

Effect of Processing Parameters to The Fish Oil from Fish Waste Via Modified Soxhlet Extraction

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The oil production and fatty acid methyl ester (FAME) contents of fish waste were studied. Solvent, temperature, and extraction time were the processing parameters involved. The oil and FAME were extracted using a modified soxhlet extraction method. The best solvent for fish oil extraction was found to be ethanol, which yielded 49.0%, followed by ethanol:heptane (1:1), methanol:heptane (1:1), and methanol. The oil extract composition contained a high yield of oleic acid (C18:1), ranging from 42% to 66%. Followed by palmitic acid (C16:0), palmitoleic acid (C16:1), and stearic acid (C18:0). According to the statistical model obtained, at the 95% confidence level, extraction time had a significant effect on both oil and oleic acid yield (C18:1). These results demonstrated that a high oleic acid fraction can be obtained from fish waste with ethanol, implying that there is a high potential for cost-effective biofuel production.

Keywords: Fish waste; oleic acid; soxhlet extraction; fatty acid methyl ester; biofuel

I. INTRODUCTION

The sustainable management of waste from the seafood processing sector is posing a major problem that has the potential to become a global issue. In the fish processing industry, the fish market is regarded as a major source of waste. The amount of head, skin, bone, viscera, fins, and scales in fish wastes is estimated at 50% of the fresh weight (Villamil *et al.*, 2017).

Fish oil derived from fish waste is said to have various potential sources including polyunsaturated fatty acids (PUFA) and bioactive peptides (Ghaly *et al.*, 2013; Inguglia *et al.*, 2020), besides being able to produce biodiesel (Costa *et al.*, 2013; Yahyaee *et al.*, 2013). Fish oil is used in various industries, including pharmaceutical manufacturing, leather tanning, glycerol, paints, soap and cosmetics (Bako *et al.*, 2017). The process of converting fish waste into biodiesel includes a transesterification step, as well as a conversion to fatty acid methyl ester (FAME) (Al Azad *et al.*, 2019; Javidialesaadi & Raeissi, 2013). FAME contains fatty acids including palmitic, palmitoleic, oleic, and stearic acids, which

increase the potential for biodiesel production (Jaiswal *et al.*, 2014).

Various extraction procedures have been selected to extract the oil from the fish waste, such as solvent extraction (Moffat *et al.*, 1993; Jayasinghe *et al.*, 2013), soxhlet extraction (Rincón-Cervera *et al.*, 2017), supercritical fluid extraction (Haq *et al.*, 2017) and wet pressing extraction (Honold *et al.*, 2016). Soxhlet extraction is the most commonly used technique in extracting fish oil because it is the simplest, cost-effective, and most acceptable method for lipid determination (Suharamli *et al.*, 2016). However, there are a few drawbacks such as the hazardous nature of the solvents used (e.g., hexane, petroleum ether, chloroform) and the disposal of solvent waste (Ideia *et al.*, 2019; Sahena *et al.*, 2010). Considering this, a quantitative study of the oil developed by improvised solvent selection will give insight into the future prosperity of soxhlet extraction.

Thus, the goal of the present research was to quantitatively identify the oil yield and FAME content of the fish waste using soxhlet extraction using less toxic organic. Other processing

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parameters such as extraction temperature and time were also investigated in the study.

II. MATERIALS AND METHOD

A. Preparation of Fish Wastes

The mixed species of fish wastes from freshwater were gathered from the Jaya Gading fish market in Kuantan, Malaysia. The heads and tails were manually removed and separated from the wastes obtained. The chosen fish wastes were washed and dried in an oven at 50°C for 24 hours (Madhu *et al.*, 2017). An electrical grinder was used to compress the dried wastes into a fine powder at 1mm particles size.

B. Modified Soxhlet Extraction

To begin, 6 g of powdered fish waste was placed in the flat bottom flask with 100 ml of solvent and connected to the condenser. Separate experiments were carried out with four different solvent systems: ethanol, methanol, ethanol:heptane (1:1), and methanol:heptane (1:1). In the beaker filled with water, the flask was heated. The temperature was set at 60°C, 500 rpm for 4 hours. In another set of experiments, the temperature (50°C, 60°C, and 70°C) and time (3, 4, and 5 hours) were conducted separately, using a one-factor-at-a-time approach. The other parameters remained at the previously specified values.

