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Simultaneous Extraction and Fractionation of Fish Oil from Tuna By-Product Using Supercritical Carbon Dioxide (SC-CO_2)

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ABSTRACT

Fish oil was extracted and simultaneously collected into six fractions based on molecular weight and the chain length of triglycerides in terms of fatty acid constituents without splitting of the triglycerides, using supercritical carbon dioxide (SC-CO_2) at optimized conditions of 40 MPa, 65°C, and a flow rate 3 mL min^{-1}. In each type of fractionation, the first fraction (F1) was rich in saturated fatty acids (SFA; 52.57 to 61.26%), followed by monounsaturated fatty acids (MUFA; 22.17 to 23.22%) and polyunsaturated fatty acids (PUFA; (0.54 to 20.37%); the sixth fraction (F6) was rich in PUFA (48.93%), followed by MUFA (33.59%) and SFA (13.61%). It was obvious that short-chain fatty acids were extracted at an earlier fraction; therefore, the latter fractions were dominant in long-chain fatty acids, especially MUFA and PUFA. Thus, omega-3 fish oil (last three fractions) was successfully separated to be used as a value-added health product.

KEYWORDS

Tuna by-product; fish oil; omega-3 fatty acids; fractionation; supercritical carbon dioxide (SC-CO_2)

Introduction

The demand for high purity and quality of fish oils, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has led researchers to find safer techniques for the fractionation of desired components. Fish oil derivatives in the form of omega-3 polyunsaturated fatty acids (PUFA) are increasingly demanded as pharmaceutical products, food additives, and health supplements (Espinosa et al., 2002). Many clinical and epidemiologic studies have shown positive roles for omega-3 PUFA in infant development; cancer; cardiovascular diseases; and more recently, in various mental illnesses—including depression, attention-deficit hyperactivity disorder, and dementia (Riediger et al., 2009). PUFA are not synthesized *de novo* in human beings and hence are termed as essential fatty acids. They must be obtained from the diet. A natural dietary source of omega-3/6 PUFA includes fish oil, marine animal oil, and vegetable oil. The oils derived from each of these sources, however, also contain substantial levels of saturated fatty acids (SFA) and other undesirable impurities. Health and nutrition experts have recently recommended increasing the dietary intake of PUFA in order to maintain an omega-6/omega-3 ratio between 5:1 and 10:1 in the diet (Food and Agriculture Organization of the United Nations [FAO], 1998). The European Food Safety Agency (EFSA, 2009) has proposed labeling reference intake values of 250 mg per day of EPA + DHA. As a result, PUFA-rich fish oil is of importance to nutritionists and physicians, and the production of omega-3 PUFA-rich fish oil as a food or pharmaceutical material has been one of the growing research areas in the last decades.

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The skin, heads, tails, and viscera of fish are regarded as wastes, which are discarded as processing leftovers in the seafood industry. Disposing of the fish waste is one of the biggest problems that greatly affect both the environment and the economy. Tuna head contains a high amount of omega-3 PUFA, especially DHA (Chantachum et al., 2000) and have drawn much attention as a functional food. Therefore, an effective use of tuna head is beneficial for maximum resource utilization and waste reduction.

Longtail tuna (*Thunnus tonggol*), locally known as Aya hitam, is the main targeted species in Malaysia due to high demand in tuna canneries, year-round availability, and relatively low cost. In the cannery, tuna were processed followed by deheading. Therefore, tuna heads are generated as by-products, which are normally used as animal feeds (Chantachum et al., 2000). Saito et al. (1999) reported that omega-3 PUFA (especially DHA) contents in the lipids of head tissue of all highly migratory tuna species are comparatively high. However, tuna head cannot be recommended for the diet as it is not commercially available as food. Accordingly, it can be chosen as a source of fish oil from where the PUFA-rich fraction can further be separated to obtain PUFA concentrates.

The fractionation of specific compounds from fish oil is difficult due to the complexity of the material. Therefore, the triglyceride mixture is hydrolyzed followed by esterification of the free fatty acids with methanol or ethanol. Transesterification of the mixture containing PUFA with lower alcohols (especially ethanol) followed by a countercurrent extraction with supercritical carbon dioxide (SC-CO_2) can give substantial separation based on the number of carbon atoms of the PUFA. There are several reports on the preparative column chromatography using supercritical fluid as the eluent (Bartle et al., 1995; Señoráns and Ibañez, 2002). However, a planned separation based on the degree of unsaturation was not possible by these techniques. On the other hand, the transesterification involved in such methods is an extra burden.

