

Physicochemical and Fatty Acid Profile of Refined Tuna Fish Oil By-Product from Canning and Meal Fish Industries

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Abstract

Fish oil by-product (FOB) recovered from the precooking process in the cannery industry and the pressing process in fish meal production are potential resources. However, this fish oil still needs a refinery process to improve the quality of fish oil. This study aimed to identify the physicochemical characteristics of the initial fish oil by-product, the physicochemical profiles, and fatty acids of the fish oil by-product after degumming neutralization and bleaching (bentonite, zeolite, and carbon adsorbents) processes from the cannery and fish meal industry. Results showed the initial physicochemical characteristics of fish oil waste from fish flour have lower quality than canned products (acid value, free fatty acids, PV, AV, TOTOX value, viscosity, and refractive index) but are inversely proportional to the iodine value. Results showed degumming (D) and neutralization (N) processes from the Fish oil canning process (FCP) and Fish oil meal process (FMP) could improve the quality parameters of fish oil by-products by reducing the acid value (7.90, 0.65, 9.17, 0.58 mg KOH/g), respectively; free fatty acid (3.97, 0.33, 4.60, 0.29 % oleic) respectively; peroxide value (PV) (15.18, 7.38, 17.64, 7.10 mEq/kg), respectively; anisidine value (AV) (14.36, 6.61, 14.64, 6.51) respectively; total oxidation (TOTOX) value (44.72, 21.37, 49.64, 20.71) respectively; and iodine (167, 153, 163, 155 mg/100 g), respectively as well as increasing the lightness (23.80, 25.70, 20.65, 26.00), respectively. In the bleaching process (B), zeolite (15 %) was an effective adsorbent to obtain oil with the best quality parameters due to zeolite possessed greater polarity and surface area at a higher concentration, which resulted in the acid value for FCP and FMP (0.26, 0.22 mg KOH/g); free fatty acid (0.13, 0.15 % oleic); PV (2.44, 5.71 mEq/kg); AV (2.5, 4.9); TOTOX value (7.16, 9.11); and iodine (138, 151 mg/100 g) as well as increasing the lightness (37.08, 36.74) that are appropriate to International Fishmeal & Oil Manufacturers Association (IFOMA), Codex Alimentarius Committee (CAC), and International Fish Oils Standards (IFOS) standards ($p < 0.05$). All adsorbents effectively increase the brightness of the FOB. The higher the concentration of adsorbents, the higher the clarity of the FOB. For zeolite adsorbents, there was an increase of 16.73 - 32.3 % for FCP and 15.39 - 32.69 % for FMP, but the viscosity decreased with a range of 24.50 - 36.50 cPs. FCP contains 14 fatty acids, while FMP contains 12 fatty acids. The number of SFA and MUFA detected in FCP is lower than in FMP. In contrast, the amount of PUFA in FCP is relatively higher than in FMP. The percentage of EPA and DHA in FCP is lower than in FMP. Overall, the refining process affects the fatty acid composition of fish oil by-products both in the canning process and in fish meal.

Keywords: Adsorption, Bleaching, Degumming, Fish canning industry, Fish oil by-product

Introduction

Indonesia holds a significant position in the primary fisheries-related economic activities, and the country's rich marine resources have led to the establishment of a thriving seafood industry [1,2]. Tuna (*Thunnus* sp.), known as Indonesia's main catch commodity, is one of the bountiful fish in Indonesia, with total production reaching 355,349 tons in 2022 [3]. One of the main tuna species caught in Indonesia is yellowfin tuna (*Thunnus albacares*). Therefore, Indonesia became one of leading exporters contributing to 4.16 % of tuna canning products worldwide [4]. The growth of tuna canning industry resulted in the high production of fish waste, including solid waste and wastewater. Around 30 - 65 % of solid by-products [5] and wastewater comprising fish oil are generated during the processing. Generally, solid wastes are further processed into fish meals and brought out fish oil by-products (FOB). According to Costa *et al.* [5], more

than 150 tons of fish oil are produced annually and washed away in wastewater during fish processing. Those FOB could be recovered to improve the value-added and to prevent nutrient losses in the food chain. In addition, the recovery process of fish oil in wastewater could hinder severe environmental pollution.

Fish oil has been widely known as a source of omega-3 (n-3) fatty acids, mainly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), attributed to many remarkable health benefits [6]. EPA effectively treats cardiovascular diseases and prevents thrombosis, atherosclerosis, and inflammatory diseases [7-9]. On the other hand, DHA has an essential role in preventing skin disorders and enhancing brain development [10-12]. However, fish oil by-products obtained fishery industry contain impurities and undesirable components affecting the products' stability, such as free fatty acids, phospholipids, and other volatile compounds, as well as have off-odor and dark color [13]. Therefore, refining is required to eliminate those undesirable compounds and generate edible fish oil products for human diet.

The chemical refining steps used to improve the characteristic of FOB are degumming, neutralization, and bleaching [14]. The degumming process aims to remove gums and phospholipids; neutralization is used to remove free fatty acid by caustic soda; and bleaching removes soap and trace elements in FOB [14-17]. The refining process of FOB from various marine sources has been studied, for example, fish waste of mackerel and crucian carp with conventional extraction [18], neritic tuna using supercritical carbon dioxide [19], tuna heads with enzymatic hydrolysis [13], Nile tilapia and hybrid sorubim, as well as ray liver using chemical refining process [14,16]. Different sources of raw materials and refining techniques affected FOB's final characteristics and quality [20,21].

Furthermore, absorbent treatment in the bleaching process also influenced fish oil's final color and odor. Monte [22] mentioned that adding 10 % activated carbon in carp viscera crude oil showed the lowest losses of carotenoid content and the highest decreases in dark oil color. Güner *et al.* [23] also reviewed that all tested absorbents effectively improved fish oil's sensory attributes. As aforementioned, the literature shows limited data about studies that revealed the FOB treatment from yellowfin tuna, Indonesia's dominant canned fish raw material. For this reason, this current study aimed to fill this gap by evaluating the physicochemical and quality of refined yellowfin tuna FOB from the canning and fish meal industry in every step of the chemical refining process, including degumming, neutralization, and bleaching, which makes it interesting to study. In this study, different types and concentrations of absorbents were applied in bleaching to observe the best treatment that will benefit the industrial production of FOB.

Materials and methods

Materials

Fish oil by-products (FOB) of tuna yellowfin (*Thunnus albacares*) from canning and fish meal industries in Banyuwangi East Java Indonesia was used as the study's primary source of raw materials. The fish canning and flour industry wastewater treatment facility samples were sent to the laboratory and then frozen at $-40\text{ }^{\circ}\text{C}$ until further research. The chemicals used in the purification and chemical properties processes (phosphoric acid, sodium hydroxide, ethanol, phenolphthalein, ammonium thiosulfate, barium dichloride, ferro sulfate, chloric acid, ferric chloride, peroxide acid, benzene, methanol, distilled water, glacial acetic acid, hexane, p-anisidine reagent, scorching reagent, chloroform, starch indicator, activated zeolite, charcoal, and bentonite with a size of 60 mesh) were purchased from chemical stores. The highest analytical grade was employed for all the solvents and reagents.

Methods

This study was divided into 3 stages. The first stage was to analyze the characteristics initial physicochemical properties of fish oil by-products (FOB), encompassing color, viscosity, refractive index, free fatty acids (FFA), PV, iodine value, AV, total oxidation number (TOTOX), acid number, and fatty acid profiles from canning and fish meal industries. The second stage was to analyze physicochemical properties after the degumming, neutralization, and bleaching process. The last step was to investigate the fatty acid profiles of refined FOB in the degumming, neutralization, and bleaching process. Bleaching was performed while 3 distinct adsorbents (bentonite, zeolite, and activated carbon) were present at 5, 10, and 15 % (w/w) concentrations.