The extracted sample was then moved to a centrifuge tube and centrifuged at 3000 rpm for 5 minutes. Later, two layers of oil and solid form were formed. The oil was then transferred into the vial and the solid form was discharged. The vial was then heated at 70°C for 24 hours or until the remaining solvent evaporated. All experiments were done in triplicate.

C. Oil Yield % Determination

The oil yield percentage (X) was calculated as in Equation (1) (Adejumo *et al.*, 2013). The percentage of oil yield is denoted as (% w/w).

$$X = \frac{\text{Weight of oil extracted (g)}}{\text{Weight of fish waste powder before extracted (g)}} \times 100\% \quad (1)$$

D. Fatty Acid Methyl Ester (FAME) Analysis

Fatty acid methyl ester (FAME) is formed from the fatty acid in the oil using the Gaikwad *et al.* (2011) method. Agilent Technologies' Gas Chromatography Mass Spectrometry (GCMS) was used to analyse the FAME (G3171A, China). The samples were inserted into a capillary column (HP-5, 30 m length, 0.25 mm ID). Helium was employed with a flow rate of 1 mL/min. The temperature in the inlet and detector were kept constant at 250°C and 280°C, respectively. The injector temperature is programmed to start at 100°C for 3 minutes and then increase by 10°C every minute until it reaches 250°C (Gaikwad *et al.*, 2011). Fatty acid molecules found include oleic acid (C18:1), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), linoleic acid (C18:2), and linolenic acid (C18:3). The entire analysis was done in triplicate.

E. Statistical Analysis

The data was presented in the form of means and standard deviations. According to Duncan's Multiple Range Test (DMRT), the differences were significant at $P \leq 0.05$.

III. RESULT AND DISCUSSION

A. Effect of Solvent on The Recovered Fish Oil

The effects of different solvents on fish waste extraction were studied, especially on the oil yield and the composition of fatty acid methyl esters (FAMES). Figure 1 depicts the screening of various polar and non-polar solvents for their ability to extract oil. The graph shows that the polarity of the solvent affects the oil yield. In comparison to the other solvents, ethanol extraction yielded a higher yield. The oil yields obtained with ethanol were 49.0% with ethanol, 28.1%, 19.0%, and 11.8%, with ethanol:heptane, methanol:heptane, and methanol, respectively. The oil yield obtained in this investigation was equivalent to that obtained in earlier studies, despite the fact that they utilised petroleum ether as the solvent extraction, which resulted in a yield of 48% (Hajeb *et al.*, 2015).

Mudalip *et al.* (2010) and Ferdosh *et al.* (2016) were found that ethanol improved the yield of fish oil. This could be attributed to a general understanding that more polar lipids

were obtained in fish waste than non-polar lipids. In this study, ethanol was introduced into the fish waste cell and extracted lipid from the cell membrane (Bligh & Dyer, 1959). The shorter the fatty acid chain in the lipids, the more oil was recovered from fish waste (Akoh & Min, 2002).

The oil compositions, on the other hand, were varied according to the solvent type, as shown in Figure 2 and Table 1. The extracted fish oil contained five fatty acid methyl esters (FAMES) compounds: oleic acid (C18:1), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), and linoleic acid (C18:2). Palmitic and stearic acids are saturated fatty acids (SFAs), while palmitoleic and oleic acids are monounsaturated fatty acids (MUFAs), and linoleic acid is a polyunsaturated fatty acid (PUFAs). The fatty acid of the fish waste revealed that the dominant fatty acid at all solvent types is oleic acid (C18:1), with a yield ranging from 42% to 66%. The yields of palmitic, palmitoleic, and stearic acids vary depending on the solvent type, ranging from 5% to 26%. While linoleic acid is detected in the smallest amount in all solvents, it does not exceed a 3% yield. Other researchers obtained similar fatty acid compositions from fish waste, claiming that oleic acid is abundant in fish waste, followed by palmitic acid (Kraiem *et al.*, 2015; Ghaly *et al.*, 2013; Khoddami *et al.*, 2009; Costa *et al.*, 2013; Yahyae *et al.*, 2013). However, the fatty acid components varies greatly depending on the fish species and the time of collection (Kasmiran, 2016).

