Extraction of Omega-3 Fatty Acid from Jade Perch (*Scortum barcoo*) Using Enzymatic Hydrolysis Technique

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Abstract: *Extraction of omega-3 fatty acid from Jade Perch (*Scortum barcoo*) using enzymatic hydrolysis techniques are expected to be more economically possible techniques due to the uses of the enzyme with the characteristic of environmentally friendly, reusable and less energy required during large-scale production. Design of Experiments (DOE) was used to study the effect of process parameters such as the concentration of alcalase (0.5–1.5%), temperature (50–70 °C) and pH (6.5–8.5) towards the yield of oil. The findings showed 16.55% of oil yield was extracted from the jade perch under an optimum condition at 50 °C, 6.5 pH with 0.5% of enzyme concentration for 2 h incubation time. The fish oil was then undergone enzymatic concentration of omega-3 FA using lipase from* Candida rugosa. *The acid value and peroxide value of the fish oil was 71.422 mg KOH/g and 0.799 meq/kg, while the acid and peroxide value of the omega-3 concentrated oil was lower to 49.074 mg KOH/g and 0.399 meq/kg. The FTIR spectrum showed the presence of C–H stretch, =C–H stretch and C=O stretch bond justified the existence of lipids as it presents of alkanes, alkene, and carboxylic acids functional group. At the same time, GC-MS analysis showed the fish oil contains higher total PUFA content and omega-3 fatty acid content than omega-3 concentrated oil.*

Keywords: Scortum barcoo*; jade perch; lipase; omega-3 fatty acid; enzymatic hydrolysis*

■ INTRODUCTION

Recently, many efforts have been done by researchers in both academy and industry in the utilization of the fish waste as the raw material and transform it into a more valuable product such as biofuel, protein, and oil [1]. For most parts, the high concentration of omega-3 fatty acid in fish oil has many applications in the pharmaceutical and nutraceutical field. The omega-3 fatty acid is a class of essential fatty acids that can help in treatment and prevention of various diseases such as cardiovascular disease, rheumatoid arthritis, autoimmune diseases, Attention Deficit Hyperactivity Disorder (ADHD) and Alzheimer's disease [2]. Moreover, an omega-3 fatty acid also helps in promoting brain health during pregnancy and early life of a baby, reduce asthma in children, reduce fat in the liver as well as prevent colon, prostate and breast cancer.

Scortum barcoo is a species of fish under the family of Terapontidae which has a common name, jade perch. It is originate come from Lake Eyre Basin, Queensland, Australia, where it normally found in the river basins of Australia with the Lake Eyre Basin and Barcoo river [3]. Jade perch have the highest concentrations of omega-3 fish oils, which are 2483 mg/100 g of fish fillet than sawfish and Atlantic salmon [4]. Jade perch is believed to be an alternative source of omega-3 as it contents a high concentration of omega-3 fatty acid.

Extraction of omega-3 fatty acid from fish fillets using enzymatic hydrolysis techniques are expected to be more economically possible techniques due to the uses of the enzyme with the characteristic of reusable, environmentally friendly and less energy required during large-scale production. In other words, enzymatic hydrolysis improves the hydrolysis process by

utilizes enzymes that disrupt tissue resulting in the release of lipids would more environmentally friendly and safe method for the efficient release of oils [5]. Nowadays, it is important to utilize the fish waste which has been cause several environment effects in another country. Another extraction method, such as supercritical fluid extraction and heat extraction required high energy consumption, which will directly increase the cost of production and safety concern. In addition, solvent extraction utilizes a carcinogenic solvent, which will cause the environment and health concern as omega-3 fatty acids for human consumption. Moreover, there is a lack of research regarding the omega-3 fatty acid extraction from jade perch using enzymatic hydrolysis method. By considering this, the present investigation is focusing on extraction of omega-3 fatty acid from jade perch using enzymatic hydrolysis techniques that would later be optimized its recovery process for the improvement in term of quality of oil and its yield.

■ EXPERIMENTAL SECTION

Sample Collection and Preparation

Jade perch (*S. Barcoo*) which cultured by Institute of Agrotechnology Lestari (INSAT), Perlis was used as the sample in the experiment. The fillet of jade perch was chopped by using knife and chopper board without the addition of solvent or water to obtain a pure fish sample. Then, the fish fillet was weighed and put into the sealed plastic bag. The sample now is ready to conduct an experiment.