Fish oil by-products (FOB) Refining Process

Degumming process

The gum removal process was carried out by stirring 500 g of FOB from canned and fish meal facilities at $70\text{ }^{\circ}\text{C}$ [24]. Following that, phosphoric acid with a concentration of 85 % (v/v) was added to the oil in a quantity equal to 1 % of its weight. The stirring operation was carried out for 30 min at a steady temperature. After that, the oil was cooled for about 15 min at room temperature. The final oil and mucus mixture was

centrifuged at 5000 rpm while the degummed oil was examined for its fatty acid, chemical, and physical contents.

Neutralization process

500 g of degummed oil (DFOB) was heated to the desired temperature at 60 °C while stirring slowly [25]. The deacidification reactor was filled with NaOH solution and heated for 10 min to 70 °C. The DFOB was cooled to ambient temperature and centrifuged for 15 min at 5000 rpm to separate it. The final oil was washed by spraying 5 % distilled from the soap for \pm 10 min. The washing process was repeated until neutral wash water was obtained. Finally, the neutral FOB was analyzed in physical, chemical, and fatty acid composition.

Bleaching process

Adsorbents were added to the mixture and stirred continuously at 80 °C for 20 min to complete the bleaching process following Pedro's approach [25]. The amount of adsorbent, which came in activated zeolite, charcoal, and bentonite, varied from 5, 10, to 15 %. Following that, the oil and adsorbent mixture was spun at 10,000 rpm for 10 min at room temperature (29 °C). The aluminium foil-wrapped container or the dark bottle where the bleached fish oil separated from the adsorbent was stored was kept at a temperature of 4 °C. In the final step, the physicochemical properties and fatty acid composition of the bleached fish were examined.

Fish oil by-products (FOB) physicochemical properties analysis

The properties of canned and fish meal waste oils were carried out prior to the refining process. Physical properties consisted of color, viscosity, and refraction brix analysis following [26,27] methods. At the same time, chemical characteristics encompassed acid value, FFA, PV, AV, total oxidation number (TOTOX), and fatty acids compositions according to Hastarini [27]. The equipment used to analyze the physicochemical properties of FWO were "Denver Instrument M 310" analytical balance, 20D spectrophotometer Plus "LaboMed", Color reader (Minolta CR 300), viscometer (Elcometer 2300 RV) and Abbe refractometer. The composition of fatty acid of FWO was analyzed using GC-MS "Shimadzu QP2010S, AGILENT DB-1 column, length 30 m, helium carrier gas, ionizing EI 70 Ev, pressure 12.0 kPa, column flow 0.54 mL/min, linear velocity 26.6 cm/s".

Data Analysis

The results of each experimental analysis were given as mean standard deviation (SD), with each experiment being run in triplicate. Quantitative information from 2 different FWO sources in the process-by-product oil from canning and flouring was included in this research and was descriptively examined. Color, viscosity, refractive index, free fatty acid content, PV, AV, iodine value, TOTOX value, acid value, and fatty acid composition were reported for both FOB in the degumming and neutralization processes in tabular form and figures. Analysis of variance (ANOVA) was also employed to observe the significance of differences using one-way, followed by Tukey post hoc testing to look at group differences. The standard for statistical significance was $p < 0.05$ using IBM SPSS Statistic Version 26, IBM Inc., Chicago.

Results and discussion

Characterization of fish oil by-products (FOB)

FOB was characterized to observe the initial fish oil properties before the refining process, as shown in **Table 1**. In the canning process, parameters of fish oil, such as acid value, free fatty acid, PV, AV, TOTOX value, viscosity, and bias index, were lower than those in fish meal processing. In addition, the color of fish oil from the pressing process in the fish meal industry was much darker by comparison. The color observation confirmed that L* value of oil obtained from the canning process was higher than L* of oil in the fish meal process. Results indicated that FOB from fish meal processing had lower qualities than the canning process.

The high acid value of FOB in the fish meal process might be influenced by the raw material consisting of fish waste, such as fish silage and viscera. Besides, the freshness of raw material is related to the acidity index [20]. The acid value is associated with free fatty acid (FFA) detected in fish oil by-products. Generally, low FFA released in fish oil contributed to a better quality of fish oil [28]. Ferdosh *et al.* [19] reported that the ranges of FFA were 1.8 - 5.0 % oleic acid, and PV was 1.2 - 2.4 % in neritic tuna fish oil products from discarded parts (head, skin, and viscera). In addition, another study conducted by Mkaem and Kaanane [29] found that the average values of acidity, PV, and AV of fish oil by-products in

the sardine canning process were 1.43 % oleic acid, 4.61 mEq/kg and 4.25, respectively. Corresponding to those researches, the PV and AV of the crude oil in this study were extremely high, ranging from 17.93-19.62 mEq/kg and 27.15 - 30.82, respectively. Fish species, oxidation states, and extraction conditions of fish oil could be attributed to differences in these quality parameters [5,29].

Conversely, the iodine value of fish oil obtained from the canning process was higher than from fish meal processing, at 166 ± 1.41 (mg/100 g) and 161 ± 1.41 (mg/100 mg), respectively. The high unsaturated fatty acid content in fish oil contributed to the high concentration of iodine value [14]. The type of processing and raw material could affect different parameter values between FOB from the canning and fish meal process. In the cannery industry, fish oil was obtained from the pre-cooking process of tuna meat. In comparison, the raw material used in fish meal processing was solid waste from fish cannery. Solid waste was then pressed to get the fish oil by-products in the fish meal processing. Moreover, high impurities in fish oil by-products obtained from processing can initiate oxidation, leading to the quality degradation of fish oil products [30-33]. Fish oil supplements are highly susceptible to oxidative degradation, which can cause the development of rancid off-flavors and degradation of essential nutrients, thus affecting sensory and nutritional qualities and shelf life [33,34]. The oxidation of n-3 PUFA, which are highly prone to oxidative degradation, makes fish oils one of the most labile supplements sold to consumers [31].

Physicochemical characteristics of refined FOB in degumming and neutralization processes

FOB obtained from the canning and fish meal process were furtherly refined with degumming and neutralization steps to improve the fish oil quality, as presented in **Table 1**. The quality parameters were observed in each refining step. In comparison, parameter qualities in the degumming process, such as acid value, FFA, PV, AV, TOTOX value, and iodine value, decreased after the neutralization step. In comparison, color parameters (lightness and hue) and viscosity were increased. Results revealed that the quality of fish oil improved during the refining process. Similar results were also obtained from the sardine oil refining process, exhibiting that FFA, PV, AV, and TOTOX values reduced after the degumming and neutralization process [35,36].

Table 1 The parameter value of fish oil by-product and refined fish oil quality obtained from the canning and fish meal process.

