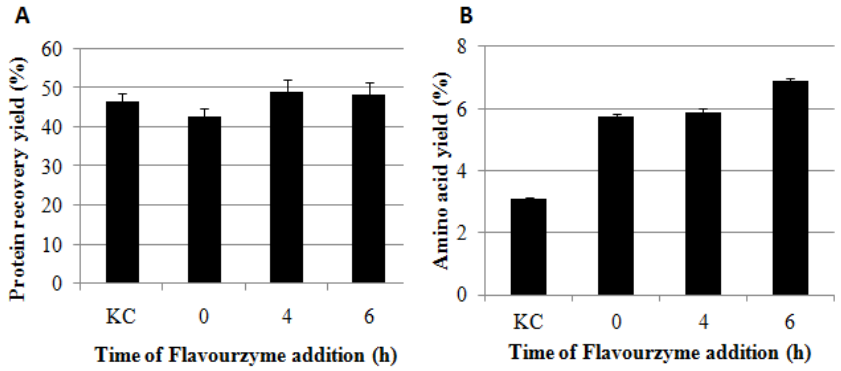

Figure 6. Protein hydrolysis of shrimp waste by combination of Alcalase and Flavourzyme. (A) KC was symbolized for protein hydrolysis by Alcalase alone; (B) KC was symbolized for protein hydrolysis by Flavourzyme alone.

4. CONCLUSION

Combination of Alcalase and Flavouzyme is needed to improve the protein hydrolysis. The time of addition Flavourzyme seemed important. The simultaneous or sequential mode of adding Flavourzyme affected the protein recovery yield as well as aminoacid yield. Whereas simultaneous adding of Flavourzyme could improve the aminoacid recovery yieldbut not the protein recovery yield. Sequential addition of first Alcalase and then Flavouzyme after 6 hrs proved to be most succesful in term of protein and aminoacid recovery. The study indicated pH7 could be used for both Alcalase and Flavouzyme to improve the protein and amino acid recovery yield.

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TÓM TẮT

NGHIÊN CỨU THỦY PHÂN PROTEIN PHẾ LIỆU TÔM BẰNG CHẾ PHẨM PROTEASE THƯƠNG MẠI

Trần Thi Linh*, Phạm Thu Thủy

Viện Công nghệ sinh học và Công nghệ thực phẩm, Trường Đại học Bách khoa Hà Nội, 1 Đại Cồ Việt, Hà Nội

*Email: *linhtt35@gmail.com*

Dịch thủy phân protein đã được thu nhận từ phế liệu tôm bao gồm chủ yếu đầu và vỏ tôm bằng cách thủy phân bởi các chế phẩm protease thương mại (Alcalase, Protamex, Flavourzyme). Phế liệu tôm khô được trộn với nước theo tỉ lệ 1:5 w/v và được thủy phân bằng protease riêng rẽ hay kết hợp cùng một lúc hoặc theo trình tự. Nhiệt độ được giữ tối ưu cho từng enzym. pH dịch thủy phân được điều chỉnh phù hợp cho từng enzym. Kết quả nghiên cứu đã chỉ ra rằng nếu enzym được sử dụng riêng rẽ, hiệu suất thu hồi protein cao nhất (YPR 48.88 %) thu nhận được

khi dùng Alcalase thủy phân với liều lượng 375 U/g ở điều kiện 50 °C, pH 8 trong thời gian 4 giờ. Bên cạnh đó, hiệu suất thu amino acid cao nhất 2,91 % có thể thu nhận khi dùng Flavourzyme với liều lượng 250 U/g nguyên liệu thủy phânở điều kiện 50 °C, pH 7 trong thời gian 4 giờ. Đặc biệt việc sử dụng kết hợp Alcalase và Flavourzyme theo trình tự Alcalase trước còn Flavorzyme sau 4 - 6 tiếng đã cải thiện quá trình thủy phân đáng kể. Hiệu suất thu hồi protein cao nhất (YPR 49,13 %) vàhiệu suất thu amino acid cao nhất (YAR 6,91 %) đã nhận được khi bổ sung Flavourzyme 6 giờ sau khi bổ sung Alcalase. Nghiên cứu này đã cho thấy khả năng thủy phân phế liệu tôm bằng các chế phẩm protease để thu hồi protein.

Từ khóa: phế liệu tôm, thủy phân bằng enzym, Alcalase, Flavourzyme, thu hồi protein.