Fatty acid composition and nutritional analysis of waste crude fish oil obtained by optimized milder extraction methods

Chiamaka Linda Mgbechidinma1, Gang Zheng2, Elnalee Buyagao Baguya1, Hanghai Zhou1, Samuel Ukpong Okon3,4, Chunfang Zhang1†

1Institute of Marine Biology and Pharmacology, Ocean College, Zhejiang University, Zhoushan 316021, Zhejiang, China
2Ocean Research Center of Zhoushan, Zhejiang University, Zhoushan 316021, Zhejiang, China
3School of Ocean Sciences, Bangor University, Gwynedd, Wales, Bangor LL57 2DG, UK
4Department of Marine Biology, Akwa Ibom State University, P.M.B. 1167, Nigeria

Received January 18, 2022 Revised March 09, 2022 Accepted March 10, 2022

ABSTRACT

The increasing environmental impact of fish waste has attracted more attention towards developing simple, greener, and effective valorization methods. This study employed optimized milder extraction methods as a potential process intensification strategy to valorize marine and freshwater fish wastes with significant proximate compositions (p < 0.05). Using response surface methodology, the optimized parameters, including lower temperature (60°C), less enzyme concentration (1%), and 1:1 ratio of less toxic solvents, had high desirability levels (> 0.92). Although identical chemical functional groups were observed in the infrared spectra, the marine fish waste produced higher oil yields. The physicochemical characteristics of the oils were within the international CXS 329-2017 standard. Palmitic acid (21.21–26.63%), oleic acid (19.78–27.11%), eicosapentaenoic acid (6.02–9.97%), and docosahexaenoic acid (6.02–9.97%) were the dominant saturated and unsaturated fatty acids in the crude fish oil. The average nutritional quality values of the fatty acids were: polyunsaturated/saturated (0.85–1.33), omega-6/omega-3 (0.18–0.22), atherogenic index (0.35–0.46), thrombogenic index (0.23–0.33), hypocholesterolemic/hypercholesterolemic ratio (2.21–2.77), nutritive value index (0.87–1.68), health-promoting index (2.19–2.82), and fish lipid quality (24.18–28.88). The present research revealed the potentiality of fish waste valorization by optimized milder extraction methods as a possible sustainable approach for targeting the era of zero waste while producing quality crude fish oil.

Keywords: Crude fish oil, Fatty acid composition, Fish waste valorization, Milder extraction, Response surface methodology, Waste management

1. Introduction

In recent years, tons of fishery wastes are generated globally, leading to inherent problems that have become an issue of public concern. Experts estimate that about 50% of the fish products meant for human consumption are discarded as waste, causing nutrient losses in the food chain and severe environmental problems such as pollution [1, 2]. Although modern microtechnological methods minimize this waste, many fish processing factories continue to use conventional methods leading to continuous waste generation [2-4]. Fish wastes consist of by-catches, fins, heads, bones, skin, and viscera that are not edible and underutilized. In addition, they contain bacteria and enzymes, which represent a risk for processing and storage through invasion of spoilage microbes and fouling, thereby creating huge economic and environmental problems [5]. The nutritional value of solid fish waste products is almost identical to that of the edible parts consisting of minerals [6], vitamins [2], protein [7], and lipids [8]. This stresses the importance of developing adequate fish waste valorization methods that could serve as a sustainable strategy for obtaining valuable products such as gelatin, chitosan, hydrolysates, collagen, peptides, and fish oil.

Crude fish oil, an extractable product from marine and freshwater fish wastes, is widely desired due to its fatty acid composition [9]. These fatty acids comprise carbon bonds in cis-or-trans config...
uration, where the cis fatty acids are preferable as they confer lower melting points and increased membrane fluidity [10]. The primary chemical constituent of fish oil is triglycerides with carboxylic acids and aliphatic chains, but they also contain variable amounts of phospholipids, glycerol ethers, and wax esters [9]. The fatty acids of fish oil can be unsaturated or saturated, depending on the presence or absence of double covalent bonds between adjacent carbon atoms, respectively. The presence of more unsaturated fatty acids, mainly polyunsaturated (PUFA), differs fish oil from animal and vegetable oils [5]. Omega-3 [eicosapentaenoic acid (EPA, C20:5, n-3), docosahexaenoic acid (DHA, C22:6, n-3)], and omega-6 fatty acids are PUFAs of interest with important structural roles in phospholipid membranes. In addition, many PUFAs also provide fluidity to triacylglycerol reserves and serve as eicosanoid precursors (prostaglandins, prostacyclins, thromboxanes, and leukotrienes) with important biological functions [11, 12].

Over the years, previous studies have enumerated the importance and applicability of fish oil in the food, cosmetic, pharmaceutical, and biofuel industries [10, 13, 14]. Although humans might not directly consume crude fish oils unless further purification is conducted, conserving their initial nutritional quality is critical. Meanwhile, fish oils are used in their crude form in aquaculture, biodiesel, and other biotechnological industries [3, 10, 15]. The addition of fish oil to microbial culture medium induces the production of bacteria enzyme (lipase) [5]. For this reason, crude fish oil is a potential growth substrate for microbes since the fatty acids are rich in carbon. The mono and polyunsaturated fatty acids, mainly omega -3, -6, -7, and -9, in fish oil possess antimicrobial effects against pathogenic Gram-positive and Gram-negative bacteria [5, 16]. Regardless of the numerous applications of fish oil, its sustainability depends on its continuous production. Factors that determine oil recovery from fish wastes include specific fish species, the fat distribution in fish parts, age, sex, nutritional status, health, and time of year [1, 17]; however, the extraction method significantly influences the oil yield and quality.

There is a wide range of methods used for extracting oil from fish waste depending on the nature of the fish waste and the final application of the fish oil. The applied techniques are either conventional or modern. High temperature/pressure, residual solvents, and the increasing cost of enzymes are disadvantages in using most conventional techniques. Wet reduction, chemical extraction by solvent, and enzymatic hydrolysis, which are conventional techniques, are the most commonly used for fish oil extraction due to their cheap techno-economic advantage [2, 4, 5, 10]. However, modern techniques like supercritical fluid, microwave, and ultrasound are associated with high cost, high dielectric solvent demand, and unstandardized sonication rates [1, 18, 19]. Despite modern techniques being more mechanically advanced, studies have demonstrated similarities in their fish oil extraction efficiency with conventional techniques [5, 20]. Therefore, optimizing conventional techniques to milder methods which are greener by using lower temperatures, less toxic solvents, and less enzyme concentrations, can be a sustainable and economical process intensification strategy [19, 21, 22]. Process intensification is any developmental technique at the unit operational level that substantially improves (bio)chemical manufacturing and processing [23]. A wide range of developmental processes in edible oil production aligns with the PI principle. For example, microwave irradiation, ultrasonically assisted reactors, enzymatic alternatives, and membrane technologies have proven effective in accelerating modern biodiesel and fish oil production [23-25]. These processes improve mass transfer, reduce the amount of reactant and catalyst required, increase the contact surface area between the alcohol/oil phases, and lower the use of utilities and material feed flows (reactants, solvents, steam, electricity) upon higher performance. Notwithstanding, process intensification principles are less explored with improving conventional fish oil extraction methods.