Procedure

Enzymatic hydrolysis of fish oil

About 50 g of fish sample was weighed in a 250 mL Erlenmeyer flask. In order to inactivate or denature the endogenous enzymes containing in the fish sample, the sample was heated at 95 °C for 5 min in the water bath. Next, 50 mL of distilled water was added into Erlenmeyer flask followed by pH adjustment to 6.5, 7.5 or 8.5 using 3 M NaOH. The flask was then transferred to preheated water bath shaker at temperature 50, 60 or 70 °C. The process of enzymatic hydrolysis was initiated when the alcalase concentration 0.5, 1.0 or 1.5% (w/w) of the fish sample was added into the Erlenmeyer flask. The hydrolysis process proceeded for 2 h at 120 rpm stirring speed in the water bath shaker. After the hydrolysis, the enzyme was inactivated or denatured by transferred the flask into the water bath for 5 min at 90 °C and then cooled into room temperature. In order to separate the oil with the solid sample, the mixture was transferred into a centrifuge tube and centrifuged at 3000 rpm for 20 min. Two layers of liquid consists of oil and emulsion layer formed on top were collected into another centrifuge tube. About 2 mL of NaCl solution with a concentration of 0.9% was added to extract impurities and centrifuged again for 10 min at 3000 rpm. The oil layer on top of the centrifuge tube was transferred into a test tube with the addition of anhydrous sodium sulfate to absorbs the water. The test tube was evenly stirred and allowed it to settle down. The oil was collected and keep at the pre-weighed universal bottle to determine the mass of oil extracted.

Preparation of fatty acid methyl ester (FAME)

Lipid extract with a volume of 100 µL was transferred into a cleaned 15 mL centrifuge tube which has been rinsed with chloroform and methanol 3 times respectively. Then, 3.0 mL of Hilditch reagent and 1.5 mL methylene chloride 0.01% v/w BHT was added subsequently into the vial. The preparation of Hilditch reagent was done by adding 1.5 mL of concentrated sulphuric acid (H_2SO_4) into the 100 mL of dry methanol. The preparation of 100 mL of dry methanol was done by transferring 100 mL methanol to a 100 mL volumetric flask, then anhydrous of sodium sulfate (Na_2SO_4) was added in sufficient amount to the methanol until the bottom of the flask was covered. The solution was mixed gently by inverting up and down for 10 min, then decanted. Next, the sample was capped and shake vigorously for 1 min. The tube was flushed with nitrogen, capped and sealed with Teflon® tape. Drying oven at 100 °C was used to incubate the sample for 1 h to make sure the transesterification reaction occurs. The tube was cooled to room temperature. 1.5 mL of hexane and 1 mL of distilled water was added into the tube followed by shake vigorously for 30 sec. The upper layer was pipetted to a new vial without disturbing the lower

layer. The step was repeated by adding 1.5 mL of hexane into the residue followed by shake vigorously for 30 sec, and the organic layer was collected. Evaporation of hexane was done with a gentle stream of nitrogen in a fume hood. 0.5 mL of hexane is added to re-suspend the fatty acid. Finally, the vial was capped with a nitrogen flush, followed by sealed with Teflon® tape.

Determination of acid value

About 0.5–2.5 g of oil sample was weighed using 250-mL glass Erlenmeyer flask. 2 mL of 1% of phenolphthalein indicator together with 125 mL of 1:1 toluene-isopropyl alcohol was poured into the 250-mL glass Erlenmeyer flask containing oil sample and mixed thoroughly in the 250 mL Erlenmeyer flask. 0.1 N KOH was used to titrate the sample until a permanent pink color appear. The acid value with a unit of mg KOH/g of sample was determined by the Eq. (1).

$$\text{Acid value}=\frac{(A-B)\times N\times 56.1}{W} \quad (1)$$

where A = Volume of KOH used in the titrating the sample (mL); B = Volume of KOH used in the titrating the blank (mL); N = normality of standard alkali; W = Mass of oil sample (g); and 56.1 = molecular weight of KOH in grams.

