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Effect of Deodorization Methods on Physico-Chemical and Fatty Acids Properties of Refined Milkfish (*Channos Channos*) By-Product Oil

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ABSTRACT

Milkfish oil is recognized for its health benefits, particularly due to its high content of omega-3 fatty acids, which include essential fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). During fish processing, by-products of milkfish can be utilized to extract these valuable omega-3-rich oils. This study aimed to evaluate the effects of different deodorization methods on preserving the omega-3 fatty acid content in milkfish by-product oil. To extract the oil, an ultrasound-assisted extraction method was employed, conducted over 68 *min* at 84°C, with a solvent-to-sample ratio of 3:1 mL/g, using d-limonene as a bio-solvent. Deodorization, a critical step in oil refining, was carried out using several techniques, including liquid-liquid extraction (LLE), steam distillation (SD), and solid-phase adsorption with activated carbon (AC), zeolite (ZT), bentonite (BT), and diatomite (DT).The results revealed that the refined milkfish by-product oil showed significant differences in physicochemical characteristics, nutritional content, and saturation levels compared to the crude oil. The LLE method was particularly effective, significantly reducing acid and peroxide values while preserving the omega-3 fatty acid composition. The low temperature used in LLE helped prevent the oxidation and degradation of the oil. Both the LLE and solid-phase adsorption methods proved advantageous due to their cost-effectiveness, efficiency, and ease of application in the deodorization of milkfish oil.

Keywords: Milkfish oil, PUFA, d-limonene, UAE, deodorization methods

INTRODUCTION

In recent years, annual marine fish catch and aquaculture production have reached 100 million metric tons. Approximately 53% of global fishery products are utilized for human consumption, while the remaining portion is allocated for non-culinary uses, such as fish feed (FAO, 2018). In Indonesian cuisine, milkfish are highly valued for their rich flavor and high-fat content, which enhance their appeal as a sought-after culinary ingredient.

Milkfish is a rich source of protein, amino acids, vitamins, and essential fatty acids. Its fatty acid profile includes a significant proportion of unsaturated fatty acids (50.74%), consisting of polyunsaturated fatty acids (34.47%) and monounsaturated fatty acids (16.27%) (Murthy *et al*., 2018).

Milkfish has recently become a popular raw material in the fillet industry, resulting in significant by-product generation, which can account for up to 40% of the original fish. Due to its nutritional benefits, there is increasing interest in milkfish oil. The processing of fresh fish for human consumption produces various edible and non-edible by-products, such as heads, offal, bones, and scales (Sarker, 2020). These by-products present a substantial opportunity for extracting omega-3-rich fish oil from milkfish.

Traditionally, fish oil for food uses is produced through processes such as heating, pressing, centrifugation, and sedimentation. Alternatively, ultrasound-assisted extraction (UAE) using d-limonene as a bio-solvent has been employed to reduce the time required for fish oil extraction. UAE has been successful in extracting

omega-3 fatty acids and other nutrients from Sparus aurata (Çavdar *et al*., 2022). This technology enhances mass transfer and accelerates the extraction kinetics of bioactive components from the matrix, facilitating their release while preserving their nutritional content for use in the food industry (Prasetyaningrum *et al*., 2023).

Fish oil, a significant source of polyunsaturated fatty acids (PUFAs), has garnered considerable scientific interest for its role in human nutrition and disease prevention over the years. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are two polyunsaturated omega-3 fatty acids that constitute the majority of PUFAs, featuring four to six double bonds (Sahena *et al*., 2009). Research has demonstrated the beneficial effects of fish oil, including its potential to reduce the risk of cardiovascular disease, immune system disorders, inflammation, hypertension, and depression (Ellulu *et al*., 2015; Yashodhara *et al*., 2009). Consequently, there has been an intensive effort in scientific research to produce high-quality fish oil.

However, crude fish oil often contains impurities and undesirable compounds, such as free fatty acids, phospholipids, and volatile compounds, which can negatively affect the stability of the oil. Therefore, a refining process is essential to ensure the oil's quality for human consumption or use in nutritional supplements. This refining process includes gum conditioning, neutralization, washing, drying, bleaching, filtration, and deodorization. Among these steps, deodorization is particularly critical as it effectively removes free fatty acids (FFAs), volatile compounds, and oxidation products, thereby improving the oil's stability and quality (Menegazzo *et al*., 2014).

The deodorization process typically involves high temperatures, which can lead to the degradation of long-chain fatty acids. To prevent damage to fish oil, precise temperature control is essential during deodorization. Steam distillation (SD) is the conventional deodorization method, operating at high temperatures (180–270°C) and low pressures (0.1–1 kPa) (Merkle *et al*., 2017). However, this method can induce the formation of methylene-interrupted ethylenic double bonds, resulting in various chemical transformations such as oxidation reactions, cis-trans isomerization, cyclization, polymerization, and double bond migration (Fournier *et al*., 2006). Therefore, exploring alternative methods that use milder conditions to remove odoriferous compounds during the refining of crude fish oil is of significant interest.