Fatty acids with a high oleic acid content (C18:1) have been reported to have good biodiesel properties due to increased oxidative stability for longer storage, better cold flow properties, cetane number, specific gravity, and viscosity (Nosheen *et al.*, 2018; Rashid *et al.*, 2008). Thus, of the five types of FAMES detected, oleic acid was chosen for further investigation due to its significant impact on biodiesel quality. Ethanol was chosen as a solvent extraction to extract the fish waste in the latter experiment due to the best oil recovery.

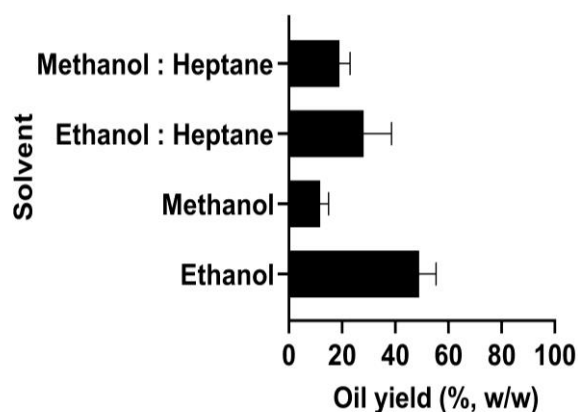


Figure 1. Effect of solvents to the fish oil yield. The extraction conditions were: Sample to solvent ratio = 0.06:1 (w/v), extraction time = 4h, temperature 60°C, mixing speed = 500 rpm.

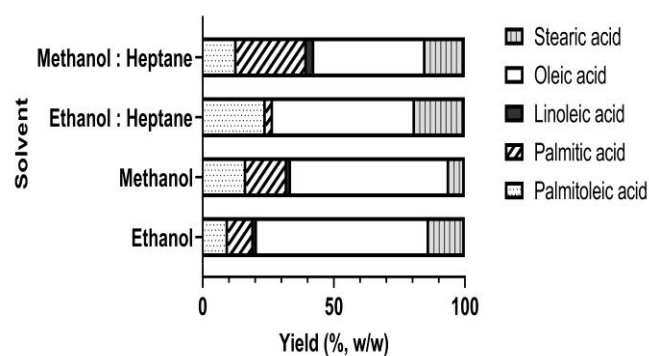


Figure 2. Effect of solvent to the FAME yield. The extraction conditions were: Sample to solvent ratio = 0.06:1 (w/v), extraction time = 4h, temperature 60°C, mixing speed = 500 rpm.

Table 1. Fatty acid composition of extracted fish oil

Fatty acid/Type of solvent	Amount of fatty acid (%)			
	Ethanol	Methanol	Ethanol: Heptane	Methanol: Heptane
C16:0	9.80	15.60	2.78	26.71
C16:1	9.68	16.64	24.10	12.96
C18:0	13.62	5.94	19.04	15.05
C18:1	65.65	60.30	54.08	42.36
C18:2	1.25	1.52	NA	2.92
SFA	23.42	21.54	21.82	41.76
MUFA	75.33	76.94	78.18	55.32
PUFA	1.25	1.52	NA	2.92

B. Effect of Temperature

The rate of oil extraction from fish waste is significantly affected by temperature. Figures 3 and 4 show the yield (%) of oil and oleic acid (%) of fish waste extracted using ethanol as the solvent extraction at various temperatures. According to the graph, when the temperature was elevated from 50 to 60°C, the amount of oil and oleic acid yields increased from 34% to 49% and 43% to 65%, respectively, and decreased dramatically at higher temperatures. The results show that the extraction temperature had a significant effect on oleic acid but not on oil yields, with P-values of 0.0214 and 0.0717, respectively, at the 95% confidence level. While the coefficient of determination, R^2 , obtained for oleic acid and oil yields, respectively, were 0.958 and 0.860. Ghazali & Yasin (2016) and Mani *et al.* (2007) discovered a similar pattern of oil extraction, claiming that the oil yield from *M. oleifera* seed diminished as the temperature rose above 60°C.

