

Effect of Processing Variables on Fish Oil Extraction

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ABSTRACT - The accumulation of fish waste from seafood processing poses significant environmental and economic concerns. Therefore, there is a need to explore an efficient and sustainable extraction process for producing biodiesel, particularly through the extraction of fish oil rich in fatty acid methyl esters (FAME). This study investigated the effects of different processing variables, such as solvent type, temperature, and extraction time on the oil yield and FAME composition. The oil and FAME were extracted using a soxhlet extraction method. The optimal extraction conditions were ethanol, 60 °C, and 4 h, which yielded 49.0% oil yield with a high concentration of oleic acid (C18:1), ranging from 42% to 66%. These findings demonstrated that the oil extracted from fish waste has significant potential for cost-effective biodiesel production.

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1. INTRODUCTION

The aquaculture and seafood processing sector is critical to the nation's economic growth. Seafood consumption has risen significantly during the last decade and continues to increase. Malaysia is one of the leading fish-producing nations in Southeast Asia, with total fish production reaching 1.8 million metric tonnes in 2022, supported by both marine capture fisheries and aquaculture activities, generating a monetary value of RM 15.97 billion (USD 3.83 billion) [1]. The increasing domestic consumption and export of seafood have intensified fish processing activities, consequently generating large volumes of waste. It is estimated that fish waste constitutes approximately 50% of the total fish weight, comprising head, skin, bone, viscera, fins, and scales [2, 3]. In addition, these wastes contain bacteria and enzymes, posing a notable threat to processing and storage by facilitating the invasion of spoilage microorganisms and causing contamination. Thus, it gives rise to substantial economic and environmental problems. The nutritional composition of solid waste derived from fish is nearly identical to that of the consumable portions, encompassing protein [4], minerals [5], lipids [6], and vitamins [7]. These emphasize the necessity of developing appropriate fish waste valorization methods that could serve as sustainable strategies for obtaining valuable products such as collagen, gelatin, peptides, hydrolysates, chitosan, and fish oil [8].

Fish oil is mainly composed of triglycerides containing carboxylic acids and aliphatic chains, but it also contains phospholipids, glycerol ethers, and wax esters in various ratios. The presence or absence of double covalent bonds between carbon atoms determines whether fish oil fatty acids are unsaturated or saturated. The unsaturated fatty acids present in fish oil are known as polyunsaturated (PUFA) [8]. Fish oil derived from fish waste has various potential sources, including bioactive peptides [9], and also be used as biodiesel [10]. The process of converting fish waste into biodiesel includes transesterification and conversion to fatty acid methyl ester (FAME) [11]. In the early stages, Samat et al. (2018) [12] and Kara et al. (2018) [13] utilized fish waste for biodiesel production through both esterification and transesterification methods. Zhang et al. (2020) [14] produced biodiesel from fish processing waste using in situ transesterification. Other researchers employed assisted methods, including co-solvent [15] and nanomagnetic catalysts [16]. FAME contains fatty acids, including palmitic, palmitoleic, oleic, myristic, and stearic acids, which increase the potential for biodiesel production [17]. Despite its numerous applications, fish oil's long-term viability is dependent on its commercial viability. Thus, it is vital to utilize extraction procedures that produce a high yield and quality fish oil to ensure its long-term sustainability. Several extraction methods have been reported for fish oil extraction, such as microwave-assisted extraction [18], soxhlet extraction [19], supercritical fluid extraction [20], and wet-pressing extraction [18]. Among these, soxhlet extraction emerges as the preferred choice for fish oil retrieval due to its simple, cost-effectiveness, and broad acceptance for lipid analysis [21]. Nonetheless, this technique is not exempt from limitations, such as hazardous solvents and the management of solvent waste [22].

Thus, the objective of this study was to quantify the oil yield and FAME content of the fish waste via soxhlet extraction, using less toxic organic solvent. Other processing variables, such as extraction temperature and time, were also evaluated. This approach supports Malaysia's broader sustainability agenda by promoting circular economy practices in the fisheries sector.