Solid-phase adsorption (SPA) and liquid-liquid extraction (LLE) are key technologies used for removing small molecules and refining oil. LLE, particularly with alkaline ethanol, can effectively separate heat-sensitive and low-molecular-weight compounds. Additionally, alkaline ethanol enhances the efficiency of cellulose enzyme hydrolysis, which is crucial in modern biorefinery processes (Cai *et al*., 2016). SPA, utilizing adsorbents such as activated clay, zeolites, diatomite, and bentonite, is employed to remove various contaminants due to its simplicity and high efficacy (Song *et al*., 2018). Despite the effectiveness of these techniques in fish oil deodorization, there is limited comprehensive information on the deodorization process for milkfish by-product oil extracted using ultrasound-assisted extraction (UAE) with green solvents like d-limonene, combined with LLE and SPA. While reports indicate that these methods work well for fish oil deodorization, their specific performance in deodorizing milkfish by-product oil remains underexplored.

This study aims to compare traditional high-temperature deodorization methods with alternative techniques for deodorizing crude milkfish oil. It will also evaluate the effectiveness of various deodorization methods in improving the quality of milkfish oil.

MATERIALS AND METHODS

D-limonene was sourced from Sigma-Aldrich (St. Louis, MO, USA). Solvents and reagents for gas chromatography and analysis were obtained from Merck (Darmstadt, Germany). Activated carbon, zeolites, diatomite, and bentonite were purchased from Brataco Chemical (Yogyakarta, Indonesia).

Milkfish by-product samples were collected from Juwana (-6.633068, 111.121094), Central Java, Indonesia. The samples were cleaned and dried in a cabinet dryer (AM-TD6, PT. Khalifah Niaga Lantabura, Yogyakarta, Indonesia) at 55°C until the water content reached 10%. After drying, the samples were ground using a Philips blender HR2116/40 (Amsterdam, Netherlands) and stored in a freezer at -25°C until extraction.

Milkfish by-product oil extraction using the UAE method

Milkfish by-product oil was extracted using the ultrasound-assisted extraction (UAE) method as described by Çavdar *et al*. (2022), with minor

modifications. The UAE process was conducted in a Bransonic Ultrasonic chamber (8510E MTH, Brookfield, Connecticut, USA) at a frequency of 25 kHz and a power of 200 W. In each experiment, 25 g of milkfish by-product and d-limonene were placed in an Erlenmeyer flask and subjected to ultrasonic extraction. After extraction, the mixture was centrifuged at 6000 rpm for 5 min, and the d-limonene was removed using a rotary vacuum evaporator set to 40°C and 40 mbar.

Sample preparation

The crude milkfish by-product oil was refined through degumming and neutralizing processes based on previously established methods (Šimat *et al*., 2019). Three different methods were employed to deodorize the crude milkfish by-product oil. First, the steam distillation (SD), in which 25 g sample of milkfish by-product oil was heated to 180°C and agitated at 300 rpm. The process was conducted under vacuum pressure (60 kPa) for 60 *min*. Second, the Liquid-liquid extraction (LLE), in which An alkaline ethanol solution was prepared by dissolving 0.5 g of potassium hydroxide in 100 mL of ethanol (45/55, v/v). Twenty mL of milkfish by-product oil was mixed with the alkaline ethanol solution and heated to 70°C. The mixture was stirred and maintained at this temperature for 15 *min*. The oil phase was then separated and washed with water until a neutral pH of 7 was achieved. Third, the solid-phase adsorption (SPA), in which Four different adsorbents-activated carbon, bentonite, diatomite, and zeolite-were used. Ten mL of milkfish by-product oil was heated to 70°C, and 10% of the total oil volume of each adsorbent was added. The mixture was stirred for 15min at the maintained temperature. Subsequently, the mixture was filtered to recover the refined oil.

Physicochemical characterization of milkfish by-product oil

Acid value (AV)

A 1.0 g sample of milkfish by-product oil was weighed and placed in an Erlenmeyer flask. Next, 50 mL of neutralized 95% ethanol and 1 mL of phenolphthalein indicator were added. The sample was then titrated with 0.1 N KOH-ethanol until the phenolphthalein indicator signaled the completion of the titration (AOAC, 2011). The acid value (AV) was calculated using Equation 1.

$$\text{AV (mg KOH/g)} = \frac{\text{KOH Vol (mL) x N KOH x 56.1}}{\text{Milkfish by-produce oil (g)}} \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Peroxide value (PV)

