Extraction and Production of Omega-3 from UniMAP Puyu (Jade Perch) and Mackarel

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Abstract. Extraction techniques to extract fish oil from various types of fish are numerous but not widely accepted because of the use of chemicals that may be harmful to health. In this study, fish oil is extracted using a technique of Microwave-Assisted Extraction, which uses only water. The optimum conditions required for the production of fish oil for extraction is carried out by examining three parameters such as microwave power (300-700W), extraction time (10-30 min) and amount of water used (70-190ml). Optimum conditions were determined after using design of experiments (DOE). The optimum condition obtained was 300 W for microwave power, 10 minutes extraction time and 190 milliliter amounts of water used. Fourier transform infrared spectroscopy (FTIR) was used to analyze the functional groups of fish oil. Two types of fish such as Jade Perch or UniMAP Puyu and Indian Mackerel were used. A standard omega-3 oil sample (Blackmores) purchased from pharmacy was also determined to confirm the presence of omega-3 oil in these fishes. Similar compounds were present in Jade Perch and Indian Mackerel as compared to the standard. Therefore, there were presence of omega-3 fish oil in the two types of fish. From this study, omega-3 in UniMAP Puyu fish was higher compared to Indian Mackerel fish. However, based on the FTIR analysis, besides the presence of omega-3, the two fishes also contain other functional groups such as alkanes, alkenes, aldehyde, ketones and many others. The yield of fish oil for the Jade Perch was low compared to Indian Mackerel which was 9% while Indian Mackerel was 10%.

1. Introduction

Omega-3 is consequential to avail avert cardiovascular disease, hypertension, inflammatory and autoimmune disorders, dejection and certain disrupted neurological functions [1]. It additionally reduced the symptoms of attention deficit hyperactivity disorder (ADHD), amend lung function, phrenic adeptness and also reduce against Alzheimer’s disease and dementia. Omega-3 consists of α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) where the ALA from the plant and DHA and EPA from the fish.

Many research studies have found that omega-3 is good for health and can obviate many diseases. In addition, it is suitable for all walkas of life to be consumed. Today, omega-3 is utilized as a supplements in the form of tablets and gel-capsules but the gel-capsules has many advantages such as omega-3 oil are quite astronomically immense and the capsules cannot be divided and can be arduous to swallow. Omega-3 in the form of tablets is used for the supplements to the people to keep...
them in good conditions. In order to make omega-3 capsule as supplements, the oil is extracted out from the fish such as salmon and sardines or from plant sources such as chia seed, to get the omega-3.

Nowadays, studies have found that the content of omega-3 is higher in fish oil compared to the plant. Additionally, it was also found that UniMAP Puyu or Jade Perch, a type of fish under family of Terapontidea which have higher omega-3 as compared to the other fish [2]. Therefore, studies were conducted by utilizing UniMAP Puyu and Mackerel to compare the contents of omega-3 in the two fishes as UniMAP Puyu is known to have the high omega-3 contents (3.87% of fish flesh) known in any fish. The mackerel fish utilized in this research is Indian Mackerel (commonly called Kembung) because they are abundant in Malaysia. The incipient engenderment of omega-3 were conducted utilizing UniMAP Puyu because the content of omega-3 is higher than mackerel.

The content of the omega-3 is quite high in fish oil compared to the fish itself. Extraction method was utilized in order to obtain the fish oil. There are several extraction methods that can be used such as microwave assisted extraction and hexane extraction. Microwave assisted extraction (MAE) was utilized in this research due to the high efficiency and yield of the final compounds, which increases the integrated value of the final products and their price in the international market. Microwave assisted extraction is non-conventional extraction method in which it works with the same basic principle but improved by heating the moisture inside the cells. In such a situation, the water compound evaporated resulting high pressure on the cell membrane. High pressure on the cell membrane enhance the porosity of the biological matrix and thus increase the extraction efficiency on fish oil releasing to the extracting solvent. Beside that, MAE enables faster extraction time, lower energy and solvent consumption as well as lower environmental pollution because water is used rather than other harmful solvents such as hexane, methanol in this extraction method. Therefore, it consideras a green technology to be explored in this research.

2. Materials and Methods
2.1. Materials
UniMAP puyu (Jade Perch) and Mackerel were used as the sample in this experiment.

2.2. Preparation of Sample
The samples were gutted, and washed with sufficient amounts of water. The fishes were stored at -20°C, and then freeze dried at a drying temperature of -47°C, under a vacuum of 0.133 bar. The dried samples were then kept in desiccators for analysis.

2.3. Extraction by using MAE
MAE used was the greatest working frequency which was 2450 MHz. 2 g of test sample was weighed and placed in round bottom flask with addition of water (70, 130 and 190 ml) was added to every vessel. The incubating periods used were 10 minutes, 20 minutes and 30 minutes at different power of the microwave of 300 W, 500 W and 700 W. The mixture was cooled and the solvent was separated from the solid material by using a centrifuge and rotary evaporator [3].

