QUALITY OF FISH OIL EXTRACTED FROM BARRAMUNDI BY-PRODUCT BY ENZYMATIC HYDROLYSIS METHOD

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ABSTRACT

Study on the fish oil extraction from barramundi by-product by enzymatic hydrolysis method was carried out. The parameters of hydrolysis process, oil recovery and chemical quality including free fatty acid content, acid value, peroxide value, iodine value and saponification value of fish oil were determined. The study results showed that a considerable amount of oil can be extracted from barramundi by-product. The optimal parameters of enzymatic hydrolysis process for recovering the fish oil were the water/material ratio of 50%, enzyme/material ratio of 0.3%, hydrolysis temperature of 60^0C and hydrolysis time of 2 hours. The fish oil obtained from hydrolysis of barramundi by - product in optimum conditions was rich in omega-3 fatty acids, especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The major fatty acids in barramundi by-product oil were palmitic acid, oleic acid and docosahexaenoic acid. This study suggested that the barramundi by-product generated from fish processing industry can be utilized as a good source for fish oil recovery. Barramundi by-product oil can be used in aquaculture feed or considered as a valuable source for human consumption.

Keywords: *Barramundi by - product, enzymatic hydrolysis, fish oil extraction, quality of oil.*

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1. INTRODUCTION

Barramundi (*Lates calcarifer*) is one of the most favorite seafood in many countries of the world. Large quantities of barramundi were processed as frozen products, and in consequence a large amount of by-products (Head, skeleton, viscera, skin, trimmings, fins) was generated. Barramundi is a rich source of lipids. There is a great potential in marine bioprocess industry to convert and utilize this by -product into value - added food ingredients. One possible way to use the barramundi by-products is to extract the fish oil for the production of aquaculture feed.

The common methods used for extraction of fish oil consist of chemical extraction, cooking and pressing and enzymatic hydrolysis. The chemical extraction of fish oil is complicated, where the solvents used for extraction have to be separated requiring excess energy. The fish oil has been produced by cooking, pressing followed by centrifugal separation. This traditional method is very harsh on lipids and can lead to faster degradation of lipids. Enzymatic hydrolysis method used for oil extraction has many advantages, such as the mild hydrolysis conditions, low energy requirement, no use of solvent. The low hydrolysis temperatures minimize the oxidation of polyunsaturated fatty acids. Thus, the enzymatic extraction of oil from barramundi by-product was used in this study.

Enzymatic tissue disruption may be a valid alternative technique for releasing natural lipids from fish. During the enzymatic hydrolysis, the combination between lipid and protein was break down, which lead to fish oil release much easier from fish by-product [1]. Enzymatic hydrolysis was used to extract the fish oil using commercial proteases. Several enzymes such as Alcalase, Neutrase, Protamex and Flavourzyme can be used to extract fish oil. However, Alcalase was the best

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enzyme for the extraction of oil from fish and fish by-product [2], [3]. Therefore, in this study, Alcalase was chosen for the extraction of oil from barramundi by-product.

In the process of enzymatic hydrolysis, the factors such as water to material ratio, enzyme to material ratio, hydrolysis temperature and time influence fish oil extraction. The purpose of this study was to determine the optimal hydrolysis conditions for maximum oil recovery from barramundi by-product using Alcalase and to value the chemical quality of fish oil with various criteria, including free fatty acid, acid value, peroxide value, iodine value, saponification value and fatty acid composition.

2. MATERIALS AND METHODS

2.1. Materials

Barramundi by-product including head and skeleton of fish were provided by a seafood processing company in Nha Trang, Vietnam. After filleting at the company, barramundi by - product were stored with ice at 0 - 4^0C and transported immediately to the laboratory. The barramundi by - product were minced using a grinder (5 mm plate size), and put into the plastic bags. These bags were vacuum packaged, frozen and kept at -18 ± 2^0C until use for the hydrolysis.

2.2. Enzyme

Alcalase is a proteolytic enzyme produced by submerged fermentation of a selected strain of *Bacillus licheniformis*. Alcalase (Endopeptidase, activity 2.4 AU/g) was produced by Novozymes (Bagsvaerd, Denmark). The optimal working conditions for Alcalase are temperatures between 55 and 70^{0}C and pH between 6.5 and 8.5.