Although conventional extraction methods significantly influence fish oil yield and fatty acid composition [4], there are limited studies on conventional techniques to milder methods conversions. Thus, the productivity efficiency of combined milder extraction methods and optimization principles as a process intensification strategy in crude fish oil production is unknown. Since the development of fish oil processes in the 1940s to 1960s, only a few improvements on the conventional methods have been made despite the possible recovery of high-quality fatty acids [26]. As a result, the fatty acid composition and nutritional analysis of waste crude fish oil obtained by optimized milder extraction methods are undefined. This study used the optimizing response surface methodology (RSM) with central composite design (CCD) and desirability function to develop a waste to sustainable crude fish oil production models. Objectively, we determined the physicochemical characteristics, qualitative structural assay, and fatty acid composition of the extracted crude fish oil. We further calculated the nutritional quality index of the fatty acids obtained. To this end, the practical applicability of milder extraction methods for crude fish oil production was recommended. The viability of these studies will promote the sustainable valorization of waste generated by marine and freshwater fishes facing high consumption and overexploitation risk across the globe [27, 28].

2. Material and Methods

2.1. Reagent and Chemicals Used

The solvents used for gas chromatography (GC) analysis and reagents (potassium hydroxide, potassium iodide, ethyl acetate, ethanol, hexane, methanol, and others) were purchased from Aladdin Industrial Co., (Shanghai, China). Alcalase enzyme (food-grade alkaline protease, enzyme activity: 200 U/mg) was bought from Yuanye Biological Technology Co., Ltd. (Shanghai, China). All the solvents and reagents used were of the highest analytical grade.

2.2. Preparation of Fish Waste and Proximate Analysis

The experimental raw materials used were fish waste from marine mackerel (Scomberomorus sinensis) and freshwater Crucian carp (Carassius auratus) obtained from a local commercial market in Zhoushan, Zhejiang Province, China. The country accounts for over 60 percent of global fish production in the world. Recent reports identified Scomberomorus sinensis as a typical fish type facing overexploitation risk in China [28], while Carassius auratus species are highly consumed among other freshwater fishes [27]. The fish wastes, including heads, viscera, and bones, were immedi-
atately transported in an ice-chest to the laboratory and vacuum-freeze-dried to a constant weight. Accordingly, equal amounts of the marine and freshwater fish wastes were macerated, mixed, and packed in labeled plastic zip-lock bags with storage at -80 °C until further use. Then, the proximate analysis of the fish waste was conducted following the Association of Analytical Chemists method to determine the moisture (105°C dryings), protein (Kjeldahl method), crude fat (Schochlet analytical method), and ash content (combustion at 550°C for 6 h) (AOAC, 2000).

2.3. Crude Fish Oil Extraction

The fish waste samples were thawed at room temperature, and equal portions were used during the crude fish oil extraction processes. The pH of the extraction systems was maintained at 7.0 by adding 3M NaOH/HCl to prevent fluctuations due to acidity or alkalinity. The extraction procedures are conventional and milder methods, which are quantitatively evaluated. Following Eq. (1), the crude fish oil yields from wet reduction (WR), enzymatic hydrolysis (EH), and solvent method (SM) were calculated.

\[
\text{Total Oil Yield} (\%) = \frac{\text{Weight of extracted oil}}{\text{the initial weight of lipid in fish waste}} \times 100 \quad (1)
\]

2.3.1. Conventional extraction methods

WR was conducted following the procedure described by Jayasinghe et al. [3]. The fish waste samples were cooked at 100°C for 1 h. After which, the fish tissues were pressured, while the water fraction and biomass were removed by centrifuging and the upper oily layer (crude fish oil) recovered. In the EH method, the thawed fish wastes were transferred into different Erlenmeyer flasks before the extraction process with 5% alcalase enzyme [17]. Alcalase was used because it generates less emulsion and has a higher degree of hydrolysis than other enzymes [2, 19]. The samples were then digested in an incubator shaker set at 55°C and 120 rpm for 2 h. After which, the enzymatic processes were terminated through heat treatment at 70°C for 10 minutes (mins). Then, the digested mass contents were centrifuged at 5,000 rpm for 45 mins to separate and collect the upper crude fish oil. The most popular way to ensure the extraction of total cellular lipids is to employ the ternary solvent composition system of polar and non-polar solvent mixtures. The standard of these solvent mixtures is chloroform-methanol, described over 50 years ago by Bligh and Dyer (B & D) and Folch, with more than 50,000 citations in the experimental recovery of lipids. Thus, the SM was conducted following the Bligh and Dyer method described by Breil et al. [21]. The fish wastes were weighed into several conical flasks containing a 1.8 fraction of water while a 2.2 chloroform and methanol ratio was added. Then, the mixtures were homogenized for 2 mins while being held in iced water. The homogenates were centrifuged for 30 mins at 2,000 rpm to form the final biphasic system; then, the crude fish oil was recovered.

2.3.2. Milder extraction methods

The milder methods are developmental modifications to the conventional procedures described in section 2.3.1 using greener extraction parameters. WR was conducted at a lower temperature (60°C) since high temperatures are harsh on lipids leading to faster degradation [29]. Cooking time and temperature are vital processing parameters in WR towards separating lipids and proteins [26]. The EH was carried out using 2% enzyme concentration at 4 h hydrolysis time to provide a greener solution to the claim by Angulo et al. [30], stating that the enzymatic method is less cost-effective, despite the recoverability of the enzyme. Milder SM was performed based on hydrophobicity using greener Bligh and Dyer method of less toxic solvents instead of chloroform, a health deteriorating solvent with no sustainability credence [10]. Ethanol and ethyl acetate are selective from the conductor-like screening model for realistic solvation (COSMO-RS) based on a computational study of bio-solvents for lipid extraction by Breil et al. [21] and was used as the polar and non-polar solvents in a 2:2 ratio. Other factors that make ethyl acetate a preferable solvent to chloroform are its low boiling point for easy removal, product recovery ease, availability, lower cost, and low toxicity. The factors considered in the milder extraction method align with the green chemistry principles that include using safer solvents, reducing product toxicity, avoiding high temperatures/pressures, and cost-effective processes [31].