2.4. Optimization via RSM
An experimental design consisted of 3 coded factors including volume of water, extraction time and microwave power was used to investigate and validate the extraction parameters for screening purposes. Software was employed for the design of experiments, model building and data interpretation. The three-dimensional response surface plots were generated by keeping one response variable at its optima and plotting the other two independent factors with the response. In all the optimization experiments using MAE, 2 g of the sample was used and the 3 coded factors generated by the software included: volume of water of 70 ml, 130 ml and 190 ml; microwave power 300 W, 500 W and 700 W. A summary of the extraction parameters with 20 runs in a quadratic design mode was employed. After the microwave treatment, the extract was analyzed by Fourier Transform
Infrared Spectroscopy (FTIR). The predicted solution by the software was confirmed by actual experimental runs in duplicate.

2.5. Functional Group Analysis by using FTIR

About 100-200 mg of sample was mixed with powdered KBr and placed in the mortar. An unknown sample was added about 2 spatula tips (not more than 1-2 mg). The samples were mixed well and ground together till uniform. As the KBr absorbed water from the atmosphere, it was done as fast as possible. KBr pallet was used to determine the functional group using FTIR technique. 30-50 mg of the mixture was added into the cavity. The second bolt was screwed and tighten with the ratchets. The bolts was removed and loosed, leaving the pallet place. To make a new pallet, the press was rinsed with water and followed by acetone and the process was repeated. A spectrum was recorded by placing pallet holder with pellet in place into the transmission cell holder in sample chamber of the FTIR instrument.

3. Result and Discussion

3.1. Functional Group Analysis by FTIR

Fourier Transform Infrared Spectroscopy (FTIR) was used to determine the functional group of the UniMAP Puyu fish and Indian Mackerel fish. To determine the presence of omega-3 in these fishes, the functional groups were compared with omega 3 bought at the pharmacy which is use as standard omega 3. Figure 1 (a) shows the spectrum functional group analysis of UniMAP Puyu using the FTIR techniques, Figure 1 (b) shows the spectrum functional group analysis of Indian Mackerel using the FTIR techniques, and Figure 1 (c) shows the spectrum functional group analysis of standard fish oil using the FTIR techniques.

![Figure 1. Spectra functional group analysis (a) UniMAP Puyu, (b) Indian Mackerel and (c) Standard fish oil.](image-url)
3.2. Screening of Fish Oil Production from UniMAP Puyu and Indian Mackerel

Design of Experiment (DoE) software was used to determine the optimum condition for the fish oil production for UniMAP Puyu and Indian Mackerel. A 2-level factorial design, with 3 factors which are A for microwave power from 300 to 700 W, B for extraction time from 10 to 30 min and C for amount of water from 70 to 190 ml were employed. The run was set for 2 replicates and 3 center point per block. These 3 factors were used to determine the relationship on a response, which is the amount of fish oil extracted, Yb. The 3 factors which was replicated twice and 3 center points corresponded to 19 experimental runs were suggested by the DoE software. Table 1 shows the factors and the range that were set in DOE software for UniMAP Puyu and Indian Mackerel.

### Table 1. Parameters and the range set in DOE software for UniMAP Puyu and Indian Mackerel

| Parameter               | Range          |
|-------------------------|----------------|
| Microwave power (W)     | 300 - 700      |
| Extraction time (min)   | 10 - 30        |
| Amount of water (ml)    | 70 - 190       |

3.3. DOE Software for UniMAP Puyu

The screening study on UniMAP Puyu was analyzed using ANOVA as shown in Table 2.

### Table 2. Analysis of Variance (ANOVA) for fish oil productions

| Source         | Sum of Square | df | Mean Square | F Value | p-Value Prob>F | Remarks   |
|----------------|---------------|----|-------------|---------|----------------|-----------|
| Model          | 20.98         | 4  | 5.17        | 47.29   | <0.0001        | Significant|
| A-power        | 9.77          | 1  | 9.77        | 89.29   | <0.0001        | Significant|
| B-time         | 1.89          | 1  | 1.89        | 17.29   | 0.0011         | Significant|
| C-Amount water | 8.27          | 1  | 8.27        | 75.57   | <0.0001        | Significant|
| AC             | 0.77          | 1  | 0.77        | 7.00    | 0.0202         | Significant|
| Curvature      | 2.07          | 1  | 2.07        | 18.97   | 0.0008         | Significant|
| Residual       | 1.42          | 13 | 0.11        |         |                |           |
| Lack of Fit    | 0.30          | 2  | 0.099       | 0.88    | 0.4840         | Not significant|
| Pure Error     | 1.13          | 10 | 0.11        |         |                |           |
| Cor Total      | 24.18         | 18 |             |         |                |           |
| R-Squared      | 0.9357        |     |             |         |                |           |

The factors used in the design functioned to analyze and determine the interactions between microwave power (A), extraction time (B) and amount of water added (C). The interaction of the factors that were significantly affected the response has been identified which were AC. Figure 2 shows the interaction of microwave power (A) and amount of water added (C) towards the yield of fish oil.
Figure 2. The interaction of microwave power (A) and amount of water added (C) and the yield of fish oil.