2.3. Determination of optimal hydrolysis conditions for oil extraction from barramundi by - product

2.3.1. Determination of optimal water/material ratio

In order to determine the optimal water/material ratio for oil recovery, the minced barramundi by - product were hydrolyzed by using 0.1% Alcalase in 1 hour at temperature of 55^{0}C and natural pH of material (6.5) with water/material ratios of 0%, 25%, 50%, 75% and 100%. After hydrolysis, the enzyme was inhibited by heat treatment at 85^{0}C for 15 minutes in a water bath. Then, the mixture was filtered through a mesh to remove the solid fraction (bones). The filtrate was centrifuged at 7000 rpm for 30 minutes. After centrifugation, the following four fractions were formed: The oil fraction on the top, the emulsion and the liquid protein hydrolysate in the middle and the sludge on the bottom. The oil fraction was recovered, then weighed to calculate the percentage of recovered oil. From results obtained, the optimal water/material ratio was selected.

2.3.2. Determination of optimal enzyme/ material ratio

With the optimal water/material ratio identified and hydrolysis conditions as above (In 1 hour at temperature of 55^{0}C and pH of 6.5), the minced barramundi by - product were hydrolyzed with 0%, 0.1%, 0.2%, 0.3%, 0.4% and 0.5% Alcalase. After hydrolysis, the same steps as above were carried out. The optimal enzyme/material ratio was selected.

2.3.3. Determination of optimal hydrolysis temperature

With the optimal water/material and enzyme/material ratios identified, the minced barramundi by - product were hydrolyzed in 1 hour at pH 6.5 and temperature of 50^{0}C, 55^{0}C, 60^{0}C, 65^{0}C, 70^{0}C. After hydrolysis, the same steps as above were carried out. The optimal hydrolysis temperature was selected.

2.3.4. Determination of optimal hydrolysis time

With the optimal water/material and enzyme/material ratios identified, the minced barramundi by - product were hydrolyzed at pH 6.5 and optimal hydrolysis temperature identified in 1 hour, 2 hours, 3 hours, 4 hours and 5 hours. After hydrolysis, the same steps as above were carried out. The optimal hydrolysis time was selected.

2.4. Chemical analyses

Lipid content was determined according to the method of Folch *et al.* (1957) [4]. The free fatty

acid content, acid value, peroxide value, iodine value, saponification value were determined according to American Oil Chemists' Society AOCS (1997) [5]. Fatty acid composition in the oil was determined by gas chromatography according to Noriega - Rodríguez *et al.* (2009) [6].

2.5. Oil recovery

The fish oil was obtained as the top layer during the extraction process, and was removed with a pipette and weighed using a digital balance. The weight was used to calculate the percentage of recovered oil. The percentage of oil recovery from raw material was defined as follows:

Oil recovery (%) = (Weight of oil recovered/ total weight of lipid in raw material) x 100

2.6. Statistical analysis

The experiments were carried out in triplicates. The data obtained were subjected to one way analysis of variance (ANOVA), followed by the Duncan's multiple range test to determine the significant difference between samples at $p<0.05$ level using the SPSS 20 programme.

3. RESULTS AND DISCUSSION

3.1. Determination of optimal hydrolysis conditions for oil extraction from barramundi by - product

3.1.1. Determination of optimal water/material ratio

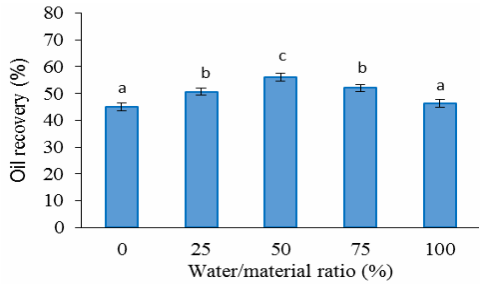

Figure 1. The influence of water/material ratio on the oil recovery from barramundi by - product. Mean values with different superscript letter are significantly different ($p<0.05$)

The influence of water/material ratio on the oil recovery from barramundi by-product is shown in figure 1.