2.4. Optimization of Extraction Parameters and Yield Evaluation

To maximize the possibility of achieving high extraction yields, we optimized the extraction parameters, which are critical steps in producing oil from fish waste [26]. This was conducted using response surface methodology (RSM), an effective mathematical and statistical tool for easy model development to determine the relationship between the target parameters and the desired product features using a regression equation at a minimized trial number [32]. The experimental design employed was central composite design (CCD) and desirability function to determine the significance of the operational parameters and their optimal levels [33, 34]. Using CCD, a factorial design model, the effect of the independent variables (extraction parameters) in optimizing the production of crude fish oil (response variable) was investigated [32]. The ANOVA and regression coefficient of the experimental design were analyzed to reveal how the input extraction parameters affect MCFO and FCFO yield alongside their single response optimization. The extraction parameters studied included temperature (20, 40 and 60°C), cooking time (1, 2.5, and 4 h), enzyme concentration (1, 1.5, 2.0% w/v), hydrolysis time (1, 2.5, 4.0 h), and the solvent ratio of 1, 1.5, and 2 for ethanol and ethyl acetate. Table S1 shows the coded levels of variables in the experimental design, where 1.414 is the axial distance from the center point. The experimental design consisted of 13 runs per extraction method, including axial, factorial, and central points (Table S2). The experiments were performed in triplicates, and the real levels of independent variables were coded according to Eq. (2):

\[
Z = Z_0 - \frac{Z_c}{\Delta Z} \quad (2)
\]

where \(Z\) = coded level; \(Z_0\) = real levels; \(\Delta Z\) = step-change and \(Z_c\) = actual value at the central point. The specific equations used for each extraction parameters are shown in Eq. (3)–(8).

\[
Z_1 = \frac{(X_1 - 40)}{20} \quad (3)
\]
Where \( X_1, X_2, X_3, X_4, X_5, \) and \( X_6 \) stands for temperature, cooking time, enzyme concentration, hydrolysis time, ethanol, and ethyl acetate, respectively.

A second-order quadratic polynomial regression model was used for predicting the \( Y \) responses, which are marine crude fish oil (MCFO) and freshwater crude fish oil (FCFO) yield as a function of the extraction parameters. The model proposed for the response of \( Y \) is shown in Eq. (9)

\[
Y = a_0 + \sum_{i=1}^{n} a_i X_i + \sum_{i=1}^{n} a_i^2 X_i^2 + \sum_{i=1}^{n} a_{ij} X_i X_j
\]

where \( Y \) = predicted response function (MCFO and FCFO yield); \( a_0 \) = constant term; \( a_i \) = linear term; \( a_{ij} \) = squared term; \( a_{ij} \) = interaction term while \( X_i \) and \( X_j \) = coded extraction parameters used.

To this end, the multiple responses were obtained using the desirability function and numerical optimization to determine the target parameter points for maximum MCFO and FCFO yield. Then, the desirability function goals were set to maximize MCFO and FCFO yield while minimizing the cooking time, enzyme, ethanol, and ethyl acetate concentration. While employing the most optimal and desired parameters, MCFO and FCFO yields were gravimetrically determined. The yields are expressed as a percentage of crude fish oil extracted to the lipid content of the minced marine and freshwater fish waste, as in Eq. (1).

2.5. Characteristic Evaluation of MCFO and FCFO

2.5.1. Qualitative structural analysis of MCFO and FCFO

Fourier transform infrared spectroscopy (FT-IR) analysis of the extracted crude fish oil was conducted using an FT-IR spectrometer (iS10, Thermo-Nicolet, America). The spectral region investigated ranged between 4,000-500 cm\(^{-1}\), at a 4 cm\(^{-1}\) resolution.

2.5.2. Fatty acid methyl esters (FAMEs) derivation and analysis

FAMEs of MCFO and FCFO (10 mg) were prepared using an alkali catalyzed transesterification reaction as previously described for the comparative analysis of the public health risks and benefits of fatty acids [14]. Briefly, 4 mL of 2 M methanolic potassium hydroxide (KOH) was added into MCFO and FCFO contained in screw-capped glass test tubes. The mixtures were heated using a digital water bath at 70°C for 60 min with simultaneous stirring at 600 rpm. After completion of the reaction, the products were cooled to room temperature. 2 mL hexane was added, and the tubes vortexed for 2 min at room temperature. After centrifugation at 4,000 rpm for 5 min, the hexane layers were collected and analyzed using gas chromatography-mass spectrometry (GC-MS QP2020, Shimadzu, Japan) equipped with an SH Rxi-5Sil MS column (30 m × 0.25 μm × 0.25 mm, Shimadzu). The GC-MS results of FAMEs were aligned in the “National Institute of Standards and Technology” mass spectral library database to evaluate MCFO and FCFO fatty acid composition. Then the percentage quantification of fatty acid compositions was determined by an area normalization method.

2.6. Determination MCFO and FCFO Quality Value

2.6.1. Physicochemical characterization and quality parameters

MCFO and FCFO were characterized based on their oxidative stability and quality parameters using established methods. Acid value (AV; mg KOH/g) by titration method [17]. Iodine value (IV) using the AOAC method described by Tavakoli et al. [35]. Peroxide values (PV) according to the AOAC method [36]. Anisidine value (p-AV) by reacting aldehydic compounds in MCFO and FCFO with p-anisidine, then the p-AV determined at 350 nm absorbance according to ISO 6885:2006 standard. Meanwhile, the total oxidation number (ToTox) depended on the peroxide and anisidine values. In all, the representative equations used are illustrated in Eq. (10)–(13)

\[
AV (\text{mg KOH/g}) = \frac{(V-V_0) \times C \times 56.1}{m}
\]

\[
IV (g/100 \text{ g oil}) = \frac{127(a-b) \times N}{10W}
\]

\[
PV (\text{meq/kg}) = \frac{(a-c) \times N \times 1000}{\text{Weight (g) of the sample}}
\]

\[
\text{ToTox} = 2 \times \text{Peroxide value} + 1 \times \text{Anisidine value}
\]

Where:

- 56.1 = molecular weight of titration solution used (g/mol)
- \( C \) = concentration of the titration KOH solution (mol/L)
- \( V \) = volume of KOH solution used for titration (mL)
- \( m \) = mass of the crude fish oil (g)
- \( a \) = volume (mL) of 0.1 mol L\(^{-1}\) \( \text{Na}_2\text{S}_2\text{O}_3 \) (blank test)
- \( b \) = volume (mL) of 0.5 mol L\(^{-1}\) \( \text{Na}_2\text{S}_2\text{O}_3 \) for IV
- \( c \) = volume (mL) of 0.1 mol L\(^{-1}\) \( \text{Na}_2\text{S}_2\text{O}_3 \) for PV
- \( N \) = normality of \( \text{Na}_2\text{S}_2\text{O}_3 \)
- \( W \) = weight of sample.