Initially, 1.0 g of milkfish by-product oil was weighed and placed in an Erlenmeyer flask. Then, 30 mL of a chloroform acid (2:3) solution was added to the sample, which was agitated until homogeneous. Next, 0.5 mL of saturated potassium iodide (KI) solution was added, and the mixture was allowed to stand for 1 minute. Following this, 1 mL of a 0.5% starch solution was added as an indicator, and the mixture was titrated with a 0.1 N sodium thiosulfate (Na2SO3) solution. The titration was stopped when the blue color of the mixture disappeared (AOAC, 2011). The peroxide value (PV) was then calculated using Equation 2.

$$\text{PV (mEq O}_2\text{/kg)} = \frac{\text{Na}_2\text{SO}_3\text{ vol (mL) x Na}_2\text{SO}_3\text{ N}}{\text{Milkfish by-produce oil (g)}} \text{x } 1000 \quad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Anisidine value (AV) and TOTOX index

A 1.0 g sample of fish oil was mixed with 25 mL of n-hexane, and the absorbance (A1) was measured using a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at 350 nm. Subsequently, 5 mL of the solution was transferred to a test tube, and 1 mL of p-anisidine in glacial acetic acid was added. The solution was shaken for 10 *min*, and the absorbance (A2) was measured at the same wavelength. The anisidine value was calculated using Equation 3 (AOAC, 2011).

$$\text{PV (mEq O}_2\text{/kg)} = 25 \text{ x } \frac{(1.2 \text{ x } A_2 - A_1)}{\text{Milkfish by-produce oil (g)}} \quad \ldots\ldots\ldots\ldots\ldots\ldots(3)$$

Oil degradation was assessed by calculating the total oxidation (totox) index using Equation 4 (AOAC, 2011).

$$\text{Totox index (mEq O}_2\text{/kg)} = 2\text{PV} + \text{Anisidine value} \quad \ldots\ldots\ldots\ldots\ldots\ldots(4)$$

Iodine value (IV)

A 0.3 g sample of milkfish by-product oil was weighed into an Erlenmeyer flask. The oil was dissolved in 10 mL of chloroform and then mixed with 25 mL of Wijs reagent (1% iodine chloride in glacial acetic acid). After 30 *min* of exposure to darkness, the sample mixture was supplemented with 50 mL of CO2-free distilled water and 10 mL of 15% potassium iodide solution. The mixture was titrated with a standard 0.1 N sodium thiosulfate solution until a faint yellow color appeared. Subsequently, 2 mL of 0.5% starch solution was added as an indicator, and titration continued until the blue color disappeared. Titration against blanks

was also performed. The iodine value (IV) was calculated using Equation 5 (AOAC, 2011).

$$IV\left(\frac{gI_2}{100g}\right) = \frac{(V.\ Na_2SO_3(blank) - V.\ Na_2SO_3) \times Na_2SO_3\ N \times 12.6}{\text{Milkfish by-produce oil (g)}} \quad \ldots\ldots\ldots\ldots\ldots(5)$$

Milkfish by-product oil color and viscosity

Color testing was conducted using a Chromameter CR 400 Series (Konica Minolta Optics, Inc., Ramsey, New Jersey, USA) to measure lightness (L), redness (a), and blueness (b). The viscosity of the milkfish by-product oil was measured with a Brookfield CT3 Viscometer (Middleboro, Massachusetts, USA) using spindle LV2 (61) at 100 rpm. The torque ranged from 15% to 25%.

Fatty acid composition analysis

The fatty acid profile was identified using a gas chromatography flame ionization detector (GC-FID, Agilent 7890B, Santa Clara, USA) in accordance with the AOAC method (AOAC, 2000), with slight modifications. The process began with the derivatization of fatty acids into fatty acid methyl esters (FAMEs). A 0.5 mL sample of milkfish by-product oil was collected, and 1.5 mL of methanolic sodium solution was added. The mixture was heated at 60°C for 10 *min*, then cooled. Afterward, 2 mL of boron trifluoride (BF3) was added, followed by another 10 *min* of heating at 60°C, and then cooled again. Next, 1 mL of saturated sodium chloride (NaCl) and 1 mL of n-hexane were added to the mixture, which was then vigorously agitated using a vortex. The upper layer was carefully transferred to a new vial. A 1 µL volume of the sample solution was injected into the GC-FID, which was fitted with a DB-WAX column (Agilent HP-88, Santa Clara, USA). The oven temperature was programmed to increase from 50°C to 230°C at a rate of 3°C per minute.

Statistical analysis

Data analysis was performed using SPSS® version 24 (New York, USA). The physicochemical characteristics of the milkfish by-product oils were evaluated using one-way Analysis of Variance (ANOVA), followed by Tukey's multiple comparison test to identify significant differences.

RESULTS AND DISCUSSION

Physicochemical characterization

The physicochemical characteristics of milkfish by-product oil deodorized using various methods-including acid value, peroxide value, anisidine value, iodine value, Totox index, color, and viscosity-were thoroughly investigated (Table I). These characteristics serve as quantitative indicators of the quality of the milkfish by-product oil.