The study result showed that the oil recovery from hydrolysis of barramundi by - product reached the highest value (56.17%) with water/material ratio of 50%. The oil recovery reduced with the increase in water/material ratio from 50 to 100%. This result was in accordance with that reported by Mbatia *et al.* (2010) who also showed that an increase in water/material ratio during the hydrolysis resulted in a decrease in oil yield [7]. Decrease in oil yield with increasing water/material ratio during the hydrolysis may be due to emulsion formation [7]. Daukšas *et al.* (2005) reported that the lipid recovery in the oil fraction after hydrolysis of different by - products ranged from 36.4% to 82.8% [8]. The previous studies indicated that the oil liberation was depended on the raw material and hydrolysis conditions [7], [8]. The study has indicated that the enzymatic hydrolysis using Alcalase with water/material ratio of 50% has brought the highest percentage of oil recovery. Therefore, the water/material ratio of 50% was optimal for oil recovery from barramundi by - products.

3.1.2. Determination of optimal enzyme/ material ratio

During the enzymatic extraction of oil from the barramundi by - product with Alcalase, enzyme/material ratio plays an important role in the recovery of oil. Figure 2 indicate the influence of enzyme/material ratio on the release of oil.

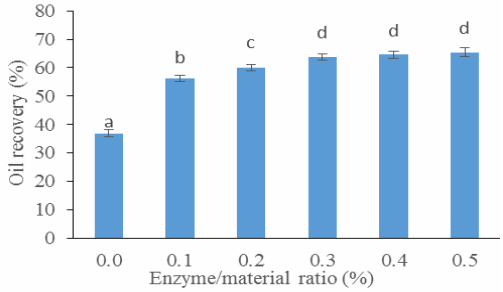

Figure 2. The influence of enzyme/material ratio on the oil recovery from barramundi by - product. Mean values with different superscript letter are significantly different ($p<0.05$)

The results demonstrated that increasing the enzyme/material ratio increased the oil recovery from barramundi by-product. The oil recovery

increased strongly from 36.85% to 63.65% with the increase of the enzyme/material ratio from 0% to 0.3%. As the enzyme concentration increases, the rate of hydrolysis increases, which leads to the increasing of oil recovery. However, there were no significant differences in oil recovery among the samples with the enzyme/material ratios of 0.3%, 0.4% and 0.5%. Mbatia *et al.* (2010) stated that maximum oil recovery from salmon head was achieved when 0.5% Bromelain was used, and showed that a higher enzyme/material ratio did not result in the further increase in oil recovery [7].

According to the results in this study, the highest oil recovery was obtained with enzyme/material ratio of 0.3%. The enzyme/material ratio above 0.3% did not improve the oil recovery. Therefore, the enzyme/material ratio of 0.3% was optimal for the oil extraction.

3.1.3. Determination of optimal hydrolysis temperature

The influence of hydrolysis temperature on the oil recovery from barramundi by-product is demonstrated in figure 3.

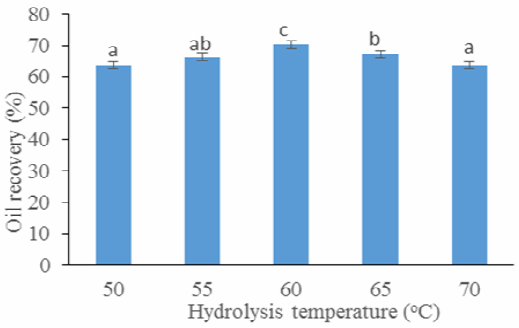

Figure 3. The influence of hydrolysis temperature on the oil recovery from barramundi by - product. Mean values with different superscript letter are significantly different (p<0.05)

The results indicated that the hydrolysis temperature had a significant effect on the oil recovery. Increasing the hydrolysis temperature increased the oil recovery from barramundi by-product. The oil recovery increased sharply from 63.60% to 70.29% with the hydrolysis temperatures in a range of 50 - 60^{0}C. The highest oil recovery was achieved at 60^{0}C. However, with the hydrolysis temperature of 65^{0}C and 70^{0}C, the oil recovery from barramundi by-product decreased in 67.07% and 63.72% respectively. This may be due to decreasing the activity of enzyme at the high temperature (65^{0}C and 70^{0}C). The results showed that the optimal temperature for oil extraction from barramundi by - product was 60^{0}C.

3.1.4. Determination of optimal hydrolysis time

The influence of hydrolysis time on the oil recovery from barramundi by-product is shown in figure 4.