Heat causes the fish waste cell to rupture and increases the mass transfer kinetics at higher temperatures (Albert *et al.*, 2015). As a result, it facilitated the opening of oil globules, resulting in the release of oil. However, increasing the temperature (> 60°C) did not improve the yield but rather decreased the extraction recovery (Figures 3 and 4). This phenomenon was most likely caused by analytes decomposition and the breakdown of fish waste fat into volatile compounds at temperatures > 60°C (Eskilsson and Björklund, 2000; Wu and Bechtel, 2008). Furthermore, too high an extraction temperature causes lipase denaturation in the samples and preventing the release of free fatty acid components (Weber *et al.*, 2008).

At lower temperatures (< 60°C), the volatility of the sample is low, reducing the solvent's ability to dissolve oil from fish waste (American Oil Chemists' Society, 1994). The free fatty acid value in fish oil was affected by temperature variations during extraction. As a result, 60°C was chosen for the next investigation.

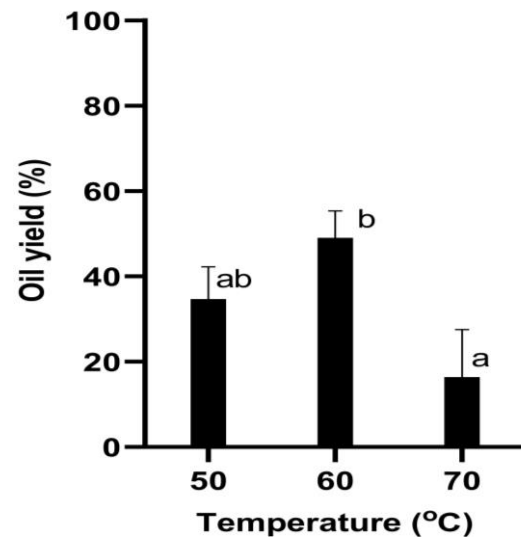


Figure 3. Effect of temperature on the fish oil yield. The extraction conditions were: Sample to solvent ratio = 0.06:1 (w/v), extraction time = 4h, mixing speed = 500 rpm. DMRT showed that bars are not statistically different ($P < 0.05$) with the same superscript letter.

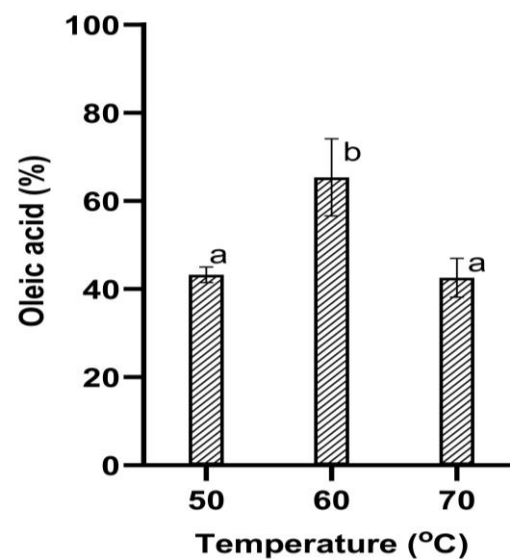


Figure 4. Effect of temperature on the oleic acid yield. The extraction conditions were: Sample to solvent ratio = 0.06:1 (w/v), extraction time = 4h, mixing speed = 500 rpm. DMRT showed that bars are not statistically different ($P < 0.05$) with the same superscript letter.

C. Effect of Extraction Time

Extraction time is a critical factor in oil extraction. Figures 5 and 6 show how extraction time affects the yield of oil and oleic acid extracted at 60°C. The yield of extracted oil increased to 37% after 4 hours of extraction and then

decreased slightly. The trend shows that the extraction time had a significant impact on the oil yield and oleic acid content. At the 95% confidence level, the P values for oil and oleic acid yields were 0.0080 and 0.0010, respectively. While the coefficient of determination, R^2 , obtained for oil and oleic acid yields were 0.9840 and 0.9981, respectively.