Crude milkfish by-product oil initially had an acid number of 1.63 ± 0.02 mg KOH/g, which was reduced through deodorization. The acid number of deodorized milkfish by-product oil ranged from 0.34 to 0.87 mg KOH/g. The acid number is commonly associated with free fatty acid (FFA) formation and undesirable flavor compounds in fats and oils (Crexi *et al*., 2010). The LLE method achieved the lowest acid number (0.34 ± 0.02 mg KOH/g), followed by bentonite (BT) at 0.60 ± 0.07 mg KOH/g, activated carbon (AC) at 0.63 ± 0.12 mg KOH/g, zeolite (ZT) at 0.83 ± 0.09 mg KOH/g, steam distillation (SD) at 0.84 ± 0.11 mg KOH/g, and diatomite (DT) at 0.87 ± 0.09 mg KOH/g. The hydrolysis of triglycerides releases fatty acids from glycerol bonds, while the oxidation of fatty acid double bonds produces free fatty acids (Crexi *et al*., 2010). An increase in free fatty acids during oxidation or hydrolysis indicates oil degradation. The acid value of milkfish by-product oil, containing free fatty acids, must meet the IFOS™ (International Fish Oil Standards) criterion of ≤ 3 mg KOH/g.

Deodorization significantly reduces the peroxide value (PV) of crude oil. The PV of milkfish by-product oils ranged from not detected (n.d.) to 0.1 ± 0.01 mEq O2/kg. The peroxide value indicates the presence of primary oxidation compounds (hydroperoxides) in oils (Tengku Mohamad & Birch, 2013). This value meets the IFOS™ criterion of less than 5 mEq O2/kg. Notably, the PV in milkfish by-product oil deodorized with diatomite was undetectable. Small amounts of peroxide were also observed in milkfish by-product oil deodorized with other adsorbents. Solid-phase adsorbents demonstrated significant reductions in PV, with zeolites showing 0.001 mEq O2/kg, bentonite 0.002 mEq O2/kg, and activated carbon 0.002 ± 0.00 mEq O2/kg. Adsorbent-based deodorization effectively reduces primary oxidation products due to favorable sorption characteristics. Previous studies have shown that increasing adsorbent concentration correlates with a decrease in peroxide value, reflecting improved oil quality (Rosmalina *et al*., 2021).

The anisidine value (AV) is an indicator of secondary oxidation. It is correlated with the peroxide value, as high peroxide values accelerate the formation of secondary oxidation products.

Table I. Physicochemical characterization of milkfish by-product oil.

Deodorization	Acid value (mg KOH/g)	Peroxide value (mEq O_2/kg)	Anisidine value (mEq O_2/kg)	Iodine value (g I_2/100g)	Totox index (mEq O_2/ kg)	Viscosity (cP)	Color		
							L	a	b
Crude oil (CO)	1.63± 0.02^{a}	4.40± 0.23^{a}	14.18± 0.19^{a}	100.56 ±1.10^{a}	22.98± 0.64^{a}	53.99± 0.7^{a}	42.3± 0.01^{a}	-3.69± 0.22^{d}	8.08± 0.06^{e}
Liquid-liquid Extraction (LLE)	0.34± 0.02^{c}	0.003± 0.00^{c}	4.57± 0.40^{cd}	99.61± 0.10^{c}	4.58± 0.40^{c}	44.13± 0.57^{de}	11.39± 0.17^{d}	0.07± 0.10^{c}	10.36± 0.1^{b}
Steam Distillation (SD)	0.84± 0.11^{b}	0.10± 0.01^{b}	2.76± 0.08^{e}	101.18 ±0.12^{a}	2.9± 0.07^{e}	51.17± 0.55^{b}	9.88± 0.15^{e}	3.07± 0.4^{a}	9.88± 0.04^{bc}
Activated carbon (AC)	0.63± 0.12^{bc}	0.002± 0.00^{c}	4.02± 0.17^{de}	100.33± 0.10^{b}	4.03± 0.17^{d}	45.63± 0.64^{d}	11.04± 0.17^{d}	0.11± 0.09^{c}	8.63± 0.28^{e}
Bentonite (BT)	0.60± 0.07^{bc}	0.002± 0.00^{c}	6.39± 0.30^{b}	99.33 ± 0.11^{cd}	6.39± 0.30^{b}	48.06± 0.80^{c}	14.12± 0.16^{b}	1.17± 0.11^{b}	12.80± 0.33^{a}
Diatomite (DT)	0.87± 0.09^{bc}	n.d	5.94± 0.27^{b}	98.96± 0.10^{cd}	6.39± 0.26^{b}	43.35± 0.70^{e}	11.42± 0.03^{d}	0.95± 0.06^{b}	9.17± 0.10^{cd}
Zeolite (ZT)	0.83± 0.09^{bc}	0.001± 0.00^{c}	3.30± 0.27^{de}	98.04± 0.10^{e}	3.30± 0.26^{de}	49.10± 0.25^{c}	13.66± 0.06^{c}	0.78± 0.05^{b}	9.09± 0.60^{de}
(IFOS, 2011)	**< 3**	**< 5**	**< 20**	**-**	**<26**				