| Parameters | Canning Process | | | Fish meal Process | | | Fish Oil Standard | | |
|------------------------------|------------------------|----------------------|-----------------------|---------------------|----------------------|--------------------|-------------------|------|------|
| | Fish Canning Oil (FCP) | Degummed Oil (DCP) | Neutralized Oil (NCP) | Fish meal oil (FMP) | Degummed oil (DMP) | Neutralized (NMP) | IFOMA | CAC | IFOS |
| Acid value (mg KOH/g) | 7.91 ± 0.10^b | 7.90 ± 0.04^b | 0.65 ± 0.01^a | 9.21 ± 0.05^B | 9.17 ± 0.10^B | 0.58 ± 0.04^A | - | < 3 | < 3 |
| Free fatty acid (% Oleat) | 3.98 ± 0.05^b | 3.97 ± 0.02^b | 0.33 ± 0.00^a | 4.63 ± 0.03^B | 4.60 ± 0.00^B | 0.29 ± 0.02^A | 1 - 7 | < 1 | < 1 |
| Peroxide value (PV) (mEq/kg) | 17.93 ± 0.32^c | 15.18 ± 0.02^b | 7.38 ± 0.02^a | 19.62 ± 0.11^C | 17.64 ± 0.06^B | 7.10 ± 0.08^A | 3 - 20 | < 5 | < 5 |
| Anisidine value (AV) | 27.15 ± 0.20^c | 14.36 ± 0.21^b | 6.61 ± 0.72^a | 30.82 ± 0.05^C | 14.64 ± 0.06^B | 6.51 ± 0.02^A | 4 - 60 | < 20 | < 20 |
| TOTOX value (2PV + AV) | 62.53 ± 0.16^c | 44.72 ± 0.18^b | 21.37 ± 0.76^a | 70.05 ± 0.17^C | 49.64 ± 0.46^B | 20.71 ± 0.18^A | 10 - 60 | < 26 | < 26 |
| Iodine value (mg/100 g) | 166 ± 1.41^b | 167 ± 0.71^b | 153 ± 1.41^a | 161 ± 1.41^B | 163 ± 1.41^B | 155 ± 0.71^A | 120 - 200 | - | - |
| L* | 22.75 ± 0.21^a | 23.80 ± 0.14^a | 25.70 ± 0.42^b | 20.40 ± 0.57^A | 20.65 ± 0.79^A | 26.00 ± 0.71^B | < 14* | - | - |
| a* | 3.05 ± 0.07^b | 2.75 ± 0.07^{ab} | 1.65 ± 0.35^a | 2.05 ± 0.07^B | 1.84 ± 0.06^{AB} | 1.55 ± 0.07^A | - | - | - |
| b* | 2.60 ± 0.14^a | 2.35 ± 0.07^a | 9.05 ± 1.06^b | 1.55 ± 0.07^A | 1.45 ± 0.07^A | 10.00 ± 0.57^B | - | - | - |
| Hue | 40.43 ± 0.88^a | 40.51 ± 0.12^a | 79.87 ± 0.78^b | 37.09 ± 0.31^A | 38.08 ± 0.29^A | 81.17 ± 0.89^B | - | - | - |
| Viscosity (cPs) | 40 ± 1.41^a | 35 ± 1.41^a | 39 ± 0.00^a | 42 ± 0.71^B | 36 ± 0.71^A | 38 ± 1.41^{AB} | - | - | - |
| Bias index | 1.484 ± 0.00 | 1.477 ± 0.00 | 1.460 ± 0.00 | 1.494 ± 0.00 | 1.477 ± 0.00 | 1.465 ± 0.00 | - | - | - |

IFOMA: International Association of Fish Meal and Oil Manufacturers

CAC: Codex Alimentarius Commission

IFOS: International Fish Oils Standards

L*: lightness of sample (100 = white, 0 = black)

a*: +ve a = redness, -ve a = blueness

b*: +ve b = yellowness, -ve b = greenness

All values are an average of means \pm SD

Different lowercase letters (a,b,c) are significantly different ($p < 0.05$) in the canning process (FCP, DCP, and NCP) and different uppercase letters (A,B,C) are significantly different in the fish meal process ($p < 0.05$)

*Gardner color scale

Regarding **Table 1**, the acid value and free fatty acid of fish oil by-products in both the canning and fish meal process were considerably stable to degumming. Phosphoric acid used in the degumming process might influence the quality parameter. In previous studies, the degumming process with phosphoric acid increased the percentage of free fatty acid in salmon oil [37] and the acidity index in Nile tilapia oil [14]. The degumming process decreased the value of PV, AV, and TOTOX, improving the quality of fish oil by-products. However, PV and TOTOX values were still far from the assigned standards (CAC and IFOS). Therefore, the following step in neutralization should be conducted to refine the fish oil by-product quality.

Results revealed that neutralization could lower the acid value, free fatty acid, AV, and TOTOX value better than the degumming process. After neutralization, the TOTOX value of oil in the canning and fish meal process was 21.37 ± 0.76 and 20.71 ± 0.18 , respectively, below the standard limit at < 20 . TOTOX value has a role in determining the presence of oxidized PUFA compounds in fish oil [36]. On the contrary, PV values ranged from 7.10 - 7.38 mEq/kg, which still needed further refinery steps to be in the edible standard for human consumption at < 5 mEq/kg. During the following stages of the chemical refinery, the lightness and viscosity of fish oil were increased and generated a clearer color and more viscous of fish oil by-products, respectively. [15] investigated the color changes of refined sardine oil, showing that neutralization could enhance the lightness (L^*), a^* , and b^* values compared to degummed oil.

Physicochemical characteristics of refined FOB in the bleaching process

Utilizing 3 different adsorbents - activated bentonite, zeolite, and carbon - the bleaching process was carried out to purify the intermediate FOB from the degumming and neutralization stages. These adsorbents were dried in an oven to remove any remaining water and then were activated by soaking them in a solution of 5 M KOH and 85 % H_3PO_4 for 24 h before filtering. The process resulted in powders with particle sizes between 60 and 100 mesh. Based on physicochemical qualities, the FOB obtained from CP and MP operations met the standards for fish oil set by IFOMA, CAC, and IFOSH by using zeolite, except for the L^* value enacted by IFOMA, which still exceeded 14.

Following the completion of the degumming and neutralization process, the effectiveness of bentonite, zeolite, and activated carbon as adsorbents to evaluate the physicochemical properties of FOB was assessed, as depicted in **Figure 1**. The unpurified FOB from CP and MP had initial L^* , a^* , b^* , and Hue values of 22.75 ± 0.21 ; 20.40 ± 0.57 ; 3.05 ± 0.07 ; 2.05 ± 0.07 ; 2.60 ± 0.14 ; 1.55 ± 0.07 ; 40.43 ± 0.88 ; and 37.09 ± 0.31 , respectively (**Table 1**). All adsorbents effectively increased the lightness of the oil.

The instrumental color values for FOB from CP and MP operations varied slightly. However, the luminosity (L value) of the addition of activated carbon was marginally lower than other adsorbents, ranging between 29 and 34. The canning method produced the sample with the greatest L value out of all the others. The raw material contents of fish meal consisting of viscera and head, which are naturally brown in color and rich in hemoglobin, were responsible for the high level of brown color and lower L value [38,39]. At the same time, there was no difference between the samples regarding the level of redness/greenness, which was associated with the a^* value. The a^* value decreased with the addition of adsorbents for both CP and MP oils. A reduction in red pigment occurred during the adsorption process in all oils with negative a^* values showing a minor green tint. Myoglobin, a colored protein that stores oxygen in tuna muscle tissue, was responsible for the FOB's color [40,41]. While it has been demonstrated that adsorbents can absorb the red hemoglobin pigments from FOB. The b^* and Hue values showed some apparent differences, with carbon active obtaining a lower value than bentonite and zeolite, albeit the range was not very broad (8 - 11 and 77 - 84). The adsorbent material raised the b^* and Hue values in FOB, creating the fish oil color to yellow and less red (fade). The color data suggested that these adsorbents had some ability to adsorb some coloring substances from the fish oil [23,42]. This phenomenon is in accordance with salmon oil waste treatment that activated earth adsorbent significantly increases the clearness of oil with yellowness color [26].

Generally, since the studied FOB was crude oil, it had intense color, and therefore major differences occurred for the color values after the adsorbent treatments. The higher the concentration of zeolite and bentonite, the higher the clearness of FOB from CP and MP sources. Vice versa, it is observed that adding more than 10 % of activated carbon caused a slump of color clarity due to a large amount of activated carbon reducing the adsorbent's surface area interacting with the adsorbate [43]. The clearest FOB was achieved at 15 % of zeolite, accounting for 37.08 ± 0.08 (CP) and 36.5 ± 0.69 (MP). Zeolite is more polar than the other 2 adsorbents, bentonite, and activated charcoal; hence, it can absorb pollutants more. In addition, zeolite had a higher SiO_2 content, significantly affecting adsorption [44,45].