The extraction rate for oil yield is fast at 3 and 4 hours of extraction time but slows down at longer times (> 4 hours) (Figure 5). The extracted oil diffuses fast from the waste to the solvent as a result of mass transfer, since the solvent contains less oil at the start of the extraction process (Sayyar *et al.*, 2009). As the extraction time goes on, the concentration of oil in the solvent rises, causing the diffusion rate to decrease. When they reached their maximum yield, the oil yield level remained constant. Mani *et al.* (2007) and Ghazali & Yasin (2016) found that increasing the extraction time (> 6 hours) for *M. oleifera* seeds did not improve the oil yield.

The extraction of oleic acid, on the other hand, increased significantly over time (Figure 6). Ramadhas *et al.* (2005) discovered a similar result, claiming that the ester yield improved slightly as the reaction time extended. Thus, the best time for oil extraction was determined to be 4 hours.

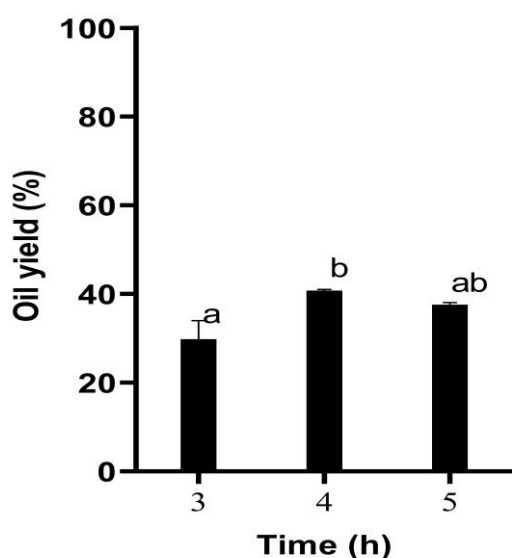


Figure 5. Effect of extraction time on the fish oil yield. The extraction conditions were: Sample to solvent ratio = 0.06:1 (w/v), temperature 60°C, mixing speed = 500 rpm. DMRT showed that bars are not statistically different ($P < 0.05$) with the same superscript letter.

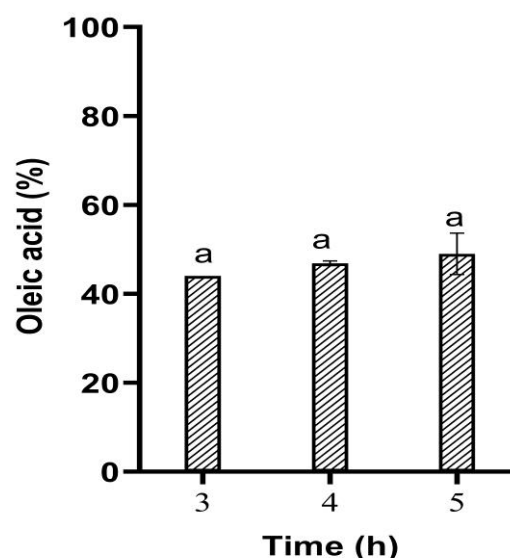


Figure 6. Effect of extraction temperature on the oleic acid yield. The extraction conditions were: Sample to solvent ratio = 0.06:1 (w/v), temperature 60°C, mixing speed = 500 rpm. DMRT showed that bars are not statistically different ($P < 0.05$) with the same superscript letter.

IV. CONCLUSION

Various solvents, temperature conditions, and extraction time were used to successfully extract fish waste via soxhlet extraction. Ethanol has been demonstrated to be the best solvent with a 2-3 fold higher oil yield than the other solvents (i.e., ethanol:heptane, methanol:heptane, and methanol). The most prevalent fatty acid recovered from fish waste was oleic acid (C18:1), which accounting 42% to 66% of the total. In solvent extraction, temperature, and extraction time are linked. Statistical models demonstrated that extraction temperature and duration had a significant effect on oleic acid yield at a 95% confidence level. The R^2 values obtained under these conditions were 0.9580 and 0.9981, respectively. The best extraction temperature and time were obtained at 60°C and 4 hours, respectively.

V. ACKNOWLEDGEMENT

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