2.6.2. Nutritional quality index of lipids

The nutritional quality values of the crude fish oils were evaluated based on their fatty acid composition, following Eq. (14) to (19) to assess the dietary applicability of MCFO and FCFO in the food and pharmaceutical industries.

\[
\text{a) Atherogenic Index (AI)} = \frac{[\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0}]}{\Sigma (\text{MUFA} + \text{PUFA})}
\]

\[
\text{b) Thrombogenic Index (TI)} = \frac{(\text{C14:0} + \text{C16:0} + \text{C18:0})}{[0.5 \times (\Sigma \text{MUFA}) + (0.5 \times \Sigma \text{SFA}) + (3 \times \Sigma \text{PFA}) + \Sigma \text{EPA} + \Sigma \text{DHA}]}
\]
c) Hypocholesterolemic /hypercholesterolemic ratio (h/H) \[38\]
\[
h/H = \frac{\text{cis-C18:1} + \text{EPA}}{\text{C12:0} + \text{C18:0} + \text{C16:0}}
\] (16)

d) Nutritive Value Index (NVI) \[39\]
\[
\text{NVI} = \frac{\text{C18:0} + \text{C18:1}}{\text{C16:0}}
\] (17)
e) Health Promoting Index (HPI) \[40\]
\[
\text{HPI} = \frac{\text{UFA}}{\text{C12:0} + \{(4 \times \text{C14:0}) + \text{C16:0}\}}
\] (18)
f) Fish Lipid Quality (FLQ) \[40\]
\[
\text{FLQ} = 100 \times \frac{\text{C22:6 (n-3)} + \text{C20:5 (n-3)}}{\text{EFA}}
\] (19)

Where: UFA = unsaturated fatty acid; PUFAs = polyunsaturated fatty acids; MUFA = monounsaturated fatty acids; C12:0 = lauric acid; C14:0 = myristic acid; C16:0 = palmitic acid; C18:0 = stearic acid; cis-C18:1 = oleic acid; C22:6 (n-3) = docosahexaenoic acid; and C20:5 (n-3) = eicosapentaenoic acid.

2.7. Statistical Analysis

All the experimental analyses were conducted in triplicates, and the results were expressed as mean ± SD (standard deviation). Using the software Design Expert version X, RSM was conducted while exploring the CCD and desirability function. Other statistical analyses were performed with SPSS software (IBM SPSS Statistics Version 24, IBM Inc., Chicago) and OriginPro 2021 software (version 9.8.0.200). One-way analyses of variance (ANOVA) were employed to determine the significance of differences followed by least significant difference (LSD) post hoc tests to examine the differences among groups. The standard for statistical significance was \(p < 0.05\). In comparison, principal component analysis (PCA) revealed the main fatty acids and nutritional quality index concerning the three milder extraction methods employed. Then, the Pearson’s correlation analysis was performed using R software.

3. Result and Discussion

3.1. Proximate Composition of the Fish Waste

The proximate composition for the marine and freshwater fish heterogeneous wastes, including heads, viscera, gut, and bones, is presented in the supplementary material (Table S3). Although the freshwater fish waste had a higher percentage of moisture (70.13±0.09) and crude protein (18.15±0.08), the ash and lipid content of the marine fish waste (16.54±0.04 and 2.85±0.05 respectively) was higher. Simultaneously, it was evident that the lipid content in marine and freshwater fish waste negatively correlates with the moisture and crude protein content. Generally, the results were statistically significant at \(p < 0.05\), indicating differences in the fish waste content and appropriate use of both representative samples in this study.

3.2. Influence of Different Extraction Methods on the Crude Fish Oil Yield

The extraction methods influenced the amount of extracted oil from the fish wastes. The crude fish oil yield obtained is presented in Fig. 1. Using WR, EH, and SM, the ANOVA result showed that the conventional method with an average range of crude fish oil yield for MCFO (33.7–66.7%) and FCFO (24.0–58.7%) was significantly different (\(p < 0.05\)) to the milder extraction methods, with 32.5–70.7% MCFO and 28.5–60.7% FCFO yields. While \(p < 0.001\) was observed for WR and SM, milder extractions by WR and EH had a better oil recovery rate, suggesting that rigorous extraction methods influence crude fish oil yield.

The lower oil yield from the conventional WR could be attributed to the formation of stable emulsions and packed unfolded proteins/trapped lipid droplets that hinder separation phase formation. Likewise, at temperatures above 90°C, intermolecular disulfide bonds form alongside protein coagulation, limiting oil recovery. This implies that in fish oil production by WR, protein degradation and denaturation are highly dependent on the chosen heating treatment and time. These vital parameters required for an effective lipid separation and removal from the solid stream into the liquid stream. However, the milder WR result revealed that the oil separation might become more effective using other cooking temperatures or time. Glowacz-Różyska et al. [41] demonstrated that high-temperature extraction results in low-quality fish oil; hence improving the oxidative stability and quality using lower temperatures is important.

While polar lipids are easily bound to proteins in solvent extractions, an appropriate solvent mixture (polar to non-polar) will enhance oil recovery. This principle is evident in the results observed in conventional and milder SM. Considerably, the type/amount of enzyme, reaction conditions, and solvents mixture should be closely matched to the kind of fish waste for possible improvement. The high dependence of marine fishes on marine planktons [20] most likely accounts for the higher yield of MCFO observed. Previous studies have also reported similar trends in fish oil yield even with sophisticated extraction methods. For example, using the supercritical CO\(_2\) extraction method, Roy et al. [20] reported a higher fish oil yield from the waste of Japanese Spanish mackerel (Scomberomorus niphonius), a highly distributed fish species in temperate waters of Korea, Japan, and China.