2. METHODS AND MATERIAL

2.1 Preparation of Fish Wastes

The fish waste, which comprised marine fish species from the Jaya Gading Kuantan fish market, was collected for the study. The waste was manually separated, cleaned, and dried for 24 h in an oven at 50 °C [23]. Next, the waste was ground into a powder with a 1 mm particle size using an electrical grinder.

2.2 Solvent Extraction

The flat-bottomed flask was filled with 100 mL of solvent and 6 g of powdered fish waste before being connected to the condenser. Four different solvent systems, including ethanol, methanol, ethanol:heptane (1:1), and methanol:heptane (1:1), were used in separate studies. The flask was heated in the water-filled beaker. The experiment was at 4 h at 60 °C and 500 rpm. The temperature (50 °C, 60 °C, and 70 °C) and time (3, 4, and 5 h) were separately measured in various sets using a one-factor-at-a-time approach. Other variables remained fixed at the predetermined values. The extracted sample was transferred to a centrifuge tube and spun at 3000 rpm for 5 min. After centrifugation, the mixture was separated into two distinct layers: the upper layer contained the solvent-oil mixture, and the lower layer consisted of solid residues. The upper liquid layer was carefully separated using a pipette into a pre-weighed glass vial. The vial was dried in a drying oven at 70 °C for 24 h to evaporate any remaining solvent [24]. Once the solvent had completely evaporated, the vial containing the recovered fish oil was cooled to room temperature and reweighed to determine the oil yield. All experiments were in triplicate.

2.3 Oil Yield % Determination

The oil yield (% w/w) was calculated as in Eq. (1) [25].

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

2.4 Fatty Acid Methyl Ester Analysis

Fatty Acid Methyl Ester (FAME) was formed from the fatty acid in the oil using the Gaikwad et al. [26] method. The FAME was analyzed using Agilent Technologies' Gas Chromatography Mass Spectrometry (GCMS) (G3171A, China). The samples were placed in a capillary column (HP-5, 30 m length, 0.25 mm ID). Helium was at a flow rate of 1 mL/min. The temperatures in the inlet and detector were kept constant at 250 °C and 280 °C, respectively. The injector temperature started at 100 °C for 3 min and then increased by 10 °C every min until it reached 250 °C. The analysis was triplicate.

2.5 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 $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Effect of Solvent on the Recovered Fish Oil

The study investigated the effect of different solvents on the oil yield and the composition of fatty acid methyl esters (FAME) during fish waste extraction. The addition of heptane to polar solvents, such as ethanol and methanol in a 1:1 ratio was to enhance extraction efficiency. This combination creates a biphasic solvent system that can dissolve a broader range of lipid classes. Polar solvents are effective at extracting phospholipids and other polar lipids, while non-polar solvents like heptane are more selective toward neutral lipids, such as triglycerides. Therefore, combining polar and non-polar solvents is often hypothesized to increase overall lipid recovery by targeting both types simultaneously.

Figure 1 illustrates the assessment of polar and non-polar solvents for oil extraction. The graph shows that the polarity of the solvent affects the oil yield. Ethanol exhibited the highest oil yield (49.0%), followed by ethanol:heptane (28.1%), methanol:heptane (19.0%), and methanol (11.8%). These findings align with a study by Ferdosh et al. (2016) [27], who reported that ethanol improved the yield of fish oil. This trend was due to the polarity of the solvents, the intermolecular interaction capabilities, and the ability to disrupt lipid–matrix interactions in the fish waste. The polarity index (PI) decreases when polar solvents, such as ethanol and methanol, are mixed with heptane. The estimated PI values for a 1:1 of ethanol:heptane is 2.65, and methanol:heptane is 2.6. While the PI values for ethanol and methanol are 5.2 and 5.1, respectively [30, 31]. Ethanol exhibits a balanced polar–nonpolar character and strong hydrogen bonding capacity, which enables it to effectively penetrate biological matrices, disrupt lipid–protein and lipid–membrane interactions, and dissolve both polar and moderate non-polar lipids. In comparison, although methanol has slightly different PI than ethanol, it proves less effective due to its smaller molecular size and reduced ability to form stabilizing hydrogen bonds with hydrophobic lipid tails. This limitation hinders its interaction with certain lipid bound within complex tissue matrices. Consequently, the low PI for solvent mixtures (ethanol:heptane and methanol:heptane) weakens the solvents' ability to disrupt lipid–protein and lipid–matrix interactions in fish tissue, resulting in low oil yields.