Perrut et al. (2001) used one or more countercurrent columns in which the SC-CO_2 and a polar cosolvent mixture served to remove impurities from PUFA-rich triglycerides. But the use of a polar organic solvent as a cosolvent is not desirable. Recently, a supercritical fluid chromatography separation of PUFA-rich ethyl esters from commercial fish oils by varying the column temperature has been reported (Gironi and Maschietti, 2006; Perretti et al., 2007). However, most of the methods discussed here involved the fractionation of PUFAs from purified and readily available PUFA-containing oils and needed to combine two or more techniques.

Extraction of fish oil by SC-CO_2 with concomitant fractionation into saturated fatty acid esters and unsaturated ones without the use of further transesterification and column chromatography has not been described yet. The aim of this study was to direct the separation of the PUFA-rich fish oil fraction, especially omega-3/6 fatty acid constituents, from fish by-product of the fish processing industry using cosolvent with SC-CO_2 as an environmentally benign, cheap, and safer technique.

Material and methods

Sample preparation

Thunnus tonggol were obtained from the fish landing center of Batu Maung, Pulau Penang, Malaysia. The fresh fish samples were collected in an insulated icebox and transported to the laboratory of Environmental Technology, School of Industrial Technology, USM, Malaysia. The fish samples were immediately de-headed manually. The heads were then freeze-dried (Model: LABCONCO, Kansas City, MO, USA) at a drying temperature of −47°C and vacuumed at 0.133 bar for 3 days. The samples were then kept in desiccators until further use. The dried heads were ground with a dry mixer (Waring Laboratory, Stamford, CT, USA) into particle sizes ranging from 0.2 to 0.5 mm by sieving.

Fractionation of oil by SC-CO_2

The experimental set-up for the SC-CO_2 extraction process was assembled according to Norulaini et al. (2009). The ISCO SFE System (ISCO Inc., Lincoln, NE, USA) consisting of a

Table 1. Fractional oil yield extracted from tuna head using SC-CO_2 (values are mean ± standard deviation).

		Fractional yield (g oil/100 g sample)		
Fraction no. (F)	Control*	Fraction Type A**	Fraction Type B***	Fraction Type C****
F-1	35.60 ± 0.52	25.37 ± 0.49	13.15 ± 0.61	7.34 ± 0.35
F-2	0	10.23 ± 0.27	15.42 ± 0.16	9.01 ± 0.54
F-3	0	0	7.03 ± 0.38	10.28 ± 0.27
F-4	0	0	0	5.17 ± 0.33
F-5	0	0	0	2.98 ± 0.42
F-6	0	0	0	0.82 ± 0.12

*Total oil yield extracted by 120 min. **Total yield was fractionated into two (each fraction 60 min). ***Total yield was fractionated into three (each fraction 40 min). ****Total yield was fractionated into six (each fraction 20 min).