Determination of peroxide value

About 2.5 g of oil sample was weighted at the beginning and added into 250 mL Erlenmeyer flask. Next, 50 mL 3:2 acetic acid-chloroform was added into the flask and the flask was stirred until the oil sample dissolved into the solution. One milliliter of saturated potassium iodide (KI) solution was added and stand in room temperature for 1 min with occasional swirling. Then, 100 mL of distilled water was added into the flask. Titration was carried out until the light-yellow color appeared using 0.01 N sodium thiosulfate. Approximately 1 mL of starch indicator solution was added and continue titration until the colorless solution formed with constant agitation. On the other hand, the blank sample was prepared without the addition of the oil sample. The peroxide value (milliequivalents peroxide/1000 g sample) was determined by the Eq. (2).

$$\text{Peroxide value}=\frac{(S-B)\times N\times 1000}{W} \quad (2)$$

where B = Volume of sodium thiosulfate used for titration of blank (mL); S = Volume of sodium thiosulfate used for titration of sample (mL); N = normality of sodium thiosulfate solution; 1000 = per 1000 g of sample; W = Mass of sample (g).

Determination of lipid yield

The lipid yield was determined by the following formula:

$$\text{Lipid yield}(\%)=\frac{\text{Mass of total lipid content}}{\text{Mass of fish sample}}\times 100\% \quad (3)$$

FTIR spectroscopy analysis

The mid-infrared spectral (4000–700 cm^{-1}) data were collected by using an FTIR portable spectrometer (Perkin Elmer) equipped with a temperature-controlled, 5-bounce ZnSe crystal ATR. The sample which has been sealed with parafilm was cooled to room temperature prior to data collection. The data were recorded in duplicate. First, oil with a volume of 80 µL was deposited using micropipette on ATR sensor and kept at 65 °C, and the spectra were performed over a range of 4000–700 cm^{-1} at 4 cm^{-1} resolution. The spectra were collected in terms of absorbance using Perkin Elmer Spectrum Version 10.5.2.

Optimization of antimicrobial activity

Fatty acids were categorized into several classes based on the carbon atom by utilizing the Agilent 7890A Gas Chromatography (GC) system equipped with mass spectrometer system (MS) of an Agilent 5975C inert MSD with triple-axis detector which is shown in Table 1 as the protocol.

Optimization of extraction oil yield

The Design-Expert® is used to study the optimization of oil yield in the extraction of fish oil using enzymatic hydrolysis method using an alcalase enzyme. By using Response Surface Methodology (RSM) coupled with Central Composite Rotatable Design (CCRD), different enzyme concentration, incubate temperature, and pH is set as a parameter. The selected range of the parameter used is stated in Table 2.

Table 1. Protocol for fatty acid analysis using GC-MS

Instrumental Parts	Particulars
Gas Chromatography system	7890A, Agilent Technologies
Detector	Mass spectrometer system Agilent 5975C inert MSD
Column	BP20 (wax) polar capillary column 30 m in length, 0.25 mm of internal diameter and 0.25 μm film thicknesses
Carrier gas	Helium
Carrier gas flow rate	1 mL/min
Oven	Initially 55 °C for 3 min
	Ramping to 250 °C at a rate of 6 °C /min for 5 min
Injector Temperature	200 °C
Detector Temperature	230–230 °C (Ion generation)
	150–200 °C (Ion acceleration)

Table 2. The ranges of the studied parameter

Parameters	Range	
	Minimum	Maximum
Concentration of enzyme (%)	0.5	1.5
Temperature (°C)	50	70
pH	6.5	8.5

■ RESULTS AND DISCUSSION

Statistical Analysis for the Optimization Study

CCRD with duplication of factorial point and three central points was used to analyze the relationship between three different parameters (concentration of enzyme, temperature, and pH) towards the percentage of oil yield. The design model consists of a total of 22 runs of an experiment which is used to develop the regression model. Table 3 shows the complete design matrix and its response to parameters in CCRD. The highest percentage of oil yield is 16.86% at running 1 (0.50% enzyme concentration, 50 °C, pH 6.5); meanwhile, the lowest percentage of oil yield obtained is 12.24% at run 22 (1.00% enzyme concentration, 60 °C, pH 7.5).