* Values are presented as means ± SD. Different letters (a-e) within the same column indicated significant differences ($p<0.05$)
n.d = not detected

The AV of crude milkfish by-product oil was 14.18 ± 0.19 mEq O2/kg, which decreased to between 3.30 and 6.39 mEq O2/kg after deodorization using various methods. This demonstrates that deodorization significantly reduces the anisidine value. The IFOS™ standard (2014) requires an AV of ≤ 20 mEq/kg. The lowest AV observed was with steam distillation (SD) at 2.76 ± 0.08, followed by zeolite (ZT) at 3.30 ± 0.27, activated carbon (AC) at 4.02 ± 0.17, liquid-liquid extraction (LLE) at 4.57 ± 0.40, diatomite (DT) at 5.94 ± 0.27, and bentonite (BT) at 6.39 ± 0.30. These results suggest that the adsorbents used are effective in adsorbing both primary and secondary oxidation compounds (Chakraborty & Joseph, 2015).

The Totox index assesses lipid oxidative damage. This study revealed that various deodorization treatments significantly impacted the reduction of the total oxidation value in crude oil. The steam distillation (SD) method achieved the lowest Totox index at 2.97 ± 0.07 mEq/kg. The LLE, SD, and adsorbent methods effectively reduced the Totox value of milkfish by-product oil to levels that meet the IFOS™ criteria. The total oxidation value is positively correlated with both primary and secondary oxidation levels. The acceptable limit for the Totox value in fish oil for human consumption is ≤ 26 mEq/kg.

The iodine value (IV) reflects the degree of unsaturation of fatty acids in fish oil, with higher values indicating greater unsaturation due to tighter iodine binding. In this study, the IV of milkfish by-product oil showed a slight reduction during deodorization refining, though the difference was not statistically significant ($p > 0.05$) compared to crude oil and oil obtained via steam distillation. The iodine values across different deodorization methods ranged from 98.04 to 101.18 g I2/100 g. Rai *et al*. (2010) reported that acceptable fish oils typically have an IV between 95 and 118 g I2/100 g. The observed decrease in IV during refining likely reflects a reduction in unsaturated fatty acids, particularly polyunsaturated fatty acids (PUFAs) (Table II). A similar decrease in iodine value during refining has also been reported for Nile tilapia oil (Menegazzo *et al*., 2014).

The iodine value (IV), peroxide value (PV), and anisidine value (AV) of refined milkfish by-product oil extracted using the d-limonene combined with ultrasound-assisted extraction (UAE) were lower compared to milkfish oil extracted using the pressing method. Specifically, the chemical characteristics of pressed milkfish oil are as follows: acid value (mg KOH/g) 0.522 ± 0.025, peroxide value (mEq O2/kg) 6.830 ± 0.095, and iodine value (g I2/100 g) 95.297 ± 0.742 (Hidayah *et al*., 2022). Variations in fish oil quality are influenced by several factors, including oil composition, extraction process, and the freshness of the raw material (Dominguez & Barbagallo, 2018).

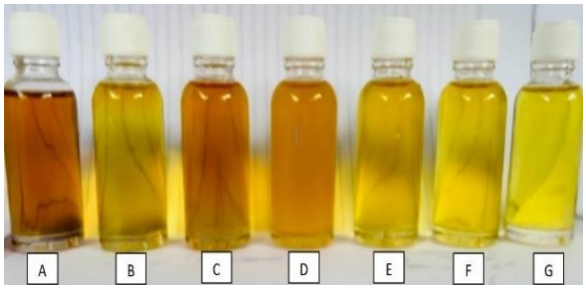

Figure 1. The colour milkfish by-product oil processed with different deodorization methods with two replications significantly different compared to crude oil ($p<0.05$), A: crude oil, B: liquid liquid extraction, C: steam distillation, D: activated carbon, E: bentonite, F: diatomite, G: zeolite

The visual attributes of oil, particularly its color, are key indicators of consumer acceptability. The L value represents lightness on a scale from 0 to 100, while the a* value indicates redness or greenness, and the b* value denotes yellowness or blueness. Crude milkfish by-product oil is darker compared to deodorized oil (Figure 1). Deodorization processes, including bleaching, affect the color of milkfish oil. The oil processed with adsorbents and using liquid-liquid extraction (LLE) exhibited a yellow and transparent hue, significantly different from the crude oil ($p < 0.05$). Adsorbents in the fish oil refining process effectively remove color pigments and contaminants, thereby improving the oil's physical and chemical quality (Suseno *et al*., 2012). Adsorbents achieve this by binding color pigments to their surfaces through physical or chemical adsorption, which helps to eliminate or reduce undesirable color pigments from the oil.