supercritical fluid extractor (ISCO, SFX 220), a controller (ISCO, SFX 200), a carbon dioxide cylinder, a chiller (Yih Der BL-730), two syringe pumps (ISCO, Model 100DX): a CO_2 pump and a cosolvent pump, and restrictor temperature controller associated with two coaxially heated capillary restrictors (ISCO). The CO_2 pump was fitted with a cooling jacket to deliver CO_2, and the cosolvent pump was fitted to deliver cosolvents as modifiers/entrainers. To cool the pump's head, an ethylene glycol-deionized water mixture (50:50, v/v) was circulated through the cooling jacket using a refrigerated bath circulator (Model 631D, Tech-Lab Manufacturing Sdn. Bhd., Selangor, Malaysia), which can chill the coolant down to 0°C. In each experiment, 2 g of ground sample (dry weight) was loaded into a 2 mL sample cartridge and placed in the ISCO extraction chamber and allowed to equilibrate at desired temperature. The restrictor was maintained at 65° C to avoid problems of restrictor plugging. The extractions were performed with CO_2 and 20% ethanol (as a cosolvent) at 3 mL min^{-1} (2.4 mL CO_2 and 0.6 mL ethanol min^{-1}, v/v), for 120 min at 65°C temperature and 40 MPa pressure. Simultaneous extraction and fractionation of yield were performed into two, three, and six fractions, which are defined as Fraction Type-A, Fraction Type-B, and Fraction Type-C, respectively. In Fraction Type-A, the extracts were collected into two fractions (F1, F2) at 60 min each (total of 60 × 2 = 120 min) at optimized condition of 40 MPa, 65°C, and 3 mL min^{-1} using the SC-CO_2 method. Similarly, the oil yield of Fraction Type-B were collected into three fractions (F1, F2, and F3) at 40 min each (total of 120 min) at optimized condition of SC-CO_2 (Table 1). In Fraction Type-C, the yields were collected into six fractions (F1, F2, F3, F4, F5, F6) at 20 min each (total 120 min) at optimized conditions (Table 1). Experiments of each fraction were conducted in triplicate, and the yields were averaged to obtain the mean value. However, the fractionation process was performed simultaneously at time base extraction where ethanol was mixed with carbon dioxide as a cosolvent to enhance the solubility of the carbon dioxide for faster extraction. Each extract with ethanol was collected separately into a preweighed blue cap bottle yield trap through a restrictor for fatty acid analysis. The trap was cooled with an ice-water mixture. The oil trap containing the extracted oil with ethanol, as a residue of the cosolvent, was evaporated under vacuum at 40°C using a rotary evaporator (Büchi-Rotavapor, Flawil, Switzerland), and then placed in the oven at 45°C for 30 min before being transferred into the desiccators.

Definition of the yields

Extractability of oil yield was defined as a percent (g oil/100 g of ground samples on dry basis), as described below:

$$\text{Extractability of oil yield}\,(\%) = \frac{\text{Total yield}\,(\text{g})\ \text{extracted by SC - } CO_2}{\text{Total yield}\,(\text{g})\ \text{extracted by Soxhlet extraction}} \times 100. \qquad (1)$$

The oil fractions obtained from different steps of extraction were weighed individually and then totaled to determine the cumulative yield, which was expressed in percent (g oil yield/100 g of sample):

$$\text{Fractionated yield}\,(\%) = \frac{\text{Fractionated yield}\,(\text{g})}{\text{Total yield}\,(\text{g})} \times 100. \quad (2)$$

Analysis of fatty acid constituent by gas chromatography

Analysis of fatty acid methyl esters (FAME) of different oil fractions was performed to determine the fatty acid profile using gas chromatography (GC-2010 Plus AOC-5000, Shimadzu, Osaka, Japan) fitted with a BPX70 (30 m × 0.32 mm × 0.25 μm film thickness) capillary column, which was purchased from Sigma-Aldrich Co., St. Louis, MO, USA. FAME was prepared by dissolving 50 mg of sample into 0.95 mL n-hexane, and 0.05 mL 1 M sodium methoxide (30% methanol in sodium methoxide). The mixture was then shaken vigorously using an auto-vortexer (Model VF2, Janke and Kunkel, Staufen im Breisgau, Germany) for 30 s and stored for another 5 min so that it formed a bilayer. The clear upper layer containing the FAMEs (1 μL) was pipetted off and injected into a GC using an external standard method, the Palm Oil Research Institute of Malaysia test method no. p3.4 (1995), which was adopted from the American Oil Chemists' Society (2003). Supelco 37 component FAME mixtures and standard menhaden oil (PUFA-3; Supelco, Bellefonte, PA, USA) was used as reference standard fish oil for the identification of peaks. The oven temperature was set at 140°C, held for 2 min, increased with a heating rate of 5°C min^{-1} up to the final temperature of 250°C and then held for 10 min at 250°C. The identification of chromatographic peaks was performed using the retention time of FAME standard. The results of fatty acids analysis were reported as an average of three analyses for each sample investigated.

Statistics

The weighted means were derived from an analysis of variance by Statistica Version 10.0 (StatSoft Inc., Tulsa, OK, USA). For tests of statistical significance between different fractions (F1 to F6) within fatty acids, the data were subjected to unequal N Tukey's HSD test for significant differences ($p < 0.05$).