In the comparative evaluation of oil yield in freshwater and marine fishes from China, Guipu et al. [42] reported that aside from the extraction methods, environmental habitat, climate, water depth, food habit/sources, and trophic level greatly influence fish oil yield. Among the several fishes studied, chub mackerel Pseudotsugia japonica in the pelagic-noritic environment, tropical climate, 20–90 water depth, omnivorous food habit, phytoplankton (diatoms)/zooplankton food sources, and 3.19 trophic level produced the highest marine fish oil (7.83±0.51 g/100 g). Meanwhile, the highest freshwater fish oil was 7.43±0.37 g/100 g from black carp Mylopharyngodon piceus in the demersal environment, subtropical climate, 5–30 water depth, carnivorous food habit, small...
snails, clams, mollusks food sources, and 3.19 trophic level [42]. Geographically, the average highest marine fish oil yield reported in this study (33.7–66.7%) is similar to those from farmed marine fish wastes in Poland using different extraction procedures but greater than those from the wild type [41]. This further emphasizes the increasing interest in marine fish waste as fatty acid-rich low-cost feedstock in food, pharmaceutical, and biodiesel industries, major fish oil-consuming sectors [4, 5, 7-12, 14, 43].

In addition to the observed yield in Fig. 1, the confidence interval values $p < 0.05$ indicated that the null hypothesis stating that the conventional and milder extraction methods are the same is not valid. Thus, the alternative hypothesis was accepted, and the production of MCFO and FCFO was optimized following the milder extraction methods.

### 3.3. Response Surface Optimization for Crude Fish Oil Production

#### 3.3.1. Fitting the model and regression coefficients

Using RSM, the maximum to minimum responses ratio for all experimental runs was less than 10, indicating that no transformation was required or conducted ($\lambda = 1.0$). Then, with the fit summary section and the sequential model sum of squares, the quadratic model with the highest polynomial standard ($p < 0.05$) and non-aliased was chosen to predict the most accurate MCFO and FCFO yield. The regression coefficients and ANOVA results were significant at $p < 0.05$, indicating that temperature, cooking time, enzyme concentration, hydrolysis time, ethanol, and ethyl acetate concentration could affect MCFO and FCFO yield (Table 1). The developed quadratic model for MCFO and FCFO yields prediction revealed $R^2$ values higher than 0.9 and nonsignificant lack of fit values ($p$-values $> 0.05$), as shown with the second-degree equations in Table 2. In the composite design for RSM, the experimental and predicted values are shown in Table S2, while the three-dimensional views of the responses are revealed (Fig. 2). In addition, the models had low standard deviation and PRESS values indicating a perfect fit for each design point observed. Hence, the best model for predicting MCFO and FCFO production.

<table>
<thead>
<tr>
<th>Regression coefficients</th>
<th>Crude Fish Oil Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WR ($X_1$ and $X_2$)</td>
</tr>
<tr>
<td></td>
<td>MCFO</td>
</tr>
<tr>
<td>Intercept</td>
<td>42.52</td>
</tr>
<tr>
<td>A-$X_1$, $X_3$, $X_5$</td>
<td>18.55***</td>
</tr>
<tr>
<td>B-$X_2$, $X_4$, $X_6$</td>
<td>3.55**</td>
</tr>
<tr>
<td>AB</td>
<td>-4.05**</td>
</tr>
<tr>
<td>$A^2$</td>
<td>-3.25***</td>
</tr>
<tr>
<td>$B^2$</td>
<td>0.8212</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9912</td>
</tr>
</tbody>
</table>

$*** p < 0.001$, $** p < 0.01$ and $* p < 0.05$. A and B represents the independent variables where $X_1$ is temperature, $X_2$ is cooking time, $X_3$ is enzyme concentration, $X_4$ is hydrolytic time, $X_5$ is ethanol, and $X_6$ is ethyl acetate.
3.3.2. Optimization of MCFO and FCFO production

The independent variables for WR, EH, and SM were optimized to ensure method sustainability for MCFO and FCFO production using the numerical and desirability function [44]. Desirability ranges between 0 to 1, where 1 is the highest desirability (supplementary material Fig. S1). The WR desirability for MCFO is 0.973, and FCFO is 0.978 with an independent variable (60°C temperature and 1 h cooking time). The optimized heating temperature and time are essential to avoid overcooking that may affect the compressibility and prevent over-evaporation of water due to suspended solids. The high desirability at 60°C suggests that the negative effect of high temperature (> 90°C) in WR, such as the degradation of thermolabile compounds through lipid peroxidation induced by extreme heat, can be avoided [1, 4, 8, 26]. Therefore, using extremely high temperatures primarily target the inactivation of parasites, viruses, and bacteria and do not necessarily consider the separation of lipids and proteins. Thus, the 95–100°C recommended optimal cooking temperature by the Food and Agriculture Organization (FAO) is questionable. A similar claim was raised by Hilmarsdottir et al. [26] while reporting the effect of varying temperatures and cooking times on the yield and quality of fish oils.

Table 2. Results for Response Surface Quadratic Model and Equation for MCFO and FCFO Production

<table>
<thead>
<tr>
<th>Crude Fish Oil &amp; Extraction methods</th>
<th>Second-degree equation obtained by RSM</th>
<th>Values</th>
<th>Lack of fit</th>
<th>Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(P values)</td>
</tr>
<tr>
<td><strong>MCFO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WR</td>
<td>MCFO Yield (wt%) = -24.7155 + 1.91558X_1 + 5.93838X_2 - 0.135X_1X_2 - 0.00813436X_1^2 + 0.364999X_2^2</td>
<td>0.9912</td>
<td>0.6660</td>
<td>0.0067</td>
</tr>
<tr>
<td>EH</td>
<td>MCFO Yield (wt%) = 45.0368 - 64.0569X_3 + 40.4066X_4 - 7.43333X_3X_4 + 29.525X_3^2 - 4.16389X_4^2</td>
<td>0.9462</td>
<td>0.9491</td>
<td>0.0005</td>
</tr>
<tr>
<td>SM</td>
<td>MCFO Yield (wt%) = 118.252 - 53.0694X_5 - 25.1571X_6 + 53.9X_5X_6 - 3.695X_5^2 - 24.095X_6^2</td>
<td>0.9954</td>
<td>0.5138</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><strong>FCFO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WR</td>
<td>FCFO Yield (wt%) = -19.5415 + 1.60963X_1 + 3.43584X_2 - 0.15667X_1X_2 - 0.00497187X_1^2 + 1.06056X_2^2</td>
<td>0.9972</td>
<td>0.6370</td>
<td>0.0002</td>
</tr>
<tr>
<td>EH</td>
<td>FCFO Yield (wt%) = 50.5764 - 73.4396X_3 + 30.8584X_4 - 6.56667X_3X_4 + 32.395X_3^2 - 2.73389X_4^2</td>
<td>0.9854</td>
<td>0.8813</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>SM</td>
<td>FCFO Yield (wt%) = 145.439 - 57.511X_5 - 69.6651X_6 + 60.7X_5X_6 - 7.15X_5^2 - 10.25X_6^2</td>
<td>0.9812</td>
<td>0.9629</td>
<td>0.0173</td>
</tr>
</tbody>
</table>