Viscosity is a crucial characteristic of milkfish by-product oil as it directly affects the overall quality of the oil. The viscosity of crude milkfish by-product oil is 53.99 ± 0.70 cP. After deodorization, the viscosity of the oil decreased, ranging from 43.35 ± 0.70 to 51.17 ± 0.55 cP. Among the deodorization treatments, the use of diatomite absorbent resulted in the lowest viscosity value of 43.35 ± 0.70 cP. This finding is consistent with previous studies that reported a reduction in viscosity of sunflower oil when refined with absorbents (Farag & Basuny, 2009). Generally, a higher viscosity indicates lower quality in fish oil. Factors influencing the viscosity of the oil include impurities, density, melting point, degree of unsaturation, and temperature (Zahir *et al*., 2017).

Fatty Acid Composition

The proportions of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in deodorized milkfish by-product oil (Figure 2). The composition of fatty acids in the milkfish by-product oil and the relative proportions of each type of fatty acid varied depending on the deodorization method used, including steam distillation, liquid-liquid extraction (LLE), and adsorption using zeolites, bentonite, diatomite, and activated carbon.

The composition and relative content of fatty acids in milkfish by-product oil varied depending on the deodorization methods used, including steam distillation (SD), liquid-liquid extraction (LLE), and adsorption with zeolites, diatomite, bentonite, and activated carbon. A total of 30 fatty acids were identified in both crude and refined milkfish by-product oil. Palmitic acid (C16:0) was found to be a major component in both the crude and refined milkfish by-product oil, comprising 47.64% and 48.17% of the total saturated fatty acids, respectively (Table II). This result aligns with reported values for various marine fish species, where palmitic acid can constitute up to 70% of the total saturated fatty acids (Özogul *et al*., 2008). Unsaturated fatty acids were predominant in the milkfish by-product oil. Specifically, oleic acid (15.62%) and linoleic acid (12.99%) were the primary unsaturated fatty acids identified in both crude and refined milkfish by-product oil (Table III).

The percentage of polyunsaturated fatty acids (PUFA) in crude milkfish by-product oil was 28.67 ± 0.3%. After deodorization, the PUFA percentages in the processed oils were as follows: liquid-liquid extraction (LLE) at 28.30 ± 0.97%, steam distillation (SD) at 25.60 ± 1.41%, activated carbon (AC) at 25.60 ± 0.72%, bentonite at 23.80 ± 0.46%, diatomite at 25.24 ± 0.61%, and zeolites at 25.10 ± 0.38%. PUFA content is a crucial indicator for assessing the nutritional value of fish oils (van den Elsen *et al*., 2013). Omega-3 fatty acids, such as EPA and DHA, are essential for human health and the prevention of various illnesses (Table II). The saturated fatty acids (SFA) profile of milkfish by-product oil processed using different deodorization methods sources of PUFA. However, PUFA can degrade when subjected to high temperatures due to the instability of their double bonds (Li *et al*., 2012). Therefore, the total PUFA content in milkfish by-product oil may be affected by deodorization methods that involve relatively high temperatures.

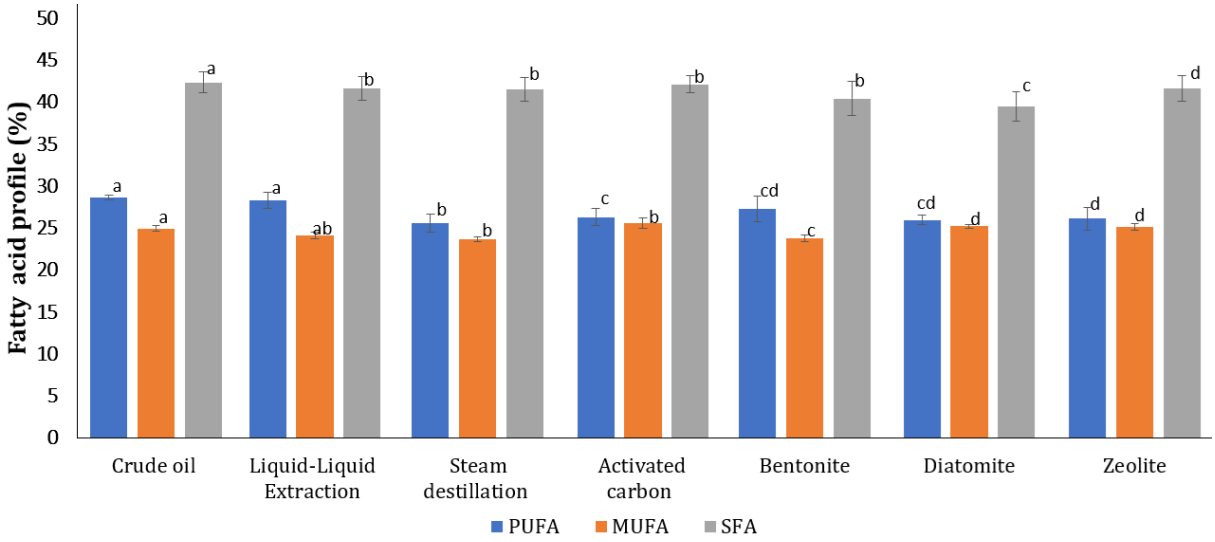

Figure 2. PUFA, MUFA, and SFA composition of crude and refined milkfish by-product oil, different letters (a-d) indicated significant differences to crude and refined milkfish by-product oil with different deodorization methods ($p<0.05$)

Table II. The saturated fatty acids (SFA) profile of milkfish by-product oil processed using different deodorization methods.

