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Research

Extraction of Oil From Solid Fat of Silver Catfish (*Pangasianodon hypophthalmus*) Waste by Centrifugation

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ABSTRACT

Oil extracted from fish waste could be a source of valuable fatty acids such as saturated (SFAs), monounsaturated (MUFAs), and polyunsaturated fatty acids (PUFAs). In this study, the solid fat from silver catfish (*Pangasianodon hypophthalmus*) waste was used to extract oil by centrifugation using different extraction solvent (distilled water, 70% ethanol, 70% acetone and 70% cyclohexane), rotation time (15, 25, 35 & 45 min), rotational speed (2000, 4000, 6000, 8000 & 10000 r.p.m) and rotational temperature (5, 10, 15, 20 & 25 °C). The compositions of fatty acids in the extracted oil were determined by gas chromatography-mass spectrometry (GC-MS) analysis. The highest oil yield of 156.7 ± 16.7 mg/g was achieved by centrifugation at 10000 r.p.m, 25 °C for 15 min using 70% acetone. The extracted oil contains 0.0223 mg/g palmitic acid, 0.0216 mg/g steric acid, and 0.0262 mg/g oleic acid. However, the essential PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) could not be detected by GC-MS analysis. This study found that the fish oil extracted from the solid fat of the silver catfish waste can be used as a potential source of palmitic acid, stearic acid, and oleic acid.

Key words: Centrifugation, fatty acids, fish oil, fish waste, one-factor-at-a-time, silver catfish

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INTRODUCTION

Generation of fish waste has been increasing every year due to the expansion of fish processing around the world. It has been estimated that 50 - 70 percent of processed fish is considered waste like head, viscera, skin, bone, and scale (FAO, 2020). Normally the waste is thrown away or is used for fishmeal and fertilizers. Many efforts have been made to convert the waste into value-added products such as oil, lipids, enzymes, proteins, collagen, and its derivatives, chitin and chitosan (Alfio *et al*., 2021; Coppola *et al*., 2021; Lionetto and Esposito Corcione, 2021). Fish oil has been produced from fish waste which accounts for 25 to 35 percent of the total volume of fish oil production due to the increase in fish oil prices (FAO, 2020).

Various techniques have been applied to extract oil from the fish waste including, enzyme extraction, supercritical fluid extraction, microwave-assisted extraction, and ultrasound-assisted extraction (Adeoti & Hawboldt, 2014; Ivanovs & Blumberga, 2017; Khawli *et al*., 2019). Extraction by centrifugation or cold extraction is the most common method to separate fish oil from the solid phase by centrifugal force (Rubio-Rodríguez *et al*., 2012; Majekodunmi, 2015). Several centrifugation parameters can be adjusted to produce a high yield of oil such as centrifugation time, rotational speed, and rotational temperature. To date, no study has been carried out to extract fish oil from silver catfish waste by the centrifugation method.

Silver catfish is known as *Patin* or its scientific name *P. hypophthalmus* is one of the most popular freshwater fish in Malaysia, especially around the state of Pahang (MYAgro, 2022). The total production of the fish was around 194,239 metric tons between 2007 and 2018 (Saba *et al*., 2020). Nowadays, fish can be easily farmed in ponds and cages due to its fast growth rate, and is also getting popular in Southeast Asia such as Vietnam (Nam *et al*., 2020) and Indonesia (Ayu *et al*., 2019). The large availability of silver catfish is one of the factors that contribute to the waste generated from fish processing. In the present study, the solid fat of *P. hypophthalmus* waste was used to extract oil by centrifugation using different extraction solvents, rotation times, rotational speed, and rotational temperature.

MATERIALS AND METHODS

Materials

Ethanol (99% purity) was purchased from HmbG Chemicals (Hamburg, Germany). Acetone (99.5% purity) and cyclohexane (99.5% purity) were purchased from Merck (Darmstadt, Germany). Fatty acid standards were purchased from Sigma-Aldrich (St. Louis, USA).

Sample preparation

Fish waste was collected at Kuantan, Pahang. The waste was cleaned with tap water to separate solid fat from unwanted waste such as viscera, fins, and head. The fat was dried at room temperature before being ground using a mortar and a pestle to obtain a fine powder.