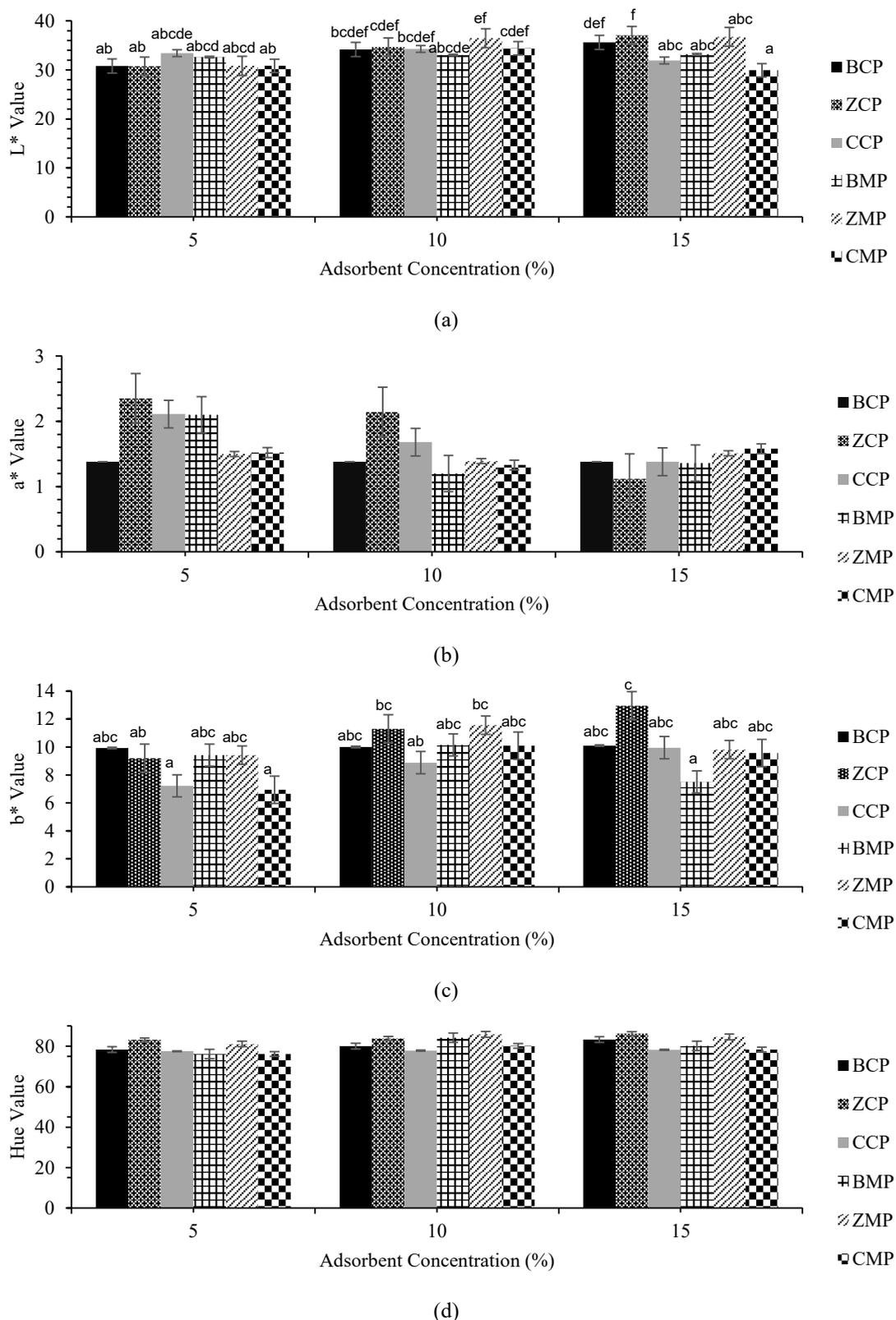


Figure 1 Post-bleaching color characteristic of FOB derived from canning and fish meal processing (B = bentonite; Z = zeolite; C = activated carbon; CP = FOB canning process; MP = FOB fish meal process; all values are an average of means \pm SD; Different lowercase letters (a, b, c, d, e, f) are significantly different ($p < 0.05$)). (a) L* value, lightness of sample (100 = white, 0 = black); (b) a* value, +ve a = redness, -ve a = blueness; (c) b*, +ve b = yellowness, -ve b = greenness; (d) Hue value.

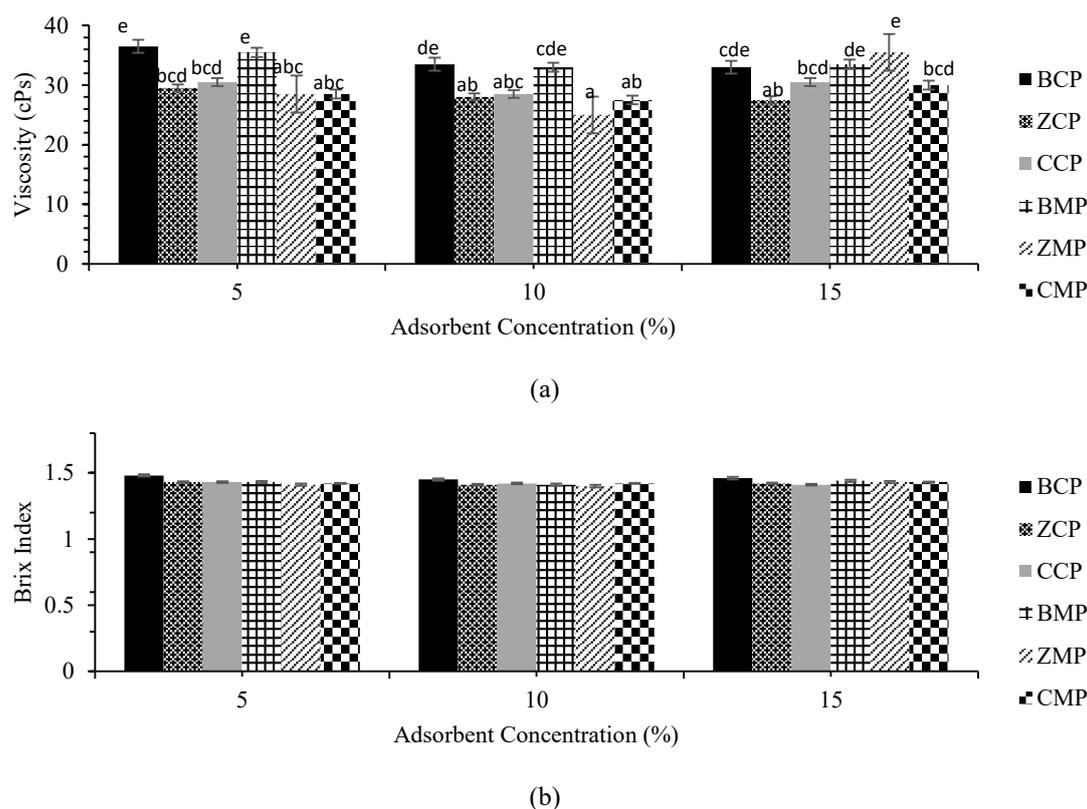


Figure 2 Physical characteristics of FOB after the bleaching process obtained from the canning and fish meal process (B = bentonite; Z = zeolite; C = activated carbon; CP = FOB canning process; MP = FOB fish meal process; all values are an average of means \pm SD; Different lowercase letters (a, b, c, d, e, f) are significantly different ($p < 0.05$). (a) Viscosity value; (b) Brix index.

The neutralized oil from canning by-products had a viscosity value of 39 cPs, and after bleaching, it dropped to a range of 24.50 - 36.50 cPs, as shown in **Figure 2**. The viscosity value of the MP oil reduced as well and ranged from 24.00 to 35.00 cPs, with the initial value being 38 cPs. Removing contaminants, free fatty acids, residues, and remnants of soap stock by the adsorbent added during the bleaching process caused the oil density and viscosity to decrease [24]. Comparing fish oil from by-products of canning to fish oil from the fish meal process, the average viscosity value of CP was greater. High viscosity values represent the proportion of fatty acids with long chains, particularly those with high double bonds, where CP oil consists of more unsaturated fatty acids [45]. The resultant viscosity in all adsorbents lessened along with the increase of adsorbent concentration (**Figure 2**) due to the enormous number of significant holes in adsorbents increasing the materials' capacity to absorb the impurities [46]. However, activated natural zeolite has the ability to reduce the lowest viscosity of FOB with a range of 27 - 29 (CP) and 25 - 28 (MP). The activation process caused a change in the Si/Al ratio, increased the surface area, and increased the zeolite's porosity.