Fatty acid profile (%)	**Deodorization method**						
	CO	**LLE**	**SD**	**AA**	**BT**	**DT**	**ZT**
Lauric acid C12:0	0.99±0.02	0.86±0.07	1.04±0.04	1.01±0.01	0.89±0.02	0.98±0.07	0.99±0.07
Tridecanoic acid C13:0	1.08±0.03	1.02±0.11	1.07±0.02	1.06±0.01	1.04±0.03	1.04±0.02	1.21±0.24
Myristic acid C14:0	3.59±0.05	2.89±0.06	2.89±0.04	2.89±0.07	2.89±0.42	2.89±0.35	2.89±0.09
Pentadecanoic acid C15:0	3.92±0.07	3.02±0.36	3.06±0.02	3.02±0.03	3.02±0.01	3.12±0.01	3.23±0.13
Palmitic acid C16:0	20.17±0.62	19.75±0.18	19.95±0.56	20.05±0.02	19.05±0.73	18.05±0.67	19.85±0.28
Heptadecanoic acid C17:0	1.79±0.04	2.89±0.07	2.46±0.31	2.79±0.12	2.48±0.28	2.56±0.07	2.65±0.16
Stearic acid C18:0	5.48±0.08	5.26±0.09	5.15±0.57	5.26±0.05	4.99±0.14	5.04±0.1	5.06±0.28
Arachidic acid C20:0	1.06±0.03	1.26±0.19	1.24±0.03	1.26±0.07	1.28±0.07	1.26±0.04	1.25±0.56
Heneicosanoic acid C21:0	1.61±0.09	1.61±0.07	1.61±0.11	1.61±0.26	1.59±0.05	1.58±0.22	1.48±0.06
Behenic acid C22:0	0.75±0.08	0.95±0.04	0.94±0.08	0.98±0.08	0.96±0.06	0.87±0.09	0.85±0.12
Trichosanoic acid C23:0	0.17±0.01	0.24±0.02	0.26±0.04	0.27±0.02	0.25±0.03	0.27±0.03	0.28±0.05
Lignoceric acid C24:0	1.74±0.13	1.89±0.07	1.85±0.09	1.94±0.12	1.98±0.15	1.89±0.10	1.86±0.03
Total SFA	42.34±1.25^{a}	41.64±1.33^{b}	41.52±1.91^{b}	42.14±0.86^{b}	40.42±1.99^{b}	39.55±1.77^{c}	41.60±2.07^{d}

* Values are presented as means ± SD, different letters (a-d) within the different column indicated significant differences ($p<0.05$)

Table III. The monounsaturated fatty acids (MUFA) profile of milkfish by-product oil processed using different deodorization methods

Fatty acid profile (%)	Deodorization method						
	CO	LLE	SD	AA	BT	DT	ZT
Myristoleic acid C14:1	0.53±0.07	0.50±0.05	0.40±0.08	0.40±0.12	0.10±0.12	0.10±0.42	0.40±0.11
Palmitoleic acid C16:1	4.40±0.06	2.70±0.07	4.70±0.01	3.40±0.22	4.70±0.02	5.30±0.01	4.50±0.01
Cis-10-heptadecanoic acid C17:1	0.66±0.02	0.70±0.07	1.10±0.01	1.30±0.14	1.50±0.04	1.70±0.02	1.00±0.06
Elaidic acid C18:1n-9t	1.74±0.03	1.70±0.01	0.10±0.02	0.90±0.01	0.10±0.04	0.30±0.01	0.20±0.04
Oleic acid C18:1n-9c	15.62±0.04	16.70±0.07	16.10±0.02	17.50±0.04	16.50±0.12	16.10±0.04	17.40±0.05
Cis-11-eicosanoic acid C20:1	0.51±0.04	0.50±0.01	0.40±0.03	0.60±0.04	0.50±0.04	0.50±0.06	0.40±0.01
Erucic acid C22:1	1.25±0.02	1.20±0.06	0.80±0.05	1.30±0.14	0.20±0.02	1.10±0.04	1.00±0.06
Nervonic acid C24:1	0.26±0.01	0.10±0.03	0.10±0.04	0.20±0.01	0.20±0.06	0.12±0.01	0.20±0.04
Total MUFA	24.97±0.29^{a}	24.10±0.37^{ab}	23.70±0.26^{b}	25.60±0.72^{b}	23.80±0.46^{c}	25.24±0.61^{d}	25.10±0.38^{d}