Extraction of oil by centrifugation

One-factor-at-a-time (OFAT) technique was used to extract oil from fish waste by centrifugation (Eppendorf 5810 R). 45 mL of solvent was added to 25 g of sample powder in a 50 mL centrifuge tube. Various extraction parameters were studied; different types of solvent (distillate water, 70% ethanol, 70% acetone & 70% cyclohexane), rotation time (15, 25, 35 & 45 min), rotational speed (2000, 4000, 6000, 8000 and 10000 r.p.m) and rotational temperature (5, 10, 15, 20 and 25 °C). The polarity index (P_m) of the organic solvent and water mixture was calculated using Equation 1, where is Ø volume fraction and P is the polarity index of the respective solvent (Musa *et al*., 2011).

Equation 1:

$$P_m = Ø_1P_1 + Ø_2P_2$$

After the centrifugation, the oil (middle layer) was separated from the solvent (top layer) and solid phase (bottom layer). The oil was weighted and yield was calculated using Equation 2.

Equation 2:
Oil yield = mass of oil (mg)/mass of sample (g)

Gas chromatography-mass spectrometry analysis

The fatty acid content in the extracted oil was determined by GC-MS (Agilent Technologies, G3171A). 1 mL oil was trans-esterified using 0.75% hydrochloric acid, 5 mL hexane, and 3 mL ethanol at 80 °C for 2 h in a Pyrex tube. 1 mL of the n-hexane (top layer) was transferred to a 1 mL vial for analysis. The sample was then injected into the HP-5 capillary column and the oven temperature was used from 40 °C to 300 °C at a rate of 10 °C/min. Fatty acids were identified by comparing the retention times of fatty acid methyl ester mixture with the standards (myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, eicosapentaenoic acid & docosahexaenoic acid) (Mat Yasin *et al*., 2021).

RESULTS AND DISCUSSION

Effect of extraction solvent on oil yield

Oil was extracted from solid fat powder by centrifugation using different types of solvent (distillate water, 70% ethanol, 70% acetone & 70% cyclohexane) at 25 °C and 10000 r.p.m for 25 min. In this study, the commonly used solvent systems in oil recovery were investigated as they provide higher extraction efficiency than pure solvents (Ivanovs & Blumberga, 2017). The differences in polarities of solvents affect the efficiency of the extraction process. The highest oil yield was obtained (95.8 ± 12.2 mg/g) using 70% acetone as shown in Figure 1. The polarity indices of the organic solvent and water mixture were calculated based on Equation 1 (Musa *et al*., 2011), where water, 70% ethanol, 70% acetone, and 70% cyclohexane are 9, 6.34, 6.27, and 2.84, respectively. The 70% acetone exhibited the best solvent in fish oil extraction compared to 70% ethanol and water. Water has the highest polarity index but obtained a low oil yield due to its low solubility. Whereas, organic solvents like ethanol and acetone were more favorable for oil diffusion. The non-polar solvent such as cyclohexane could not extract oil because of its low polarity. The 70% acetone was further used to maximize oil yield in extraction time.

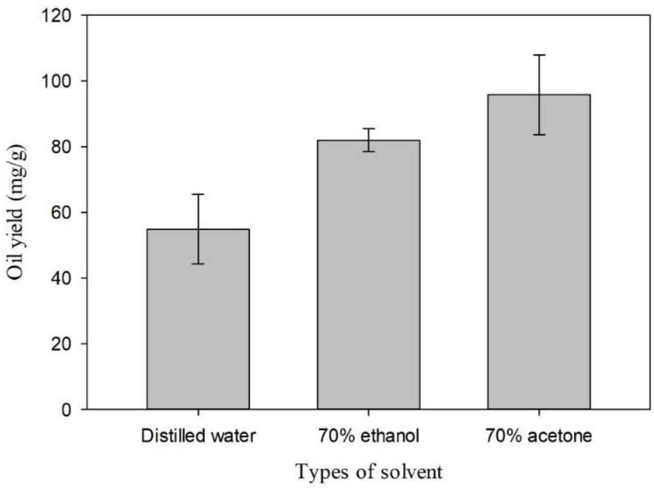

Fig. 1. Effect of extraction solvent on oil yield by centrifugation. Data are presented as the mean value ± SD.