The result of the Analysis of Variance (ANOVA) for response surface quadratic model on percentage oil yield was showed in Table 4. From Table 4, model F-value of 12.19 suggested that the model was significant due to the P-values of the model were less than 0.05, and thus, they were significant. In addition, the "Prob > F" of variable B were less than 0.05 means that the model terms were significant. In another word, factor B, which is temperature have a significant effect on the model with the highest F-value of 56.91. Therefore, the temperature is one of the significant factors that affect the enzyme activities towards hydrolysis process. Moreover, the mean of the model was 14.61, while the standard deviation was 0.68. The R^2 value indicates the correlation coefficient between the actual and predicted value was obtained, which is 0.8823. Therefore, the actual value was almost achieved the predicted value when the R^2 value is near to 1. The "lack of fit F-value" of 3.68 suggested that the lack of fit is not significant relative to the pure error. There was only a 5.09% chance that a model F-value could occur due to noise. Non-significant lack of fit is good and acceptable. The final model equations of actual factors were expressed as the following equation:

In term of actual factors:

$$\begin{aligned}\text{Oil yield}(\%) = {} & +26.17812 - 23.90937 * \text{Conc. of enzyme} \\ & +0.42347 * \text{Temp.} - 2.62208 * \text{pH} + 0.059375 * \text{Conc. of} \\ & \text{enzyme} * \text{Temp.} + 0.14875 * \text{Conc. of enzyme} * \text{pH} + \\ & 0.041063 * \text{Temp.} * \text{pH} + 9.79375 * \text{Conc. of enzyme}^2 \\ & -7.65313\text{E} - 003 * \text{Temp.}^2\end{aligned} \quad (4)$$

Temperature (B) which has a highest F value affect the percentage of oil yield obtained significantly, while the concentration of enzyme and pH showed an insignificant effect in ascending order toward the hydrolysis process at a 95% confidence level ($p < 0.05$). By compare run #10 and #14 as well as run #4 and #11 (or other comparable runs) respectively, an increase in temperature or pH caused a decrease in the percentage of lipid yield. High temperature and alkaline condition change the specific structure of the enzyme, disrupts the

Table 3. Complete design matrix and its response of parameters in CCRD

Run	Concentration of Enzyme (%)	Temperature (°C)	pH	Percentage of Oil Yield (%)	
				Actual	Predicted
1	0.50	50.00	6.50	16.86	16.98
2	0.50	50.00	6.50	16.83	16.98
3	1.50	50.00	6.50	17.16	16.6
4	1.50	50.00	6.50	16.80	16.6
5	0.50	70.00	6.50	12.94	13.02
6	0.50	70.00	6.50	13.86	13.02
7	1.50	70.00	6.50	13.67	13.82
8	1.50	70.00	6.50	13.69	13.82
9	0.50	50.00	8.50	16.12	15.99
10	0.50	50.00	8.50	16.14	15.99
11	1.50	50.00	8.50	15.08	15.90
12	1.50	50.00	8.50	15.96	15.90
13	0.50	70.00	8.50	13.47	13.67
14	0.50	70.00	8.50	13.10	13.67
15	1.50	70.00	8.50	15.23	14.77
16	1.50	70.00	8.50	14.58	14.77
17	1.00	40.00	7.50	12.90	12.90
18	1.00	60.00	5.50	13.56	13.43
19	1.00	60.00	9.50	14.51	13.39
20	1.00	60.00	7.50	14.01	13.41
21	1.00	60.00	7.50	12.73	13.41
22	1.00	60.00	7.50	12.24	13.41

Table 4. ANOVA for response surface quadratic model on percentage oil yield

Source	Sum of Square	DF	Mean Square	F Value	Prob > F	Remark
Model	44.6	8	5.58	12.19	< 0.0001[a]	significant
A	0.51	1	0.51	1.11	0.3114[b]	not significant
B	26.04	1	26.04	56.91	< 0.0001[a]	significant
C	0.0022	1	0.0022	0.0048	0.9457[b]	not significant
AB	1.41	1	1.14	3.08	0.1027[b]	not significant
AC	0.089	1	0.089	0.19	0.6473[b]	not significant
BC	2.70	1	2.70	5.90	0.0304[a]	significant
A^2	23.68	1	23.68	61.77	< 0.0001[a]	significant
B^2	6.46	1	6.46	14.13	0.0024[a]	significant
Residual	5.95	13	0.46			
Lack of fit	3.12	3	1.04	3.68	0.0509	not significant

R^2 = 0.8823; adjusted R^2 = 0.8099; standard deviation = 0.68; mean = 14.61

[a] Significant at 95% confident interval. [b] Not significant at 95% confident interval.