*Values are presented as means ± SD, different letters (a-d) within the different column indicated significant differences ($p<0.05$)

Table IV. The polyunsaturated fatty acids (PUFA) profile of milkfish by-product oil processed using different deodorization methods

Fatty acid profile (%)	Deodorization method						
	CO	LLE	SD	AA	BT	DT	ZT
Linolelaidatic acid C18:2n-9t	2.74±0.01	2.70±0.01	2.30±0.10	3.10±0.14	2.90±0.02	2.90±0.01	3.20±0.02
Linoleic acid C18:2n-6c	12.99±0.08	12.70±0.07	12.40±0.08	12.80±0.21	12.60±0.12	12.70±0.16	11.00±0.62
γ-linolenic acid C18:3n-6	0.26±0.07	0.30±0.05	0.20±0.49	0.30±0.09	0.30±0.01	0.20±0.06	0.30±0.07
Linolenic acid C18:3n-3	0.83±0.02	0.90±0.06	0.60±0.01	0.90±0.08	0.90±0.12	0.80±0.08	0.70±0.06
Cis-11. 14-eikosedienoat acid C20:2	0.43±0.01	0.40±0.03	0.40±0.007	0.40±0.001	0.40±0.02	0.30±0.03	0.50±0.007
Cis-8.11.14-eikosetrienoic acid C20:3n-6	1.21±0.02	1.30±0.02	1.00±0.007	1.20±0.13	1.20±0.1	1.20±0.03	1.20±0.04
Arachidonic acid C20:4n-6	0.15±0.03	0.20±0.05	0.10±0.08	0.10±0.03	1.30±0.78	0.20±0.04	0.20±0.01
Cis-13.16-doxosadinoic acid C22:2	0.68±0.01	0.80±0.06	0.50±0.11	0.70±0.08	0.70±0.06	0.40±0.05	0.60±0.12
Cis-5.8.11.14.17-eicosapentaenoic acid C20:5n-3	0.13±0.03	0.10±0.02	0.20±0.06	0.20±0.02	0.30±0.03	0.20±0.03	0.20±0.05
Cis-4.7.10.13.16.19-dokosahexaenoic acid C22:6n-3	9.25±0.02	8.90±0.57	7.90±0.47	6.60±0.21	6.50±0.28	7.10±0.06	8.10±0.21
Total PUFA	28.67±0.3^{a}	28.30±0.97^{a}	25.6±1.41^{b}	26.3±0.99^{c}	27.30±1.54^{cd}	26.00±0.55^{cd}	26.12±1.21^{d}

*Values are presented as means ± SD, different letters (a-d) within the different column indicated significant differences ($p<0.05$)

In refining milkfish by-product oil, the deodorization method typically results in a decrease in the total polyunsaturated fatty acids (PUFA). This reduction occurs due to the use of chemical reagents and high temperatures during the process, which can cause minor deterioration of these valuable compounds (Vaisali *et al*., 2015). While refining is necessary to produce oils that are acceptable for human consumption, optimizing the process conditions is crucial to minimizing the loss of essential components such as PUFA (Table IV).. Omega-3 PUFA includes eicosatrienoic acid (C20:3n-6), eicosapentaenoic acid (C20:5n-3), and docosahexaenoic acid (C22:6n-3). Omega-6 PUFA comprises linoleic acid (C18:2n-6), gamma-linolenic acid (C18:3n-6), eicosadienoic acid (C20:2), and arachidonic acid (C20:4n-6).

Trans fatty acids (C18:1 trans) were detected across all deodorization methods, with the highest concentrations found in the LLE and activated carbon (AC) treatments, at 1.7% and 0.9%, respectively. The presence of trans isomers is likely due to heat-induced isomerization. The temperatures and heating durations applied during deodorization are sufficient to cause the native cis double bonds of PUFA to isomerize into the more thermodynamically stable trans configuration (Li *et al*., 2012). The World Health Organization (WHO) recommends limiting trans-fat consumption to less than 1% of total caloric intake. Therefore, optimizing deodorization process conditions and selecting the appropriate method are crucial for preserving essential fatty acids and nutrients in fish oil.

CONCLUSION

The physicochemical properties of refined milkfish by-product oil, particularly odor and low levels of free fatty acids, are crucial quality parameters. Deodorization methods using liquid-liquid extraction (LLE), steam distillation (SD), and adsorbents effectively produce milkfish oil that meets IFOS™ standards for acid value, peroxide value, and anisidine value. Among these methods, LLE stands out for significantly reducing free fatty acids ($p<0.05$) while preserving the composition of omega-3 and PUFA fatty acids. Compared to traditional methods, LLE is considered simpler, more cost-effective, and efficient.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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