Results and discussion

Recovery of oil

The moisture content of freeze-dried ground fish head was 2.3%, while the moisture levels up to 15% had almost no effect on the extractability of oils in SC-CO_2 extraction (Snyder et al., 1984). Devittori et al. (2000) also found that a moisture reduction of less than 8% does not affect the extraction of oil in SC-CO_2 extraction. In our previous study, we reported that the moisture content of less than 6.33% does not affect or interfere with the solvent to recover the complete yield (Sahena et al., 2010a). However, freeze-dried samples might be rich in polar lipids, due to their amphiphilic properties leading to strong interactions with peptides and proteins, which are completely insoluble in carbon dioxide. Although the phospholipids (PLs) are lipid soluble in anhydrous condition of the freeze-dried sample, carbon dioxide was used as a solvent in its supercritical state, where the phosphate groups of PLs have no chance to be dissolved in the nonpolar carbon dioxide. In contrast, 20% ethanol (polar solvent) was mixed with the carbon dioxide (nonpolar solvent) where PLs could have more chance to be dissolved in ethanol. However, the extraction was performed only at supercritical conditions (65°C and 40 MPa) of carbon dioxide, which is the subcritical phase for the ethanol. At this condition, ethanol is completely unable to extract either PLs or any other

compound lipids due to its subcritical temperature (65°C), where supercritical temperature and boiling point of ethanol are 240.75 and 78.4°C, respectively. The role of ethanol was to enhance the solubility of triglycerides in carbon dioxide. However, the total oil yields in terms of fatty acid constituents in triglycerides form extracted using SC-CO_2 was 35.6% (g/ 100 g sample on dry basis).

In Fraction Type-A, the highest yield was obtained at F1 (25.37 g/100 g sample), followed by F2 (10.23 g/100 g sample) with 71.3 and 28.7% of total oil yield, respectively (Table 1). In Fraction Type-B, the solvent had less time to penetrate into the sample matrixes due to 20 min lower extraction period of time compared to the Fraction Type-A, thus the highest yield was obtained at F2 (15.42 g/100 g sample), followed by F1 (13.15 g/100 g sample) and F3 (7.03 g/100 g sample) and were found to be 43.3, 36.9, and 19.7% of the total yield, respectively (Table 1). Whereas in Fraction Type-C, the yield was the highest at F3 (10.28 g/100 g sample), followed by F2 (9.01 g/100 g sample), F1 (7.34 g/100 g sample), F4 (5.17 g/100 g sample), F5 (2.98 g/100 g sample), and F6 (0.82 g/100 g sample). However, in each type of fractionation, a higher yield was obtained in the earlier fractions than the later fractions and drastically dropped down to the last fraction. The findings are in agreement with the report of Zuta et al. (2003), who extracted 38.1% (g/100 g sample) fish oil from mackerel process wastes of skin, which comprised the highest yield among any other wastes of mackerel. At high pressures and temperatures, density and the solubility increased exponentially due to the exponential increase in the vapor pressure of the oil (Zaidul et al., 2006). Moreover, when the CO_2 penetrated intrinsically into the fish sample and dissolved into the oil matrix, most probably there would be considerable swelling of the liquid phase that could reduce the viscosity of the fish oil, promoting the mass transfer and enhancing the extractability of the oil yield (Zaidul et al., 2007). The authors also reported that the solubility of CO_2 in oil increases with both pressure and temperature. However, the cosolvent was used as an enhancer that might help to swell the sample matrixes. Thus, the CO_2 had more chances to dissolve with the sample, which might cause increase of the solubility of the CO_2 into the oil and improving the mass transfer of the oil (Sahena et al., 2010a). However, fish oil has a higher amount of long-chain fatty acids and PUFA which are more viscous than the fish oil that contains lower PUFA. The continuous flow of CO_2 with cosolvent helps to complete the extraction with the least amount of CO_2. Thus, using cosolvent with CO_2 provides the advantages of reducing CO_2 consumption in simultaneous extraction and fractionation of PUFA-rich oil from fish wastes.