Fig. 2. Response surface plots for MCFO and FCFO as a function of (a) and (b) temperature and cooking time, (c) and (d) enzyme concentration and hydrolysis time, (e) and (f) ethanol and ethyl acetate ratio.
heat treatments on lipid composition during pelagic fishmeal production. In the enzymatic hydrolysis, the desirability is 0.942 for MCFO and 0.928 for FCFO at 1% enzyme concentration and varied time (3.96 h for MCFO and 4 h for FCFO). The actual optimized level for ethanol and ethyl acetate for solvent extraction is 1, with the desirability of 0.978 for MCFO and 0.984 for FCFO. The desirabilities obtained were greater than 0.92 for all combined responses (Fig. S1); thus, the optimized methods are cost-effective strategies. Similar desirability has also been reported in food waste valorization (Fig. S1); thus, the optimized methods are cost-effective strategies.

3.3.3. Verification of RSM model used and optimized extraction parameters

The optimized extraction parameters of the milder methods were verified through experimental investigation to check the model’s suitability for maximum MCFO and FCFO production. The observed yield for MCFO and FCFO agreed with the predicted response values (Fig. 3 and Table S4), confirming that RSM can effectively optimize crude fish oil extraction methods. However, more crude fish oil was produced from marine fish waste, similar to previous studies [4, 15, 17, 38, 46, 47]. The MCFO and FCFO yields (77.2±0.10% and 63.7±0.09%) were higher with EH than other milder extraction methods used. Considering the intrinsic and extrinsic factors associated with fishes, the higher yield of MCFO (60.4±0.03 to 77.2±0.10%) compared to FCFO (54.9±0.12 to 63.7±0.09%) for WR, EH, and SM can be attributed to the fish type and time of the year since marine fishes’ experience seasonal increase in fat storage [4, 10]. Fish oil yield is greatly impacted by the extraction method [48]. The highly improved yields with the optimized SM show that if previous attempt to substitute the hazardous solvents of Bligh and Dyer had optimized their extraction parameters using RSM, better yields might have been reported.

According to Breil et al. [21] and Caprioli et al. [49], some of such attempt is the use of mixtures like hexane/isopropanol, hexane/aceton, methanol/methyltet-butylether, hexane/ethanol, dichloroethane/methanol, dichloroethane/ethanol, acetic ester/ethanol, acetone/dichloromethane, which are more harmful than ethanol/ethyl acetate mixture. Similar to the optimized polar and non-polar solvent ratio (1:1, v/v) confirmed in this study, Lee et al. [50] showed that using dichloroethane/methanol (1:1, v/v); dichloromethane/ethanol (1:1, v/v); and acetone/dichloromethane (1:1, v/v), respectively resulted in about 75% lipid recovery compared to chloroform/methanol. The oil yields reported by Lee et al. [50] can be increased by process optimization using RSM with CCD and desirability function. Therefore, our results suggest that process development through optimization using RSM and method modification (milder methods) can increase oil recovery in a chloroform-free system. Lifgren et al. [51] had previously reported total lipid extraction from animal tissue, but the process requires the use of safer solvent alternatives, as evident in this present study. Achieving greater MCFO and FCFO yields by optimized milder extraction methods shows that combining milder operational parameters and RSM is a promising process intensification (PI) strategy for sustainable crude fish oil production. According to Monsivais-Alonso et al. [23], PI entails implementing a developmental process that offers substantial improvements in (bio)chemical manufacturing and processing compared to their conventional counterpart. Nevertheless, PI has the philosophy of being smaller, cheaper, safer and slicker processes, as in this study.

3.4. Fatty Acid Evaluation: Characteristic Analysis

3.4.1. FT-IR analysis of MCFO and FCFO

In infrared spectra of oils, the position and magnitude of bands are affected when the ratio of fatty acid changes. Fig. 4 (a) shows that the infrared spectra had no obvious differences between MCFO and FCFO, except for minor transmission and band position shift. Thus, MCFO and FCFO from the milder extraction methods have similar characteristic functional groups (Table S5). The band positions of =C–H (cis stretching), =C=C– (cis double bond stretching), =C=H (cis bending out of plane), and =CH2 (wagging) indicates the presence of unsaturated fatty acids in MCFO and FCFO, as also reported by Amorim et al. [13]. The ratio between the bands due to =CH-cis group (3,012) and -CH2- group (2,920–2,925) can be used as a marker for analyzing PUFAs in fish oil samples. Distinctively, the cis double bond stretching of –C=C– differed, as MCFO had a higher transmission (%) than FCFO, which implies the presence of more double bonds in MCFO. However, the bands near 1,413 cm–1 correlate with the C–O ester group stretching vibration and the level of saturated acyl groups in MCFO and FCFO. The correlation coefficient between the WR, EH, and SM in MCFO ranged between 0.992–0.997, and FCFO ranged between 0.994 and 0.997. This indicates that the lipid functional groups of MCFO and FCFO, when considered individually, were not significantly different, despite the difference in the extraction method.