Besides, the solvent polarity also correlates with the dielectric constant values. At 20 °C, the dielectric constant values for ethanol, methanol, and heptane are 25.02, 33.30, and 1.91, respectively [28, 29]. A higher dielectric constant indicates

a greater solvent polarity. In this study, the oil yield from fish waste using ethanol was 4 times higher than when using methanol. The difference in yield could be attributed to specific interactions that enhance the dissolution of lipids in fish waste. Ethanol's larger molecular size and greater potential for hydrogen bonds effectively dissolve lipids and disrupt the interactions between lipids and the tissue matrices. This process facilitates the efficient extraction of lipid components. In contrast, methanol exhibits dipole-dipole interactions that are weaker than hydrogen bonding. This finding was in agreement with Kumoro et al. (2022) [30] who reported the solvent's ability to break the strong interaction forces between lipids and other cellular components plays a crucial role in extraction efficiency.

The oil compositions, however, varied according to the solvent type, as shown in Figure 2. The extracted fish oil contained five FAME compounds: a) palmitic acid (C16:0) and stearic acid (C18:0), known as saturated fatty acids (SFA); b) oleic acid (C18:1) and palmitoleic acid (C16:1), known as monounsaturated fatty acids (MUFA); c) linoleic acid (C18:2), known as PUFA. The fatty acid of fish waste revealed that oleic acid (C18:1) was the predominant fatty acid across all solvent types, ranging from 42% to 66%. The yields of palmitic, palmitoleic, and stearic acids vary depending on the solvent type, ranging from 5% to 26%. On the other hand, linoleic acid was the smallest amount in all solvents, which does not exceed 3% yield. Other researchers obtained similar fatty acid compositions, claiming that oleic acid is abundant in fish waste, followed by palmitic acid [10, 31–33]. However, according to Shamsudin and Salimon [34], the fatty acid composition of Aji-aji fish oil, obtained through soxhlet extraction, showed palmitic acid as the dominant component (31.5±1.4%), followed by oleic acid (27.7±0.1%) and stearic acid (11.8±0.6%). The sequence of SFA>MUFA>PUFA also contrasts with the order derived from the current investigation (MUFA>SFA>PUFA) due to the distinct compositions of fatty acids present in the different samples.

Fatty acids that contain a significant proportion of oleic acid have been documented to possess favorable attributes for biodiesel production. These attributes enhanced oxidative stability for extended storage duration, elevated cetane number, optimized specific gravity, improved cold flow and viscosity properties [35, 36]. In the subsequent phase of the experiment, ethanol was chosen for fish waste extraction due to its effectiveness in maximizing oil recovery.

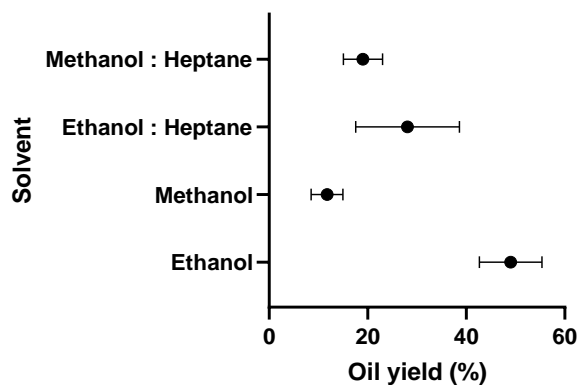


Figure 1. Effect of solvents on the fish oil yield. The extraction conditions were: Sample to solvent ratio = 0.06:1 (w/v), extraction time = 4 h, temperature 60 °C, mixing speed = 500 rpm