A reduced bias index resulted from the oil becoming clearer and purer during the bleaching process due to the adsorbent's ability to absorb contaminants. Menegazzo *et al.* [14] showed that the oil bias index decreased as oil purity increased. Nevertheless, there was no discernible variation in the effects of the adsorbents' types and concentration, with a range of 1.4 - 1.48.

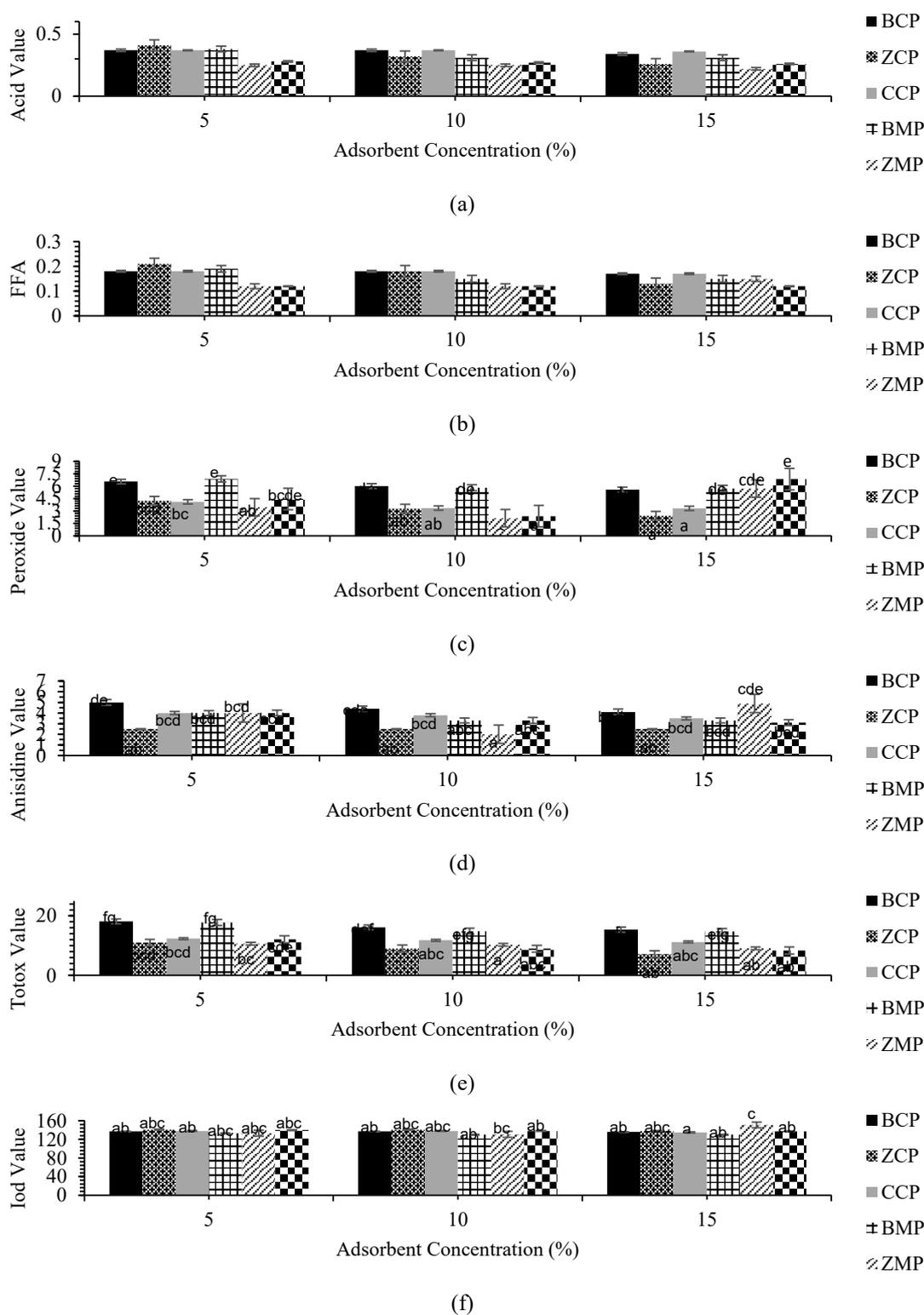


Figure 3 Chemical characteristics of FOB after the bleaching process obtained from the canning and fish meal process (B = bentonite; Z = zeolite; C = activated carbon; CP = FOB canning process; MP = FOB fish meal process; all values are an average of means ± SD; Different lowercase letters (a, b, c, d, e, f) are significantly different ($p < 0.05$)). (a) Acid value; (b) FFA; (c) Peroxide value; (d) Anisidine value; (e) Tototox value; (f) Iod value.

The acid number indicated the amount of free fatty acid (FFA) contained in fish oil by calculating this value based on the molecular weight of the fatty acid or fatty acid mixture. As a result of oxidation and the breakdown of the fat double bonds, FFA is produced, lowering fish oil quality [47]. After the bleaching procedure, all samples' acid values decreased overall using 3 adsorbents. For both CP and MP, fish oil containing 15 % zeolite was the best treatment with the lowest acid value of 0.26 ± 0.17 mg KOH/g (CP) and 0.22 ± 1.4 mg KOH/g (MP) consecutively, as shown in **Figure 3**. Fish oil impurities, such as protein, water, carbohydrates, and the colors linked to more FFA, contributed to the high acid number. Because the adsorbent utilized was able to adsorb FFA, the low acid number was achieved. Chasani *et al.* [48] claim that the higher alkali concentration used to neutralize the free fatty acids in fish oil leads to a larger number being created.

The FFA analysis was conducted to ascertain how much the oil had oxidized. According to Panagan *et al.* [49], FFA is formed as a consequence of Triacylglycerol (TAG) hydrolysis. Crude fish oil has a maximum FFA content of 12 % (as oleic acid), although average values are lower, between 1 – 7 %, and typically between 2 - 5 % [38]. FFA is regarded as a major impurity in tuna oil. It is essential to maximize the removal of FFA. The initial FFA concentration of unpurified FOB was 3.98 ± 0.05 (CP); 4.63 ± 0.03 (MP), and this was decreased by the degumming and neutralization process (**Table 1**). According to CAC (Codex Alimentarius Commission), and IFOS (International Fish Oils Standards), the allowable percentage of FFA in purified fish oil is 0.15 % and < 1%, respectively. Following acid value trends, it is also found that either degumming, neutralization, or bleaching process removed the FFA content in FOB from CP and MP.

In comparison to other adsorbent treatments, 15 % of zeolite exhibited a larger level of FFA reduction. The adsorbent adsorbed the FFA present in fish oil, causing the adsorption process to physically take place and impact each particle of trapped adsorbate [50]. Because zeolite has a greater polarity and has been activated to improve its large specific surface area and particle size, zeolite has a significant adsorption capacity. The rate at which solute molecules diffuse into adsorbent holes increases with decreasing particle size [47].

Peroxide value is the most important number to determine the degree of damage to the oil or fat [51]. The oxidation process in fish oil can be sped up by oxygen, peroxidase enzymes, heat, radiation (light), and monovalent ions, some of the components considered [52]. In this study, FOB from CP and MP successfully decreased after the bleaching process with the lowest PV found at 15 % of zeolite addition and met the IFOMA, CAC, and IFOS criteria equal to 2.44 ± 0.12 and 2.05 ± 0.46 . The more minor PV results in better quality fish oil. The high PV occurs due to the oxidation process by oxygen from the air of the unsaturated fatty acids contained in fish oil during the bleaching process. Unsaturated fatty acids are more reactive to oxygen as double bonds in the molecular chain increase, forming peroxides.