Notes: A: Concentration of enzyme; B: Temperature; C: pH

three-dimensional shape of functional alcalase as enzyme caused denaturation and inhibits the enzyme activity [7-8]. By comparing run #1 and #3 (or other comparable runs) in which an increase of the concentration of enzyme caused an increase in the percentage of lipid yield. Unfortunately, a 3-fold increase in the

concentration of enzyme contributes to a small increment in lipid yield [9]. Therefore, 0.5% w/w of enzyme load consider as an optimum point because increase the enzyme concentration does not significantly increase in lipid yield. Moreover, low enzyme concentration used to reduce the cost associated with the enzyme during production scale.

Optimal Design

Validation was carried out in order to validate the predicted responses designed by the software and figure out the optimal conditions by varying the parameters (concentration of enzyme, temperature, and pH) to produce the highest percentage oil yield. The optimal conditions obtained at 0.5% w/w concentration of alcalase enzyme, the temperature of 50 °C and pH of 6.5 with a desirability value of 0.964 and 16.98% predicted value which is within the range of parameters selected on hydrolysis of jade perch fish using alcalase. The validation was carried out by conduct triplicate experiments with the optimum variables. The average percentage of oil yield obtained is 16.545%, which is relatively lower than the predicted value. The percentage error has been calculated, which is 2.57%. Based on the experimental results obtained, the optimum response condition designed by RSMCCRD is valid and suitable for the enzyme-assisted extraction of oil because the percentage error is less than 10%.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The fish oil samples before and after treated with lipase from *Candida rugosa* were tested with FTIR spectroscopy to determine the type of bonding and functional group presence in the sample based on FTIR spectra detected. The FTIR spectra were measured within the range of 4000–700 cm^{-1} wavelength. Fig. 1 shows the comparison of FTIR spectra between fish oil and omega-3 concentrated oil. The spectra of the sample showed the presence of similar functional group and compounds in both samples such as alkanes, alkenes, carboxylic acids, ester, ether, aromatics, amide, etc. The FTIR spectra showed the absorption peaks at 3100–3000 cm^{-1} is attributed to the C–H stretch from aromatics group and =C–H stretch from alkene group. These absorption band observed at 3007 cm^{-1} (fish oil) and 3006 cm^{-1} (omega-3 concentrated oil) indicated the presences of unsaturated fatty acid. The finding proved by the previous study which proportion of mono and polyunsaturated acyl groups present at peaks approximately 3008 cm^{-1} due to carbon double bond [10].

In addition, absent of peaks at approximately 3290 cm^{-1}, which is a strong O–H stretching modes of water represents both samples are free from the moisture [11]. The absorption peaks at 3000–2850 cm^{-1} shows the