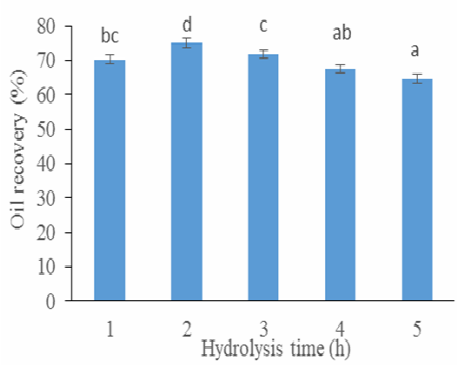

Figure 4. The influence of hydrolysis time on the oil recovery from barramundi by – product. Mean values with different superscript letter are significantly different (p<0.05)

The results indicated that there was a significant increase in oil recovery in the first 2 hours, followed by a decrease during the next 3 hours. The oil recovery from barramundi by - product reached the highest value (75.23%) after 2 hours of hydrolysis. However, when the hydrolysis time prolonged over 2 hours, the free oil recovery decreased significantly. After 5 hours of hydrolysis, the oil recovery only attained 64.65%. These results implied that the hydrolysis time of 2 hours was sufficient to release a large amount of free oil from barramundi by - product. Mbatia *et al.* (2010) also reported that the initial stage of hydrolysis could be sufficient to release the lipids. The long hydrolysis time did not improve the oil recovery but resulted in a colour change of hydrolysate solution to brow [7]. Šližyte *et al.* (2005) reported that the reduced release of lipids may be due to the formation of lipid - protein

complex [9]. The decrease in amount of free oil after 2 hours may be due to interaction of released oil with hydrolyzed proteins during hydrolysis. According to Dumay *et al.* (2009), it is not beneficial to perform a long hydrolysis to obtain the highest oil release. Indeed, the tissue disruption obtained at the beginning of the proteolysis appears sufficient to release the lipids [10]. Batista *et al.* (2009) reported that the lipid recovery was 35% after 1 hour of hydrolysis of sardine by - product with Protamex and only a small increase of 5% was observed in the next 3 hours [3]. The results in this study suggested that the optimal hydrolysis time for oil extraction from barramundi by - product was 2 hours.

In brief, the optimal hydrolysis conditions for oil extraction from barramundi by-product were the water/material ratio of 50%, enzyme/material ratio of 0.3%, hydrolysis temperature of 60^0C and hydrolysis time of 2 hours.

3.2. Chemical criteria of fish oil extracted from barramundi by - product

The chemical criteria of fish oil extracted from barramundi by - product with the optimal hydrolysis conditions is shown in table 1.

Table 1. Chemical criteria of fish oil extracted from barramundi by - product

Chemical criteria	Content
Free fatty acid (%)	1.25±0.12
Acid value (mg KOH/g)	2.49±0.24
Peroxide value (meq O_2/kg)	1.47±0.12
Iodine value (g I_2/100g)	121.85±1.87
Saponification value (mgKOH/g)	192.64±2.15

The free fatty acid content in oil is one of the most important quality parameters to value the quality of oil because the free fatty acid are more susceptible to oxidation than esterified fatty acids. The free fatty acid content should range between 1 and 7% but usually ranges between 2 and 5% [11]. The result in this study demonstrated that the amount of free fatty acid in the barramundi by - product oil was low (1.25%). The lower free fatty acid content showed higher quality and lower further oxidation.

The acid value is a measure of the hydrolysis that had occurred in the oil and is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids in 1 g of oil. The acid value of barramundi by-product oil was found to be 2.49 mg KOH/g, which was below the acceptable limit of 7 - 8 mg KOH/g reported by Bimbo and Crowther (1991) [12].

The peroxide value is commonly used to determine the rancidity of oil and is expressed in milli equivalent of active oxygen per kg of oil. The maximum limit of peroxide value of crude oil is 8 meq O_2/kg to be acceptable for human consumption [13]. The oil extracted from barramundi by-product had a peroxide value of 1.47 meq O_2/kg, which was still within the acceptable quality limit. This indicated that the fish oil extracted from barramundi by-product had low lipid oxidation rate.

The iodine value is a measure of degree of unsaturation of the oil and is defined as grams of iodine absorbed by 100 g of oil. Barramundi by - product oil had a iodine value of 121.85 g I_2/100 g, which was lower than that of mackerel oil (134 g I_2/100 g) [14].