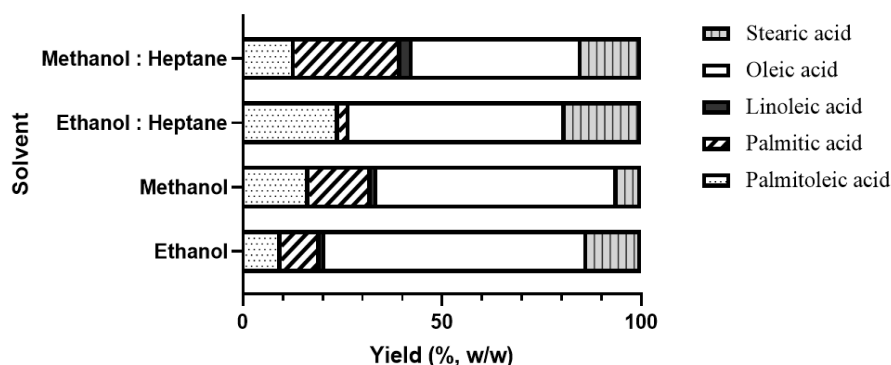


Figure 2. Effect of solvent on the FAME yield. The extraction conditions were: Sample to solvent ratio = 0.06:1 (w/v), extraction time = 4 h, temperature 60 °C, mixing speed = 500 rpm

3.2 Effect of Temperature

The temperature affects the yield of oil extraction from fish waste, as shown in Figure 3. The selection of the optimal temperature is crucial to minimizing lipid oxidation during the extraction process. As the temperature rose from 50 °C to 60 °C, the amount of oil yield increased from 34% to 49%, but it decreased dramatically at higher temperatures (Figure 3). This trend can be attributed to the temperature-induced changes in the solubility, viscosity, and mass transfer characteristics of the solvent system. As the temperature approaches the optimum, the molecular interactions between

ethanol and lipid components become stronger, enhancing the solvent's capacity to dissolve intracellular fats [37]. The temperature rise promotes the disruption of lipid–matrix interactions and improves lipid release into the solvent. Shahie et al. (2018) [37] also noted that temperature affects solvent density and oil solubility, where high temperatures decrease solvent density, resulting in reduced solubilization capacity. At an optimal temperature of 60 °C, solvent viscosity decreases, facilitating faster diffusion of lipids from the cellular interior into the bulk solvent phase, thus improving overall mass transfer efficiency. Reduced viscosity also contributed to greater solvent penetration into the cellular structure, increasing contact with lipid-rich regions. These effects collectively explained the increase in oil yield at 60 °C. However, under lower temperature (<60 °C), the reduced volatility and limited solvent diffusion hinder lipid solubilization from the sample matrix [38].

However, increasing the temperature (> 60 °C) did not improve the yield but instead decreased the extraction recovery (Figure 3). This phenomenon can likely be attributed to two primary mechanisms. First, the components of interest or analytes tend to undergo decomposition when exposed to high temperatures, leading to the transformation of fish waste fat into volatile compounds. This transformation can cause the loss of desirable components [39, 40]. Secondly, at higher temperatures, there is a likelihood of intermolecular disulfide bonds forming, which is associated with protein coagulation [8]. This molecular interaction hinders the release of free fatty acid components. Ghazali & Yasin (2016) [41] and Mani et al. (2007) [42] discovered a similar pattern in oil extraction, claiming that the oil yield from *M. oleifera* seed diminished as the temperature rose above 60 °C. This is further supported by Gowacz-Róyska et al. (2016) [43], who reported that high-temperature extraction results in low-quality fish oil.

The fatty acids composition, however, was affected by temperature variations, as shown in Figure 4. The results of this study revealed that the optimal extraction temperature was 60 °C. When the temperatures exceeded 60 °C, a reduction in the concentrations of specific fatty acids, particularly MUFA and PUFA were observed. For instance, the amount of oleic acid (C18:1) increased as the temperature rose from 50 to 60 °C, with yields of 43% to 65%, respectively, and decreased dramatically at higher temperatures. A similar pattern was observed for stearic acid (C18:0). The amount of stearic acid improved as the temperature increased from 50 to 60 °C, yielding 7% to 16%, respectively. However, it decreased dramatically at higher temperatures. Moreover, deficient linoleic acid (C18:2) was observed at all temperature variations. The reduction in the components at higher temperatures was due to the thermal reduction and oxidation effect.