3.4.2. Fatty acid composition

The total fatty acid composition of MCFO and FCFO through transesterification and gas chromatography analysis is shown in the supplementary material (Table S6). The quality of MCFO and FCFO
Environmental Engineering Research 28(2) 220034

Fig. 4. (a) The FTIR Spectra of MCFO and FCFO in the range of 4000-500 (cm⁻¹). (b) Fatty acid pattern for MCFO and FCFO. (c) Percentage prevalence of fatty acids within the major groups. C16:0 in SFA, C18:1 (n-9) in MUFA, C20:5 (n-3) + C22:6 (n-3) in n-3 PUFA and C18:2 (n-6) in n-6 PUFA for MCFO and FCFO.

are strongly related to their fatty acid composition, whereas PUFAs are the most important fatty acids. Previous studies show that the fatty acid composition of fish oil widely varies according to geographic location, type of water (marine or freshwater), feed, season, maturity, and post-capture handling and processing [10]. In MCFO and FCFO, 31 and 30 fatty acids were identified, respectively. There was no statistically significant difference in the total major fatty acid groups (SFAs, MUFAs, and PUFAs) at confidence level \( p < 0.05 \) among WR, EH, and SM for MCFO and FCFO, respectively. MCFO showed a PUFA > SFA > MUFA fatty acid pattern (Fig. 4 (b)) as in marine sardines and mackerel [38]. Meanwhile, FCFO showed a weak definite pattern with higher MUFA than PUFA in contrast to MCFO (Fig. 4 (b)). The higher MUFA in FCFO can be attributed to the fatty acid composition of the vegetation and plant materials as the major feed in freshwater. This is the opposite of the high PUFA in MCFO, linked to algae and plankton presence in marine waters [26]. However, the most prevalent fatty acids were palmitic acid (C16:0) in SFA, oleic acid [C18:1 (n-9)] in MUFA, eicosapentaenoic acid [EPA - C20:5 (n-3)], docosahexaenoic acid [DHA - C22:6 (n-3)] and linoleic acid [C18:2 (n-6)] in PUFA, as illustrated in Fig. 4 (c).

The fatty acid analysis results suggest that the lipid integrity of MCFO and FCFO from the optimized milder extraction methods is highly conserved. This claim aligns with the report by Hilmarsdotti et al. [26], stating that overall best results were obtained at treatments with lowering temperatures in the study to understand the effect of different cooking temperatures on the water, lipid, free fatty acid, and phospholipids composition of fish oil. Considering the proportion of saturated and unsaturated fatty acids in Fig. 4 (b), using MCFO and FCFO as feedstock in biodiesel production is feasible with the prospect of obtaining desirable caloric values and hydrogen to carbon (H/C) ratio [43]. Nevertheless, channeling its applicability for potential use in the health-related industries is more rational following the enormous presence of highly valued omega-3 polyunsaturated fatty acids (EPA and DHA) in Fig. 4 (c). Interestingly, the increasing demand in recent decades for Omega-3 concentrates has led to significant advances in fish oil extraction, purification, and stabilization methodologies. Although, the industrial-scale production of desirable fatty acids still faces huge developmental challenges due to the co-occurrence of complex mixtures and susceptibility to oxidative/thermal deterioration [23]. Fatty acid derivation from fish waste is by far the most economical approach to reducing the production cost of fish oil. Even though marine fish oils are highly recommended for consumption due to their widely established health benefits, consuming freshwater fish oil still has possible benefits. Following the fatty acid composition of FCFO, freshwater species contain considerable amounts of beneficial long-chain unsaturated fatty acids; thus, evaluating the quality value of MCFO and FCFO is important.

3.5. Quality Analysis of MCFO and FCFO

3.5.1. Physicochemical properties of the crude fish oil

Table S7 shows that MCFO and FCFO were within the SCT 3502-2016 standard for China’s first-grade crude fish oil and the international CNS 329-2017 standard [52]. This provides a qualitative justification that optimized milder extraction parameters employed during WR, EH, and SM does not impede the physicochemical properties of the crude fish oil. Thus, MCFO and FCFO could be refined into a more assured quality fish oil for human consumption or used directly in the industrial sectors. For this reason, Table S7 ascertains the applicability of MCFO and FCFO as healthy food additives.
and substrate for biodiesel production due to its potential quality cetane number, cold flow properties, flash point, and oxidation stability [53]. The decreasing values of AV (mg KOH/g), PV (meq/kg), P-AV, ToToX, and the increasing value of IV (g/100 g oil) as in Table S7 are similar to the observation reported for extracted quality fish oil in Mauritania [54]. PV (meq/kg) decreases with decreasing temperature [41]. The observed AV (mg KOH/g) indicates no significant hydrolytic changes during the fish oil extraction procedure.

Table 3. Comparison of Nutritional Quality Indices of Lipids in the Crude Fish Oils

<table>
<thead>
<tr>
<th>Index</th>
<th>MCFO Extraction methods</th>
<th>FCFO Extraction methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WR</td>
<td>EH</td>
</tr>
<tr>
<td>Σ SFA</td>
<td>31.03 ±0.01</td>
<td>30.94 ±0.02</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>27.67 ±0.04</td>
<td>27.79 ±0.02</td>
</tr>
<tr>
<td>Σ PUFA</td>
<td>41.30 ±0.04</td>
<td>41.27 ±0.03</td>
</tr>
<tr>
<td>Σ UFA</td>
<td>68.97 ±0.04</td>
<td>69.06 ±0.02</td>
</tr>
<tr>
<td>Σ MUFA/Σ SFA</td>
<td>0.89 ±0.03</td>
<td>0.90 ±0.02</td>
</tr>
<tr>
<td>Σ PUFA/Σ SFA</td>
<td>1.33 ±0.02</td>
<td>1.33 ±0.02</td>
</tr>
<tr>
<td>Σ n-3 PUFA</td>
<td>33.94 ±0.05</td>
<td>34.80 ±0.02</td>
</tr>
<tr>
<td>Σ n-6 PUFA</td>
<td>7.36 ±0.02</td>
<td>6.38 ±0.03</td>
</tr>
<tr>
<td>Σ n-3/Σ n-6 ratio</td>
<td>4.61 ±0.03</td>
<td>5.47 ±0.03</td>
</tr>
<tr>
<td>Σ n-6/Σ n-3 ratio</td>
<td>0.22 ±0.04</td>
<td>0.18 ±0.03</td>
</tr>
<tr>
<td>EPA + DHA</td>
<td>26.88 ±0.05</td>
<td>28.88 ±0.04</td>
</tr>
<tr>
<td>ΑI</td>
<td>0.43 ±0.01</td>
<td>0.43 ±0.00</td>
</tr>
<tr>
<td>ΤI</td>
<td>0.23 ±0.01</td>
<td>0.24 ±0.01</td>
</tr>
<tr>
<td>h</td>
<td>62.03 ±0.02</td>
<td>63.55 ±0.02</td>
</tr>
<tr>
<td>H</td>
<td>26.47 ±0.00</td>
<td>27.37 ±0.01</td>
</tr>
<tr>
<td>h/H</td>
<td>2.34 ±0.01</td>
<td>2.32 ±0.01</td>
</tr>
<tr>
<td>NVI</td>
<td>0.91 ±0.01</td>
<td>0.94 ±0.02</td>
</tr>
<tr>
<td>HPI</td>
<td>2.35 ±0.01</td>
<td>2.34 ±0.01</td>
</tr>
<tr>
<td>FLQ</td>
<td>26.88 ±0.05</td>
<td>28.88 ±0.04</td>
</tr>
</tbody>
</table>