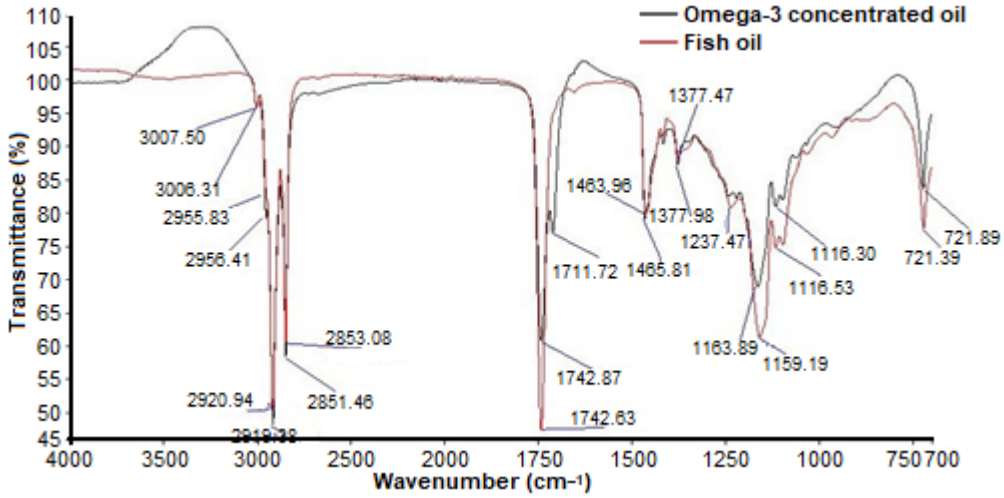

Fig 1. Comparison of FTIR spectrum between fish oil and omega-3 concentrated oil

presence of alkane group due to the C–H stretch while absorption peaks in the range of 3300–2500 cm^{-1} indicated the presence of carboxylic acids due to O-H stretch bond. Based on the result, the absorption band in oil sample before and after lipase treated observed at 2956, 2921, 2852 and 2956, 2919, 2851 cm^{-1} respectively shows the presence of alkane in the sample. Besides, absorption peaks at 1760–1665 and 1760–1690 cm^{-1} represented the presence of C=O stretch bond from carbonyls, carboxylic acids, ester, and saturated aliphatic group respectively. Those bonds detected justified the existence of lipids in the sample. In the previous study, it was reported that the absorption peaks were asymmetrical C–H stretch from methyl ($–CH_3$) group of lipids at ~2970 cm^{-1}, asymmetrical C–H stretch from methylene ($–CH_2$) group of lipids at ~2920 cm^{-1} and symmetrical C–H stretch from methylene ($–CH_2$) group of lipids at ~2850 cm^{-1} which have the same result obtained in the experiment [11].

Moreover, the finding also comes to an agreement that major bands can be observed resulting from lipids near 2930 cm^{-1} while from protein at near 1640 and 1550 cm^{-1} and carbohydrate near 1030 cm^{-1} [12]. Therefore, the sample is free from protein and carbohydrate molecule due to the absence of peaks in that region. Although the fish oil sample spectra having similarity especially in wavelength 3100–1600cm^{-1}, it shows differences in term of the amount of the specific type of bonding which reflected by the % transmittance due to the difference in nature and composition of fish oils [2].

Determination of acid value. The acid value used to determine the presence of FFAs and other non-lipid acid compounds in the oil [13-14]. Based on the result obtained, both oil sample before and after lipase activity having a high acid value which is 71.42 mg/KOH g and 49.07 mg/KOH g, respectively, as compared to the recommended value (≤ 5 mg/KOH g). This could be due to the high temperature, and acid/base condition increase the degree of unsaturation, caused the physicochemical changes of fish oil during incubation. Therefore, prolonged high temperature or exposed to acids or bases caused oil rancidity, which conversion of triacylglycerides occur, resulting in the formation of fatty acid and glycerol, which increase the acid value [15].

Determination of peroxide value. Peroxide value is the measurement of hydroperoxides and used to determine the primary oxidation of oil [13]. Based on the result obtained both oil sample before and after lipase activity having a low peroxide value which is 0.799 meq/kg and 0.399 meq/kg, respectively, as compared to the recommended value (≤ 5 meq/kg). The previous finding stated that low pH range decrease the lipid oxidation rate resulting in low peroxide value obtained. In addition, increase temperature caused the increase in peroxide value due to high temperature accelerated the lipid oxidation [7]. The finding was justified in which low peroxide value was obtained due to low pH (6.5–7) and low temperature (35–50 °C) condition during incubation.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

GC-MS analysis is used to analyze the fatty acid composition in the fish oil and also omega-3 concentrated oil [16]. About 20 fatty acids have been identified in crude jade perch fish oil using the GC analysis. Table 5 shows the summarized fatty acid composition of crude fish oil and omega-3 concentrated oil. Three main fatty acid composition such as saturated FA, monounsaturated FA, and polyunsaturated FA were obtained from crude oil which extracts from jade perch using enzymatic hydrolysis technique. Saturated FA is a fatty acid that saturated with hydrogen which forms a straight hydrocarbon chain. Monounsaturated FA consists of one carbon-carbon double bond in a different position which also known as monoenes. Polyunsaturated FA consists of two or more carbon-carbon double bond. omega-3 is the type of polyunsaturated FA in which the first double bond found between the third and fourth carbon atom while omega-6 also type of polyunsaturated FA where the first double bond found between the sixth and seventh carbon atom [17]. Based on Table 5, the highest concentration of fatty acids in both fish oil and omega-3 concentrated oil was elaidic acid (18:1 n-9) followed by palmitic acid (16:0). The predominant fatty acid content in both samples is monounsaturated fatty acids (MUFA)

Table 5. Comparison of the fatty acid composition of fish oil and Omega-3 concentrated oil