Anisidine value is the value obtained from the measurement of secondary products from fat oxidation by determining the amount of aldehyde (especially 2-alkenyl and 2,4-recognized) in oil [42,53]. Anisidine will react with aldehydes to produce a yellow color, forming a chromogen whose absorbance value was measured at a wavelength of 350 nm using spectrophotometry. AV from all samples was still detected and lower per IFOMA, CAC, and IFOS standards (4 meq/kg). AV is not always in line with the high value of peroxide [52], but the high value of peroxide can cause a high value of AV if the process given allows fish oil to go further degradation. In this experiment, the high PV aligns with the AV.

Total Oxidation (TOTOX) is the sum of the peroxide and p-anisidine values. The total oxidation can be used to measure the progression of deterioration that occurs in oil and provide information on the formation of primary and secondary oxidation products [26,38]. The total oxidation decreased with increasing adsorbent concentration for bentonite, zeolite, and activated carbon. At 15 % zeolite, the lowest TOTOX was found for CP and MP, which were 7.16 ± 2.2 and 9.11 ± 1.6 .

The iodine number is the amount (grams) of iodine that can be absorbed by 100 g of oil. The iodine number can indicate the degree of unsaturation of the oil or fat. The greater the iodine number, the higher the degree of unsaturation. All samples achieved the IFOMA standard with an iodine value of 120 - 200. It is also obtained that 15 % zeolite was responsible for the lowest iodine number for oils from CP and MP due to its high polarity.

Fatty acid profiles

The fatty acid composition was analyzed for fish oil by-products and refined fish oil by-products both from the canning process and fish meal process (FCP, Fish oil from the canning process; DCP, Degummed fish oil from the canning process; NCP, Neutralized fish oil from canning process; BCP, Bleached fish oil from canning process; FMP, Fish oil from fish meal process; DMP, Degummed fish oil from fish meal process; NMP, Neutralized fish oil from fish meal process; and BMP, Bleached fish oil from fish meal

process). Results are presented in **Table 2**. FCP was composed of 14 fatty acids, mainly in linoleic acid (42.26 %), whereas FMP comprised 12 fatty acids highly distributed in oleic and palmitic acid, at 22.31 % and 19.87 % in respective. [19] reported that around 25 - 27 % palmitic acids were the dominant constituent in total lipids extracted from neritic tuna wastes, including head, skin, and viscera. Similar results were also identified in Nile tilapia oil (25.54 %), sardine oil (21.65 %), hoki oil (17.08 %), and tuna oil (20.73 %) [6,14,15]. Overall, sums of SFA and MUFA detected in FCP were lower than in FMP. In contrast, the sum of PUFA in FCP was relatively higher than in FMP, at 50.7 and 27.69 %, respectively. The percentage of EPA and DHA in FCP was lower than in FMP because PUFA found in FMP abundantly consisted of linoleic acid. Sánchez-Zapata *et al.* [40] investigated the sum of EPA and DHA of tuna head oil, with 33.20 % in crude oil and 26.36 % in refined oil. Reported results were remarkably higher than those found in the studied fish oil in the canning process (8.44 in oil by-products and 21.18 % in neutralized oil). Furthermore, the total of EPA and DHA calculated in the fish meal process was comparable with the previous findings, with 22.95 % in oil by-products and 26.59 % in neutralized oil.

Table 2 Fatty acid (FFA) profiles of fish oil by-products and refined fish oil by-products from canning and fish meal process.

| Fatty acids | Fatty acid composition (%)* | | | | | | | |
|--|-----------------------------|-------|-------|-------|------------------------|-------|-------|-------|
| | Canning process (CP) | | | | Fish meal process (MP) | | | |
| | FCP** | DCP** | NCP** | BCP** | FMP** | DMP** | NMP** | BMP** |
| C12:0 (lauric) | 0 | 0 | 0.75 | 0.49 | 0 | 0 | 0 | 0 |
| C14:0 (myristic) | 1.44 | 1.87 | 9.28 | 10.73 | 7.04 | 6.68 | 7.46 | 6.5 |
| C15:0 (pentadecanoic) | 0.41 | 0.61 | 0.60 | 0.65 | 1.09 | 0.87 | 0.61 | 0.57 |
| C16:0 (palmitic) | 16.97 | 16.9 | 21.42 | 20.94 | 19.87 | 20.1 | 22.19 | 23.63 |
| C17:0 (margaric) | 0.58 | 0.97 | 0.52 | 0.58 | 1.17 | 0.96 | 0.56 | 0.61 |
| C18:0 (stearic) | 7.42 | 6.69 | 4.42 | 4.46 | 7.62 | 6.85 | 4.86 | 4.88 |
| C20:0 (arachidic) | 0.68 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C22:0 (behenic) | 0.75 | 0.23 | 0 | 0 | 0 | 0 | 0 | 0 |
| C24:0 (lignoseric) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C16:1 ω -7 (palmitoleic) | 1.89 | 2.5 | 8.55 | 9.18 | 9.35 | 8.63 | 8.19 | 7.76 |
| C16:3 ω -3 (hexadecatrimonioic) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C18:1 ω -7 (octadenoic) | 0.25 | 0.37 | 0 | 0 | 0 | 0 | 0 | 0 |
| C18:1 ω -9 (oleic) | 18.24 | 18.01 | 22.74 | 20.47 | 22.31 | 22.17 | 23.7 | 20.18 |
| C18:2 ω -6,9 (linoleic) | 42.26 | 38.86 | 9.53 | 6.92 | 3.77 | 3.11 | 2.83 | 2.82 |
| C18:3 ω -3 (α -linolenic) | 0 | 0 | 0 | 0 | 0.97 | 0.96 | 1.6 | 0.63 |
| C20:1 ω -9 (eicosenoic) | 0.67 | 1.14 | 0.96 | 1.37 | 2.18 | 2.1 | 1.41 | 1.79 |
| C20:2 ω -9 (eicosadienoic) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C22:1 ω -9 (erucic) | 0 | 0 | 0.65 | 0.75 | 1.68 | 3.21 | 0 | 0 |
| C20:5 ω -3 (EPA) | 2.34 | 3.59 | 9.58 | 10.81 | 8.35 | 9.59 | 11.51 | 12.03 |
| C22:6 ω -3 (DHA) | 6.1 | 8.01 | 11.6 | 12.04 | 14.6 | 14.77 | 15.08 | 18.25 |
| Σ SFA ^a | 28.25 | 27.27 | 36.39 | 37.85 | 36.79 | 35.46 | 35.68 | 36.19 |
| Σ MUFA ^b | 21.05 | 22.02 | 32.9 | 31.77 | 35.52 | 36.11 | 33.3 | 29.73 |
| Σ PUFA ^c | 50.7 | 50.71 | 30.71 | 30.38 | 27.69 | 28.43 | 31.02 | 34.08 |
| Σ MUFA + PUFA | 71.75 | 72.73 | 63.61 | 62.15 | 63.21 | 64.54 | 64.32 | 63.81 |

*All values are an average of means \pm SD

**FCP, Fish oil from the canning process; DCP, Degummed fish oil from the canning process; NCP, Neutralized fish oil from the canning process; BCP, Bleached fish oil from the canning process; FMP, Fish oil from the fish meal process; DMP, Degummed fish oil from the fish meal process; NMP, Neutralized fish oil from the fish meal process; BMP, Bleached fish oil from the fish meal process.