SFA exhibited higher thermal stability due to the absence of carbon–carbon double bonds in their hydrocarbon chains, which reduced the susceptibility to oxidative cleavage and structural rearrangement. In contrast, MUFA and PUFA possess one or more double bonds, which act as reactive sites and prone to degradation under thermal stress. The presence of allylic hydrogen atoms adjacent to these double bonds facilitates hydrogen abstraction, which triggers free radical chain reactions that lead to lipid peroxidation. The extent of unsaturation directly influenced oxidative susceptibility; as the number of double bonds increased, the reactivity and thermal instability also increased. The fatty acid stability generally follows the order of SFA > MUFA > PUFA. These findings are consistent with the report by Choe and Min (2006) [44], who highlighted that the oxidative stability of fatty acids is strongly influenced by both molecular structure and processing temperature.

The composition of fatty acids may cause significant changes in the biodiesel properties. FAME containing carbon atoms (C16–C18) was considered for biodiesel production [45]. According to Ramos et al. (2009) [46], the ideal biodiesel feedstock should have a high MUFA, a small amount of SFA, and a moderate amount of PUFA. A high oleic acid content (C18:1) leads to excellent biodiesel properties due to increasing oxidative stability, extending storage, improving cold flow properties, influencing cetane number, specific gravity, and viscosity [35, 36]. Therefore, optimizing the extraction temperature is essential not only to achieve maximum oil yield but also to maintain the structural integrity of bioactive lipid components, which is important for biodiesel production.

3.3 Effect of Extraction Time

Extraction time is a critical factor in oil extraction. Figures 5 and 6 show varying extraction times impact both the oil yield and FAME composition. The extracted oil showed an escalating trend, reaching 37% after 4 h of extraction, followed by a minor decline. The kinetics of oil yield extraction demonstrated rapid advancement at 3 and 4 h and subsequently transitioned to a decelerated rate at extended periods (> 4 h) (Figure 5). Prolonged heating time could increase the amount of water-soluble proteins. The extracted oil diffuses fast from the waste material into the solvent due to mass transfer dynamics, particularly since the solvent initiates the extraction process with a lower oil content. As the extraction time extended, the oil concentration in the solvent proportionally increased, while the diffusion rate decreased. Upon reaching their maximum attainable yield, the oil yield level stabilizes. This phenomenon aligns with Mani et al. (2007) [42] and Ghazali & Yasin (2016) [41], who noted that extending the extraction time beyond 6 h for *M. oleifera* seeds did not improve the oil yield.

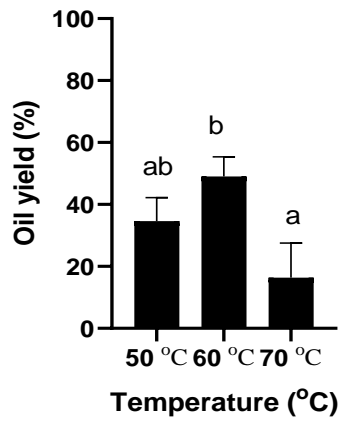


Figure 3. Effect of temperature on the fish oil yield

The extraction conditions were: Sample to solvent ratio = 0.06:1 (w/v), extraction time = 4 h, mixing speed = 500 rpm. Duncan's Multiple Range Test showed that bars are not statistically different ($P < 0.05$) with the same superscript letter

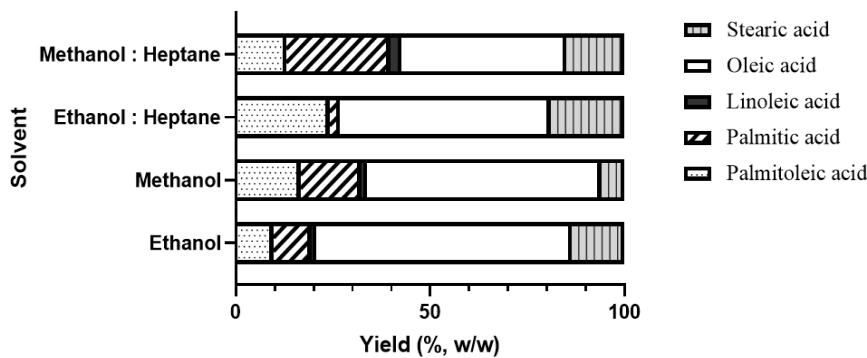


Figure 4. Effect of temperature to the FAME yield

The extraction conditions were: Sample to solvent ratio = 0.06:1 (w/v), extraction time = 4 h, mixing speed = 500 rpm