WR = wet reduction method, EH = Enzyme hydrolysis, and SM = solvent extraction. All values are an average of means ± SD computed from the fatty acid composition in Table S6.
between unsaturated and saturated fatty acids while providing insight into the effect of specific fatty acids on cholesterol metabolism and cardiovascular diseases [40]. The higher the value of h/H, the more desirable. The h/H content in MCFO (2.21±0.04 to 2.34±0.01) and FCFO (2.29±0.03 and 2.77±0.02) are similar to those reported for chub mackerel (Scomber japonicus) [47] but more preferable than the h/H values of king mackerel (Scomberomorus cavalla) (1.56) and Chilean jack mackerel (Trachurus murphyi) (1.73) [38].

Furthermore, NVI as an additional indicator of fatty acid’s effect on cholesterol metabolism is more beneficial for human health at a higher value. MCFO and FCFO from EH had the most preferred NVI, which suggests more stearic acid (C 18:0) and oleic acid (C 18:1) content compared to the percentage of palmitic acid (C 16:0). Also, higher HPI values are more desirable and reveal the potential impact of MCFO and FCFO in preventing cardiovascular diseases [40]. The HPI value of the crude fish oil is preferable to several dairy products (0.16 and 0.68) [40] but similar to the values in fish oil capsules and syrup in Turkey [14]. Meanwhile, the FLQ value of MCFO and FCFO ranged between 13.01 and 36.37, as observed in several fish species [40]. This reflects the general dietetic quality of lipids in MCFO and FCFO alongside their potential effects on chronic disease development. Hence, the optimized milder extraction methods demonstrated are effective and health-wise.

3.6. PCA and Correlation Analysis of the NQI
The PCA result in Fig. 5 (a) shows the close relatedness in the nutritional value of MCFO and FCFO regardless of the extraction method. Although, FLQ had a unique pattern suggesting variation in the EPA and DHA fraction of MCFO and FCFO. The result in Fig. 5 adds more credence to MCFO and FCFO nutritional values than the illustration reported in Fig 4. In this regard, the nutritional quality value of crude fish oil cannot be entirely determined by its prevalent fatty acid content alone. Therefore, MCFO denoted as A+FLQ in Fig 5 (a) had more EPA and DHA. This can be attributed to the presence of algae and planktons (primary omega-3 producers) in the marine food webs [47, 59], as well as seasonality and food availability [10, 60]. Thus, the high focus on marine fishes in oil-producing industries [59]. The correlation pattern of the NQI in MCFO and FCFO is illustrated in Fig. 5 (b) and (c). Although AI negatively correlated with h/H, NVI, and HPI for MCFO and FCFO, as previously reported for quality fish oil [40]. It is important to note that the correlation between TI and h/H has the highest significant confidence level at p < 0.001 for FCFO. The Σ n-6/Σ n-3 ratio also proved crucial in regulating several indices (h/H, HPI, FLQ, AI, and TI), suggesting the applicability of MCFO and FCFO in preventing cardiovascular diseases [39].

4. Conclusions and Recommendation
Fatty acids are vulnerable constituents of crude fish oil, yet a significant determining factor for fish oil application potential. The increasing demand for PUFA-enriched fish oil can be mitigated by properly using fishery resources, especially waste. Milder extraction methods were proven as a low-cost sustainable strategy for achieving higher yields and quality crude fish oil. Based on our experimental observation in this present study, we conclude with the following remarks:

- Conventional and milder extraction methods for crude fish oil production are significantly different (p < 0.05). Although oils extracted from fish waste are much cheaper than those from whole fish, optimizing the milder extraction methods is a path towards process intensification. RSM is an essential optimization tool that can be used with CCD and desirability function to maximize MCFO and FCFO production.
- The highest yield of crude fish oil was obtained through EH. MCFO and FCFO from WR, EH, and SM had no significant differences (p < 0.05) in their chemical functional groups and fatty acid compositions. Hence, optimized milder extraction methods for crude fish oil production conserves quality. Nevertheless, after catching, an immediate fish processing practice is encouraged to reduce the detrimental effects of spoilage microbes and enzymes.
- The pattern of fatty acid composition in MCFO and FCFO does not differ significantly.
not reveal the entire nutritional quality of the oils; thus, NQI, PCA, and Pearson’s correlation analysis are vital. In practice, the optimized milder extraction methods can help meet the increased global nutritional demand of fish oils while reducing the environmental pollution associated with fish waste. Hence, MCFO and FCFO, when purified, can serve as functional oil in several food and pharmaceutical industries. These oils can also be used as green substrates for biotechnological applications in microbial growth, fish meal, cosmetics, and biodiesel production.

• Future studies could be directed towards analyzing protein quality changes during processing with the optimized milder extraction parameters to develop new lines of inquiry on protein modification. Also, a life cycle assessment (LCA) is required to investigate the socio-economical impact of the optimized milder extraction methods for easy integration into the circular economy of fish oil production.

Acknowledgments
This study was financially supported by the Zhoushan Science and Technology Department Project (2019C81056, 2020C41082), the Fundamental Research Funds for the Central Universities (2020QNA4045), and by the Research Funds of the Guangxi Key Laboratory of Theory and technology for Environmental Pollution Control (No.2001K004).

Conflict-of-Interest
The authors declare that they have no conflict of interest.

Author Contributions
C.L.M. (Postgraduate student) conducted all the experiments, conceptualization, methodology, software, validation, writing - original draft, reviewing, editing, formal analysis, visualization. G.Z. (Professor) reviewing, editing, investigation. E.B.B. (Postgraduate student) reviewing, editing, formal analysis, investigation, validation. H.Z. (Postgraduate student) investigation and validation. S.U.O. (Senior lecturer) reviewing, editing, investigation, validation. C.Z. (Professor) supervision, conceptualization, methodology, resources, validation, writing, reviewing, editing, visualization.

References