Effect of rotation time on oil yield

The effect of rotation time (15, 25, 35, & 45 min) on the oil yield at 25 °C and 10000 rpm using 70% acetone is indicated in Figure 2. The result shows that the centrifugation method allowed high recovery of oil from the fish waste in less than 15 min. Additionally, the longer rotational time may lead to the degradation of a fish oil compound. However, the effect of time varies depending on the extraction method used. A longer time was required to achieve higher oil yield from by-products of fish in wet rendering (Suseno *et al*., 2021), enzyme hydrolysis (Bruno *et al*., 2019), and Soxhlet extraction (Mat Yasin *et al*., 2021).

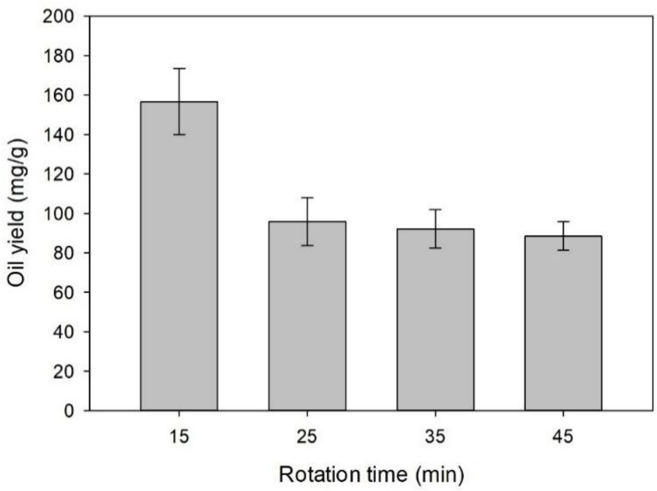

Fig.2 . Effect of rotation time on oil yield by centrifugation. Data are presented as the mean value ± SD.

Effect of rotational speed on oil yield

The effect of the rotational speed on oil yield was further investigated. The speeds were varied at 2000, 4000, 6000, 8000, and 10000 r.p.m at 25 °C using 70% acetone for 15 min. The highest centrifugation speed was needed to achieve the highest yield of extracted oil as illustrated in Figure 3. When the sample was rotated at 10000 r.p.m, a high centrifugal force was applied to the particles (Majekodunmi, 2015). The particles were then moved radially away from the rotational axis, which promotes the oil release from the solid phase. In contrast, the lower speeds (2000 r.p.m & 4000 r.p.m) were unable to extract oil due to the low effect of centrifugal force.

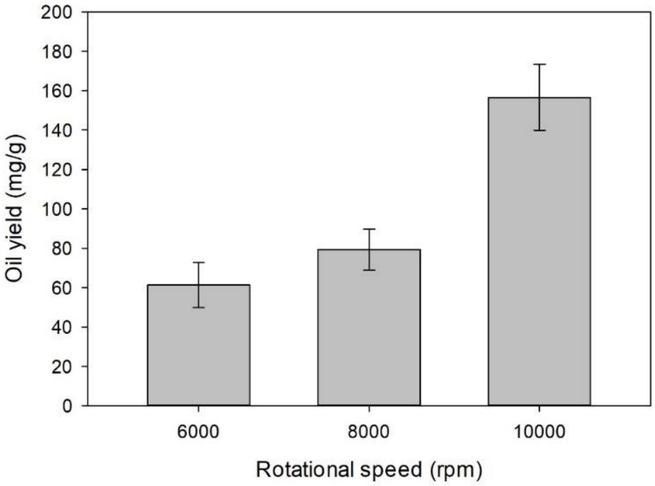

Fig. 3. Effect of rotational speed on oil yield by centrifugation. Data are presented as the mean value ± SD.