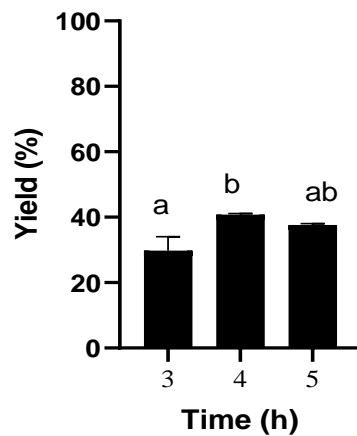


Figure 5. Effect of extraction time on the fish oil yield

The extraction conditions were: Sample to solvent ratio = 0.06:1 (w/v), temperature 60 °C, mixing speed = 500 rpm. Duncan's Multiple Range Test showed that bars are not statistically different ($P < 0.05$) with the same superscript letter

While the FAME composition, especially oleic acid and stearic acid, the yield increased slightly over time (Figure 6). Ramadhas et al. (2005) [47] discovered a similar result, claiming that the ester yield improved as the reaction time extended. After all, the FAME composition contained deficient linoleic acid (1-5%), resulting in a higher ratio of oleic acid to linoleic acid. This ratio is essential for evaluating oil quality. A high ratio confers better stability and longer shelf life. Additionally, no linolenic acid (C18:3) was found throughout the study, indicating its suitability as biodiesel feedstock. The European Standard EN 14 214 stated that the acceptable limit for linolenic acid in biodiesel must be less than 12% [48].

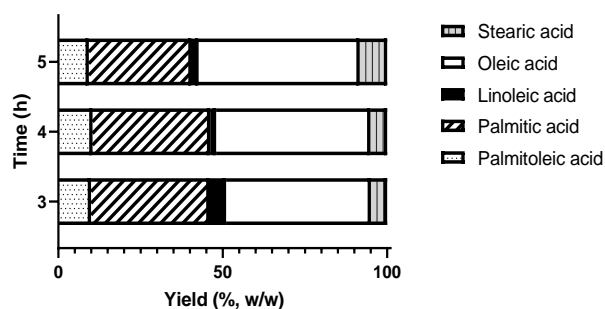


Figure 6. Effect of extraction temperature to the FAME yield

The extraction conditions were: Sample to solvent ratio = 0.06:1 (w/v), temperature 60 °C, mixing speed = 500 rpm

4. CONCLUSION

The study successfully explored various solvents, temperature conditions, and extraction times for extracting fish waste using the soxhlet extraction technique. The highest oil yield recovery was 49% using ethanol as the solvent, at extraction temperature of 60 °C and extraction time of 4 h. Besides, the FAME profile obtained dominated by oleic acid (C18:1) ranging from 42% to 66%, and smaller amounts of palmitic acid (C16:0), palmitoleic acid (C16:1), and stearic acid (C18:0). The least abundant fatty acid was linoleic acid (C18:2) in the range of 1% to 5%. The FAME composition extracted aligns with the stipulated criteria for biodiesel set by the European Standard. This outcome signifies the suitability of the extracted oil as a promising feedstock for biodiesel production. Nevertheless, the present study is constrained by the absence of oxidative stability analysis and storage performance of the extracted oil. To enhance the practical relevance of the work, future research should focus on comprehensive assessments of oxidative stability to facilitate the commercial implementation of fish waste valorization.

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CONFLICT OF INTEREST

The authors declare there are no conflicts of interest in this study.

AUTHORS CONTRIBUTION

N.F.S. Daud (Writing – original draft; Data curation)

F.M. Said (Conceptualization; Writing – review & editing; Project administration; Visualisation; Supervision)

N.H.M. Yasin (Methodology; Visualisation)

Naila Aishath (Writing – review & editing)

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