3.4. DOE Software for Mackerel
The Analysis of Variance (ANOVA) from the experimental results was determined. Table 3 shows the Analysis of Variance (ANOVA) for fish oil productions.

Table 3. Analysis of Variance (ANOVA) for fish oil productions

| Source            | Sum of Square | df | Mean Square | F Value | p-Value Prob>F | Remarks   |
|-------------------|---------------|----|-------------|---------|----------------|-----------|
| Model             | 52.19         | 6  | 8.70        | 49.92   | <0.0001        | Significant|
| A-power           | 18.06         | 1  | 18.06       | 103.66  | <0.0001        | Significant|
| B-time            | 20.25         | 1  | 20.25       | 116.22  | <0.0001        | Significant|
| C-Amount water    | 7.56          | 1  | 7.56        | 43.40   | <0.0001        | Significant|
| AB                | 1.00          | 1  | 1.00        | 5.74    | 0.0355         | Significant|
| AC                | 3.06          | 1  | 3.06        | 17.58   | 0.0015         | Significant|
| ABC               | 2.25          | 1  | 2.25        | 12.91   | 0.0042         | Significant|
| Curvature         | 0.13          | 1  | 0.13        | 0.76    | 0.4015         | Not Significant|
| Residual          | 1.92          | 11 | 0.17        |         |                |           |
| Lack of Fit       | 0.000         | 1  | 0.000       | 1.000   |                | Not significant|
| Pure Error        | 1.92          | 10 | 0.19        |         |                |           |
| Cor Total         | 54.24         | 18 |             |         |                |           |
| R-Squared         |               |    |             |         | 0.9646         |           |

The factors used in this design were analysis to determine the interactions between microwave power (A) and extraction time (B) and microwave power (A) and amount of water added (C). The
interaction of the factors that were significantly affected the response were analysed, which were AB. Figure 3 shows the interaction of microwave power (A) and extraction time (B) towards the yield of fish oil.

**Figure 3.** The interaction of microwave power (A) and extraction time (B) and the yield of fish oil.

In this study, the contents of omega-3 of fish oil from UniMAP Puyu and Indian Mackerel was compared and showed that UniMAP Puyu contains higher amount of omega-3 compared to Indian Mackerel. FTIR analysis proved that UniMAP Puyu contains more carboxylic acid than Indian Mackerel. Omega-3 consists of eicosapentaenoic (EPA) acid and docosahexaenoic (DHA) acid in the fish oil which is types of carboxylic acid. Standard sample of omega-3 (Blackmore) purchased from pharmacy was compared to the fish oil obtained from UniMAP Puyu and Indian Mackerel. Similar compounds were obtained for the standard and the fish oil extracted from the two types of fishes. Therefore, UniMAP Puyu and Indian Mackerel contained omega-3. Consumption of fishes such as UniMAP Puyu and also the cheaper variety Indian Mackerel could reduce or prevent heart disease, alleviate cardiovascular disease. Previous research done prove that omega-3 improve neural health, resolution of chronic inflammatory and prevent certain disrupted neurological functions due to their antioxidant activity where the antioxidants act as scavenging molecules [4]. So, it can aid prevention and treatment for Alzheimer’s disease, autoimmune diseases and Attention Deficit Hyperactivity Disorder (ADHD). Omega-3 also explained the free radicals that are composed in the body during the cellular processes. Otherwise, elevated levels of free radicals can damage major cell components, such as DNA, proteins, and cell membranes. Damage caused by these free radicals, categorically to DNA, plays a role in the development of cancer. Since UniMAP Puyu contain high amount of omega-3 oil it could be commercialized in capsule form for easy consumption.

The omega-3 of fish oil was extracted using microwave-assisted extraction (MAE) for both fishes (UniMAP Puyu and Indian Mackerel). The method used was suitable for the extraction of the fish oil and safe for the consumer to intake orally the product produced. Microwave-assisted extraction method only used water for the extraction. This method is cost effective as only water was used instead of chemicals but it is but also environmental friendly. It could be considered as a green extraction technique.

From the DOE for the UniMAP Puyu, only the interaction of microwave power and amount of water added was significant. The yield generated was increased when the value for microwave power was decreased and high amount of water added. Highest yield was generated to maximum amount, which was approximately 9 % when the microwave power was at 300W and the amount of water
added was 190 ml. However, for Indian Mackerel, the interaction of microwave power and extraction time and interaction of microwave power and amount of water added were significant. When the value for microwave power was reduced and the extraction time was lower, yield was increased. The yield was highest at approximately 10% when the microwave power was set at 300 W and the extraction time 10 min uses. For the second interaction, the yield generated was increased as the value for microwave power was decreased and high amount of water added. Approximately 9% yield produced when the maximum amount used was at microwave power of 300 W and the amount of water added was 190 ml.

In conclusion, MAE method considers as a green technology for the extraction of omega-3. The parameter of microwave power, extraction time and amount of water added for yield of fish oil was evaluated by using Design of Experiment (DOE). The optimum condition for the yield of fish oil produced was 9.75 % when the microwave power was at 300 W, extraction time was 10 min and the amount of water added was 190 ml.

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