^a Σ SFA, total saturated fatty acid; ^b Σ MUFA, total monounsaturated fatty acids; ^c Σ PUFA, total polyunsaturated fatty acid

Table 2 shows that the refining process influenced the fatty acid composition of fish oil both in the canning and fish meal process. Several fatty acids of fish oil in the canning process (FCP) disappeared after refinings, such as C15:0, C:20:0, C22:0, and C18:1 ω -7. In addition, some other fatty acid was generated,

for example, C12:0 and C22:1 ω -9. According to [19] the limitation of the GC column, assigned temperature, and GC standards during the analysis could affect some undetected fatty acids in fish oil.

Moreover, fatty acid profiles of fish oil in the fish meal process (FMP) remained available with fluctuated values. Fatty acid changes in fish oil might be related to the heating and chemical reagents applied in refining [54]. Vaisali *et al.* [54] reported that thermal processing increased the amounts of lipids and reduced triglyceride amounts in tilapia fillets. Nevertheless, refining steps are necessary for producing edible fish oil from fish by-products. High temperature and chemical reagents must be controlled to prevent the removal of desirable components during the refining process. Additionally, the bleaching process did not majorly affect the composition of fatty acid, supported by a minor change in **Table 2**.

Conclusions

The initial characteristics of the fish waste oil showed that the free fatty acids, peroxide value (PV), anisidine value (AV), total oxidation (TOTOX) value, viscosity, and refractive index of the canned product were lower than that of the fish meal. Iodine from fish waste oil from fish flour is higher than canned fish. Degumming decreased the value of acid value, free fatty acid, PV, AV, TOTOX value, and iodine, but inversely the color parameters (lightness and hue) and viscosity increased so that the quality of fish oil by-products increased. However, the PV and TOTOX values are still far from the established standard (CAC and IFOS). The neutralization process reduced the weight of acids, free fatty acids, AV, and TOTOX better than the degumming process, but the PV value did not meet the CAC and IFOS standards. The bleaching process with 3 adsorbents lowers the acid value, free fatty acids, PV, AV, and TOTOX value with a PV that complies with CAC and IFOS standards. All adsorbents effectively increase the brightness of the FOB. The higher the concentration of zeolite and bentonite, the higher the clarity of the FOB from CP and MP sources, but the viscosity decreases. FCP contains 14 fatty acids, mainly linoleic acid (42.26 %), while FMP contains 12 fatty acids, primarily oleic and palmitic acids, 22.31 and 19.87 %, respectively. Overall, the number of SFAs and MUFAs detected in FCP was lower than in FMP. In contrast, the amount of PUFA in FCP was relatively higher than in FMP, amounting to 50.7 and 27.69 %, respectively. The percentage of EPA and DHA in FCP is lower than in FMP because the PUFA found in FMP contains a lot of linoleic acid.

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References

- [1] RN Dewi. Occupational health and safety risk analysis using AS/NZS standard 4360:2004 in the fish meatball industry. *J. Ind. Eng. Res. Appl.* 2023; **25**, 31-41.
- [2] M Firdaus and R Rahadian. Peran sektor perikanan pada wilayah pesisir perbatasan kalimantan barat. *Jurnal Sosial Ekonomi Kelautan dan Perikanan* 2018; **13**, 15.
- [3] Ministry of Marine Affairs and Fisheries. *Data volume produksi perikanan tangkap laut menurut komoditas utama (Ton)*. Ministry of Marine Affairs and Fisheries, Indonesia, 2023.
- [4] International Trade Centre. *Trade statistics for international business development*. International Trade Centre, Indonesia, 2021.
- [5] JF Costa, MF Almeida, MCM Alvim-Ferraz and JM Dias. Biodiesel production using oil from fish canning industry wastes. *Energy Convers. Manag.* 2013; **74**, 17-23.
- [6] TM Tengku-Rozaina and EJ Birch. Physicochemical characterisation and oxidative stability of refined hoki oil, unrefined hoki oil and unrefined tuna oil. *Int. J. Food Sci. Tech.* 2013; **48**, 2331-9.
- [7] J Fialkow. Omega-3 fatty acid formulations in cardiovascular disease: Dietary supplements are not substitutes for prescription products. *Am. J. Cardiovasc. Drugs* 2016; **16**, 229-39.
- [8] S Lorente-Cebrián, AGV Costa, S Navas-Carretero, M Zabala, JA Martínez and MJ Moreno-Aliaga. Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: A review of the evidence. *J. Physiol. Biochem.* 2013; **69**, 633-51.
- [9] H Ohnishi and Y Saito. Eicosapentaenoic acid (EPA) reduces cardiovascular events: Relationship with the EPA/Arachidonic acid ratio. *J. Atheroscler. Thromb.* 2013; **20**, 861-77.
- [10] J Balakrishnan, S Kannan and A Govindasamy. Structured form of DHA prevents neurodegenerative disorders: A better insight into the pathophysiology and the mechanism of DHA transport to the brain. *Nutr. Res.* 2021; **85**, 119-34.

- [11] F Echeverría, R Valenzuela, MC Hernandez-Rodas and A Valenzuela. Docosahexaenoic acid (DHA), a fundamental fatty acid for the brain: New dietary sources. *Prostaglandins Leukot Essent Fatty Acids* 2017; **124**, 1-10.
- [12] KA Youdim, A Martin and JA Joseph. Essential fatty acids and the brain: Possible health implications. *Int. J. Dev. Neurosci.* 2000; **18**, 383-99.
- [13] DASBD Oliveira, MG Minozzo, S Licodiedoff and N Waszczynskyj. Physicochemical and sensory characterization of refined and deodorized tuna (*Thunnus albacares*) by-product oil obtained by enzymatic hydrolysis. *Food Chem.* 2016; **207**, 187-94.
- [14] ML Menegazzo, ME Petenuci and GG Fonseca. Production and characterization of crude and refined oils obtained from the co-products of Nile tilapia and hybrid sorubim processing. *Food Chem.* 2014; **157**, 100-4.
- [15] K Chakraborty and D Joseph. Production and characterization of refined oils obtained from Indian oil sardine (*Sardinella longiceps*). *J. Agr. Food Chem.* 2015; **63**, 998-1009.
- [16] DL Lamas and AE Massa. Ray liver oils obtained by different methodologies: Characterization and refining. *J. Aquat. Food Prod. Tech.* 2019; **28**, 555-69.
- [17] G Song, Z Dai, Q Shen, X Peng and M Zhang. Analysis of the Changes in volatile compound and fatty acid profiles of fish oil in chemical refining process. *Eur. J. Lipid Sci. Tech.* 2018; **120**, 1700219.
- [18] CL Mgbechidinma, G Zheng, EB Baguya, H Zhou, SU Okon and C Zhang. Fatty acid composition and nutritional analysis of waste crude fish oil obtained by optimized milder extraction methods. *Environ. Eng. Res.* 2022; **28**, 220034.
- [19] S Ferdosh, ZI Sarker, N Norulaini, A Oliveira, K Yunus, AJ Chowdury, J Akanda and M Omar. Quality of tuna fish oils extracted from processing the by-products of three species of neritic tuna using supercritical carbon dioxide. *J. Food Process. Preserv.* 2015; **39**, 432-41.
- [20] DL Lamas. Effect of enzymatic degumming process on the physicochemical and nutritional properties of fish byproducts oil. *Appl. Food Res.* 2022; **2**, 100170.
- [21] Å Oterhals and G Vogt. *Impact of extraction, refining and concentration stages on the stability of fish oil.* In: C Jacobsen, NS Nielsen, AF Horn and ADM Sørensen (Eds.). Food enrichment with omega-3 fatty acids. Woodhead Publishing, Sawston, United Kingdom, 2013, p. 111-29.
- [22] ML Monte, ML Monte, RS Pohndorf, VT Crexi and LAA Pinto. Bleaching with blends of bleaching earth and activated carbon reduces color and oxidation products of carp oil. *Eur. J. Lipid Sci. Tech.* 2015; **117**, 829-36.
- [23] M Güner, E Yılmaz and Y Yüceer. Off-odor removal from fish oil by adsorbent treatment with selected metal-organic frameworks. *Flavour. Fragr. J.* 2019; **34**, 163-74.
- [24] VT Crexi, ML Monte, LADS Soares and LAA Pinto. Production and refinement of oil from carp (*Cyprinus carpio*) viscera. *Food Chem.* 2010; **119**, 945-50.
- [25] PJ García-Moreno, A Guadix, L Gómez-Robledo, M Melgosa and EM Guadix. Optimization of bleaching conditions for sardine oil. *J. Food Eng.* 2013; **116**, 606-12.
- [26] J Huang and S Sathivel. Purifying salmon oil using adsorption, neutralization, and a combined neutralization and adsorption process. *J. Food Eng.* 2010; **96**, 51-8.
- [27] E Hastarini. *Karakteristik minyak Ikan dari Limbah Pengolahan Filet Ikan Patin Siam (Pangasius hypophthalmus) dan Ikan Patin Jambal (Pangasius djambal).* IPB University, Bogor Regency, Indonesia, 2011.
- [28] Y Fang, S Gu, J Zhang, S Liu, Y Ding and J Liu. Deodorisation of fish oil by nanofiltration membrane process: Focus on volatile flavour compounds and fatty acids composition. *Int. J. Food Sci. Tech.* 2018; **53**, 692-9.
- [29] H Mkadem and A Kaanane. Recovery and characterization of fish oil from by-products of sardine (*Sardina pilchardus*) in the canning process. *J. Aquat. Food Prod. Tech.* 2019; **28**, 1037-50.
- [30] AA Jairoun, M Shahwan and SH Zyoud. Fish oil supplements, oxidative status, and compliance behaviour: Regulatory challenges and opportunities. *PLoS One* 2020; **15**, e0244688.
- [31] D Cameron-Smith, BB Albert and WS Cutfield. Fishing for answers: Is oxidation of fish oil supplements a problem? *J. Nutr. Sci.* 2015; **4**, e36.
- [32] R Mozuraityte, V Kristinova, IB Standal, AK Carvajal and M Aursand. *Oxidative stability and shelf life of fish oil.* In: M Hu and C Jacobsen (Eds.). Oxidative stability and shelf life of foods containing oils and fats. AOCS Press, Illinois, 2016, p. 209-31.
- [33] J Pickova. *Fish oils.* In: RA Moreau and A Kamal-Eldin (Eds.). Gourmet and health-promoting specialty oils. AOCS Press, Illinois, 2009, p. 515-26.
- [34] J Wang, L Han, D Wang, Y Sun, J Huang and F Shahidi. Stability and stabilization of omega-3 oils: A review. *Trends in Food Sci. Tech.* 2021; **118**, 17-35.