Effect of rotational temperature on oil yield

The effect of the rotational temperature on the yield of oil by centrifugation is shown in Figure 4. The centrifugation process was carried out at 10000 r.p.m, using 70% acetone for 15 min. It was observed that the oil was not easily escaped from the particles at cold rotational temperatures (4 to 20 °C). Whereas, a different result was obtained from the previous study where a temperature less than 15 °C was very efficient in extracting oil from salmon skin in an aqueous solvent by cold extraction method (Głowacz-Różyńska *et al*. 2016). To provide high-quality oil from fish waste, a moderate temperature is required to reduce the oxidative stability of oil during the extraction process (Rubio-Rodríguez *et al*., 2012). In extraction that uses high temperature and pressure such as supercritical fluid, a shorter time is required to produce high-quality oil with high recovery of PUFAs (Sahena *et al*., 2010; Ozogul *et al*., 2021).

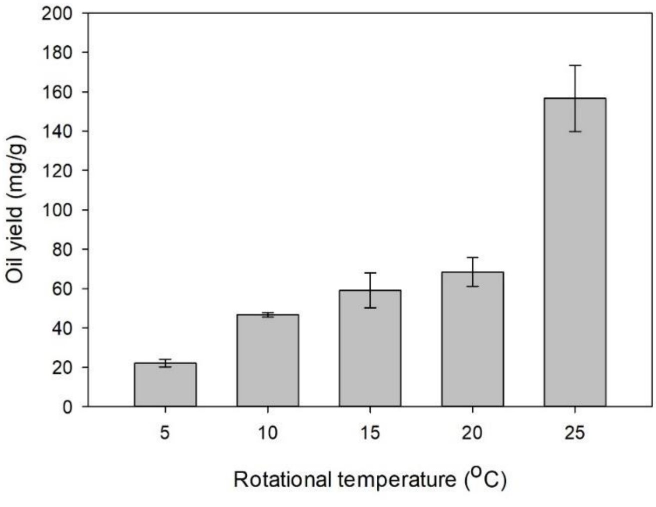

Fig.4 . Effect of rotational temperature on oil yield by centrifugation. Data are presented as the mean value ± SD.

Fatty acid profile

In this study, the maximum yield of fish oil achieved from the OFAT technique was 156.7 ± 16.7 mg/g under centrifugation conditions of 10000 r.p.m at 25 °C for 15 min using 70% acetone. The extracted oil from the solid fat was further analyzed for its fatty acid content by GC-MS analysis. It was found that the oil only contains SFAs (0.0223 mg/g palmitic acid & 0.0216 mg/g steric acid) and MUFA (0.0262 mg/g oleic acid). A previous study showed that the extracted oil from the solid fat of *P.*

hypophthalmus waste consists of SFAs (0.108 mg/g palmitic acid & 0.0802 mg/g steric acid) and MUFA (0.0875 mg/g oleic acid) using ethanol Soxhlet extraction (Mat Yasin *et al*., 2021). It is expected that the Soxhlet extraction provides a higher yield than the centrifugation method. However, both methods were unable to extract EPA and DHA in the solid fat. In another study, 30 mg/g of oil yield was obtained from *P. hypophthalmus* frames by Alcalase hydrolysis (Amiza *et al*., 2013). In a different study, all by-products including head, viscera, trimmings, frames, and skin of *P. hypophthalmus* contain SFAs (54.79 mg/g), MUFAs (7.99 mg/g) and PUFAs (24.07 mg/g) in oil after the Alcalase hydrolysis (Nam *et al*., 2020). In the same study, low yields were obtained for EPA (2.21 mg/g) and DHA (0.57 mg/g) in the oil. It can be concluded that the fish oil yield and fatty acid compositions are dependent greatly on the by-products of fish waste and the extraction method.

CONCLUSION

In this study, the effects of the centrifugation parameters on the oil yield from silver catfish waste were investigated. The highest yield of oil was achieved (156.7 ± 16.7 mg/g) at the centrifugation conditions of 10000 r.p.m at 25 °C for 15 min using 70% acetone. The extracted oil contains 0.0223 mg/g palmitic acid, 0.0216 mg/g steric acid, and 0.0262 mg/g oleic acid. The essential PUFAs such as EPA and DHA, could not be detected by GC-MS analysis. The extracted oil can be used as a potential source of palmitic acid, stearic acid, and oleic acid.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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