Saponification is the process of breaking down a neutral oil into glycerol and fatty acids by alkali treatment. Saponification value represents the number of milligrams of potassium hydroxide required to saponify 1 g of oil. The oil extracted from the barramundi by-product had a saponification value of 192.64 mg KOH/g, which was higher than that of sardine oil (186.85 mg KOH/g) [6]. Saponification values of the hilsa fish oils from different parts were found to be arranged from 180.28 to 194 [15].

3.3. Fatty acid composition of fish oil extracted from barramundi by - product

Fatty acid composition of the oil recovered from barramundi by - product at optimum hydrolysis conditions is shown in table 2.

Table 2. Fatty acid composition of fish oil extracted from barramundi by - product

Fatty acids	Content (% total fatty acids)
C14:0 (myristic)	2.65±0.12
C16:0 (palmitic)	24.34±0.21
C18:0 (stearic)	8.05±0.14
C20:0 (arachidic)	0.50 ±0.08
C24:0 (linoceric)	1.24±0.13
C16:1 ω 7 (palmitoleic)	4.75±0.21
C18:1 ω 9 (oleic)	9.65±0.25
C18:1 ω 7 (vacenic)	3.38±0.13
C20:1 ω 9 (gadoleic)	1.56±0.06
C22:1 ω 9 (erucic)	2.57±0.10
C24:1 ω 9 (nervonic)	2.84±0.08
C18:2 ω 6 (linoleic)	2.94±0.16
C18:3 ω 3 (linolenic)	2.26±0.10
C20:4 ω 6 (arachidonic)	6.26±0.15
C20:5 ω 3 (eicosapentaenoic, EPA)	4.67±0.15
C22:4 ω 6 (docosatetraenoic)	2.34±0.11
C22:5 ω 3 (docosapentaenoic)	2.25±0.13
C22:6 ω 3 (docosahexaenoic, DHA)	17.75±0.23
Total saturated fatty acids	36.78±0.28
Total monounsaturated fatty acids	24.75±0.35
Total polyunsaturated fatty acids	38.47±0.33
Axit béo ω 3	26.93±0.35
Axit béo ω 6	11.54±0.17

The results indicated that the content of saturated fatty acids in the oil recovered from barramundi by - product was 36.78% of total fatty acids. Palmitic acid was the highest among the saturated fatty acids with the content of 24.34% followed by stearic acid with the content of 8.05%. The content of monounsaturated fatty acids was 24.75%. The most abundant monounsaturated fatty acid was oleic acid with the content of 9.65%. The content of polyunsaturated fatty acids was 38.47% of total fatty acids. Néchet *et al.* (2007) reported that the contents of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids in the oil from liver of ray (*Himantura bleekeri*) were 45.32%, 22.84% and 31.85% of total fatty acids, respectively [16]. The results in this study demonstrated that the fatty acids with high contents in barramundi by - product oil were palmitic acid, docosahexaenoic acid and oleic acid. The oil recovered from barramundi by - product had the omega-3 fatty acid content of 26.93% and omega-6 fatty acid content of 11.54%.

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) content of barramundi by - product oil were 17.75% and 4.67%, respectively. This showed that the barramundi by - product oil was a good source of DHA and EPA. DHA and EPA are known as essential fatty acids for human. DHA has an important role in the development of the brain and the nervous system. EPA plays an important role in the prevention of cardiovascular diseases. The contents of DHA and EPA in liver oil of ray (*Himantura bleekeri*) were found to be 15.62% and 4.10%, respectively [16]. Sun *et al* (2006) reported that the DHA and EPA contents in the oil extracted from salmon viscera were 6.99% and 7.91%, respectively [17]. Khoddami *et al.* (2012) showed that the DHA and EPA contents in the tuna head oil were 15.7% and 1.48%, respectively [18].

4. CONCLUSION

Barramundi by - product generated from fish processing industry could be utilized as a good source for recovery of fish oil. The oil recovery from barramundi by - product using Alcalase was significantly affected by the hydrolysis conditions including water to material ratio, enzyme to material ratio, hydrolysis temperature and hydrolysis time. The optimal parameters for oil extraction from barramundi by - product were the water/material ratio of 50%, enzyme/material ratio of 0.3%, hydrolysis temperature of 60^0C and hydrolysis time of 2 hours. Under these optimum

conditions, the fish oil extracted from barramundi by - product had a high content of omega-3 fatty acids (26.93%), especially DHA (17.75%). The oil from barramundi by-product could be used in aquaculture feed or used as an ingredient in food industries after it is refined.

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