- [35] K Chakraborty, D Joseph and D Joseph. Changes in the quality of refined fish oil in an accelerated storage study. *J. Aquat. Food Prod. Tech.* 2016; **25**, 1155-70.
- [36] B Soldo, V Šimat, J Vlahović, D Skroza, I Ljubenković and IG Mekinić. High quality oil extracted from sardine by-products as an alternative to whole sardines: Production and refining. *Eur. J. Lipid Sci. Tech.* 2019; **121**, 1800513.
- [37] W Klaypradit, W Worawattanamateekul, T Taksima and W Intharapongnuwat. Characterization of refined oils from Atlantic salmon belly as affected by degumming. *Kasetsart Univ. Fish. Res. Bull.* 2014; **38**, 1-15.
- [38] SH Suseno, N Nurjanah, AM Jacob and S Saraswati. Purification of *Sardinella sp.*, Oil: Centrifugation and bentonite adsorbent. *Adv. J. Food Sci. Tech.* 2014; **6**, 60-7.
- [39] EFSA Panel on Contaminants in the Food Chain, HK Knutsen, J Alexander, L Barregård, M Bignami, B Brüschweiler, S Ceccatelli, B Cottrill, M Dinovi, L Edler, B Grasl-Kraupp, LR Hoogenboom, CS Nebbia, IP Oswald, A Petersen, M Rose, AC Roudot, T Schwerdtle, C Vleminckx, G Vollmer, ..., C Hogstrand. Assessment of a decontamination process for dioxins and PCBs from fish meal by replacement of fish oil. *EFSA J.* 2018; **16**, 5173.
- [40] E Sánchez-Zapata, M Amensour, R Oliver, E Fuentes-Zaragoza, C Navarro, J Fernández-López, E Sendra, E Sayas and JA Pérez-Alvarez. Quality characteristics of dark muscle from yellowfin tuna *Thunnus albacares* to its potential application in the food industry. *Food Nutr. Sci.* 2011; **2**, 22-30.
- [41] RL Wolke. *What color is your tuna?* Washington Post, Washington DC, 2004.
- [42] JR Bonilla and JLH Concha. Methods of extraction, refining and concentration of fish oil as a source of omega-3 fatty acids. *Ciencia y Tecnología Agropecuaria* 2018; **19**, 645-68.
- [43] T Estiasih, K Ahmadi, FC Nisa and F Kusumastuti. Optimasi Kondisi Pemurnian Asam Lemak Omega-3 dari Minyak Hasil Samping Penepungan Tuna (*Thunnus sp.*) dengan Kristalisasi Urea. *Jurnal Teknologi dan Industri Pangan* 2009; **20**, 135-42.
- [44] Syuhada, R Wijaya, Jayatin and R Saeful. Modifikasi bentonit (clay) menjadi organoclay dengan penambahan surfaktan. *Jurnal Nanosains dan Nanoteknologi* 2009; **2**, 48-51.
- [45] SAMS Tandewi and E Hambali. Refining of fish oil from fish meal processing by-product using zeolite and bleaching earth. *IOP Conf. Ser. Earth Environ. Sci.* 2022; **1034**, 012050.
- [46] RC Vaishya and SK Gupta. Arsenic(V) removal by sulfate modified iron oxide-coated sand (SMIOCS) in a fixed bed column. *Water Qual. Res. J.* 2006; **41**, 157-63.
- [47] RT Rosmalina, W Kosasih and S Priatni. The effects of adsorbent materials on the lipid quality of lemuru fish oil and the enrichment of omega-3 using lipase. *Jurnal Teknologi dan Industri Pangan* 2021; **32**, 16-26.
- [48] M Chasani, VH Nursalim, S Widyaningsih, IN Budiasih and WA Kurniawan. Synthesis, purification and characterization methyl ester sulphonate as core material detergent from seed oil of calophyllum inophyllum L. *Molekul* 2014; **9**, 63-72.
- [49] AT Panagan, H Yohandini and JU Gultom. Analisis kualitatif dan kuantitatif asam lemak tak jenuh omega-3 dari minyak ikan patin (*Pangasius pangasius*) dengan metoda kromatografi gas. *Jurnal Penelitian Sains* 2011; **14**, 38-42.
- [50] H Wang, E Oguz, B Jeong and P Zhou. Life cycle and economic assessment of a solar panel array applied to a short route ferry. *J. Clean Prod.* 2019; **219**, 471-84.
- [51] SH Suseno, JE Tambunan, B Ibrahim and AF Izaki. Improving the quality of sardine oil (*Sardinella sp.*) from Pekalongan-Indonesia using centrifugation and adsorbents (Attapulgit, Bentonite and Zeolite). *Adv. J. Food Sci. Tech.* 2014; **6**, 622-8.
- [52] MD Guillén and N Cabo. Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. *Food Chem.* 2002; **77**, 503-10.
- [53] D Deepika, VR Vegneshwaran, P Julia, KC Sukhinder, T Sheila, M Heather and M Wade. Investigation on oil extraction methods and its influence on omega-3 content from cultured salmon. *J. Food Process. Tech.* 2014; **5**, 1000401.
- [54] C Vaisali, S Charanyaa, PD Belur and I Regupathi. Refining of edible oils: A critical appraisal of current and potential technologies. *Int. J. Food Sci. Tech.* 2015; **50**, 13-23.