Fatty Acids	Composition (%)	
	Fish Oil	Omega-3 Concentrated Oil
Saturated Fatty Acids		
Dodecanoic acid (12:0)	0.5192	0.3795
Myristic acid (14:0)	2.601	2.422
Palmitic acid (16:0)	29.41	31.56
Arachidic acid / Eicosanoic acid (20:0)	0.1744	0.1271
Subtotal	32.70	34.49
Monounsaturated Fatty Acids		
Palmitoleic acid (16:1n-7)	4.139	4.167
Elaidic acid (18:1n-9)	37.75	37.98
Subtotal	41.89	42.15
Polyunsaturated Fatty Acids		
Omega-3 Fatty Acid		
α-linolenic acid, ALA(C18:3n−3)	0.7612	0.7025
Eicosapentaenoic acid, EPA(C20:5n−3)	-	0.173
Heneicosapentaenoic acid, HPA(21:5n-3)	0.2596	-
Docosahexaenoic acid, DHA(C22:6n−3)	0.8851	0.6619
Subtotal	1.906	1.537
Omega-6 Fatty Acids		
linoleic acid (18:2n-6)	11.58	10.86
γ-linolenic acid (18:3n-6)	1.104	1.240
Eicosadienoic acid (20:2n-6)	0.2315	0.5127
Dihomo-γ-linolenic acid (20:3n-6)	0.6817	0.5454
Subtotal	13.60	13.15
Omega-9 Fatty Acids		
Isolinoleic acid (18:2n-9,12)	1.345	1.440
Subtotal (PUFA)	16.85	16.13
Others compound	8.565	7.229
Total	100	100

followed saturated fatty acids. The percentage of total MUFA in the oil sample was in the range of 41.89 to 42.15%, which mainly consists of elaidic acid and palmitoleic acid.

Omega-3 concentrated oil contains higher elaidic acid (37.98%) than in the fish oil, which contains only 37.75%. Meanwhile, the highest fatty acids among saturated fatty acids group were palmitic acids, which contain 29.41% in a fish oil sample and 31.56% in omega-3 concentrated oil. However, the PUFA, which consists of omega-3, omega-6, and omega-9, were identified in the study. By referring to Table 5, the total PUFA content sample in fish oil is 16.85%, which is higher than in the omega-3 concentrated oil (16.13%). The result shows total omega-3 in fish oil was 1.906%, which consists of ALA, HPA, and DHA with the percentage of 0.7612, 0.2596, and 0.8851% respectively, while the percentage of omega-3 decreased to 1.5374% in omega-3 concentrated oil. The omega-3 presence in omega-3 concentrated oil consists of ALA, EPA, and DHA with the percentage of 0.7025, 0.173, and 0.6619% respectively. The decrease in omega-3 concentration may be due to the stirring effect during incubation. The finding proved by previous research in which low

stirring resulting in low omega-3 fatty acid content as enzyme are not oil soluble. Low stirring inhibits the formation of the enzyme-substrate complex and directly affect the rate of reaction [7]. In addition, auto-oxidation more flavor to occur in the presence of high quantities of n-3 PUFAs due to the high number of double bonds in the structure of PUFAs, which leads to successive degradation.

■ CONCLUSION

As a conclusion, extraction of fish oil has been successfully done using alcalase enzymatic hydrolysis technique. The parameters chosen are concentrations of enzyme, temperature, and pH, which play a major role in regulating the enzymatic hydrolysis process. The hydrolysis process optimum at 50 °C with pH 6.5 and 0.5% w/w enzyme concentration. The oil yield obtained from optimum condition was 16.55%. This model is applicable in industrial or research reference as the percentage error less than 10%. In FTIR analysis, the function groups alkanes, alkenes, carbonyl, and carboxylic acids are detected in both fish oil and omega-3 concentrated oil. Furthermore, the acid value for both fish oil and omega-3 enriched oil was excess the acceptable limits (< 5 mg KOH/g) which is 71.42 mg KOH/g and 49.07 mg KOH/g respectively. However, the peroxide value of fish oil and omega-3 enriched oil was 0.799 meq/kg and 0.399 meq/kg, respectively, which is within the allowed range (≤ 5 meq/kg). In qualitative and quantitative analysis, 1.906% of ALA, HPA, and DHA in the fish oil sample was analyzed using GC-MS. Lastly, ALA, EPA, and DHA were present in the omega-3 concentrated oil sample with a total percentage of 1.54%. The lipase concentrated process gives a negative effect toward the omega-3 content due to auto-oxidation. Therefore, jade perch was believed to be an alternative way for the source of omega-3 due to the presence of omega-3 fatty acid in fish fillet which has been successfully extracted using enzymatic hydrolysis technique in the experiment.

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