Fatty acid composition

The fatty acid constituents in terms of triglycerides of Fractionations Type-A are shown in Table 2. Most of the SFA constituents were extracted at F1 (52.57%), followed by F2 (19.19%). The total amounts of monounsaturated fatty acids (MUFA) were about 22.5 and 30% at F1 and F2, respectively; whereas, the total PUFA were about 20.4 and 46.8% at F1 and F2, respectively. The extraction of MUFA and PUFA at F1 was lower than the SFA due to both MUFA and PUFA containing relatively higher molecular weight and longer chain fatty acids than the SFA. As a result, MUFA and PUFA required longer time than SFA to be eluted. However, a lower amount of PUFA was extracted at F1compared to that of F2 (Table 2).

Similar trends in the fatty acid analysis were also observed (Table 3), where the fatty acid constituents in terms of triglycerides of fish oil were simultaneously extracted and fractionated into three fractions using SC-CO_2 technique. In Fractionation Type-B, more SFA constituents were eluted at F1, followed by F2 and F3. The total amounts of SFA were about 53.22, 43.61, and 15.58% at F1, F2, and F3, respectively. In contrast, MUFA and PUFA started eluting higher at F2, and the highest elution was observed at F3 (Table 3). The total amounts of MUFA were about 22, 25, and 29% at F1, F2, and F3, respectively; whereas PUFA were about 17, 27, and 51% at F1, F2, and F3, respectively. The trends were similar to Table 1, where the elution of both MUFA and PUFA was lower than the SFA at F1 and was found to be higher than F2.

Table 2. Fatty acid compositions of fractionated (Fraction Type-A) tuna head oil using SC-CO_2 (values are mean ± standard deviation).

Fatty acids (%)	Control	F-1	F-2
C14:0	5.31 ± 0.70^{b}	11.16 ± 0.17^{a}	1.07 ± 0.08^{c}
C15:0	1.55 ± 0.41^{b}	3.89 ± 0.06^{a}	0.88 ± 0.03^{b}
C16:0	29.06 ± 1.24^{b}	31.18 ± 0.83^{a}	4.14 ± 0.21^{c}
C17:0	1.14 ± 0.38^{b}	1.61 ± 0.11^{b}	2.82 ± 0.13^{a}
C18:0	8.37 ± 1.13^{a}	4.15 ± 0.08^{b}	8.53 ± 0.35^{a}
C22:0	1.13 ± 0.07^{ab}	0.58 ± 0.12^{b}	1.75 ± 0.77^{a}
16:1n7	5.12 ± 0.33^{ab}	5.28 ± 0.28^{a}	4.92 ± 0.19^{b}
17:1n7	2.36 ± 0.52^{a}	1.86 ± 0.10^{b}	2.71 ± 0.31^{a}
18:1n9	13.42 ± 0.97^{b}	14.19 ± 0.74^{b}	16.36 ± 0.37^{a}
20:1n9	1.07 ± 0.04^{b}	0.72 ± 0.07^{c}	2.89 ± 0.13^{a}
22:1n9	0.69 ± 0.10^{b}	0.44 ± 0.12^{b}	3.11 ± 0.09^{a}
18:2n6	1.17 ± 0.03^{b}	3.32 ± 0.36^{a}	2.96 ± 0.07^{a}
18:3n3	0.51 ± 0.07^{b}	2.51 ± 0.14^{a}	2.81 ± 0.11^{a}
20:4n6	2.58 ± 0.11^{b}	2.37 ± 0.06^{b}	4.06 ± 0.04^{a}
20:5n3	1.38 ± 0.08^{bc}	2.07 ± 0.04^{b}	6.11 ± 0.13^{a}
22:5n3	0.73 ± 0.06^{c}	1.44 ± 0.03^{b}	5.73 ± 0.08^{a}
22:6n3	18.9 ± 0.93^{b}	8.66 ± 0.26^{c}	25.15 ± 0.44^{a}
ΣSFA	46.56 ± 1.27^{b}	52.57 ± 1.65^{a}	19.19 ± 0.87^{c}
ΣMUFA	22.66 ± 1.03^{b}	22.49 ± 0.86^{b}	29.99 ± 0.64^{a}
ΣPUFA	25.27 ± 0.92^{b}	20.37 ± 1.34^{c}	46.82 ± 0.75^{a}
Others FAs	5.51 ± 0.09^{a}	4.57 ± 0.22^{ab}	4 ± 0.17^{b}

*Different superscript letters in the same row indicate significant difference ($p < 0.05$).

Table 3. Fatty acid compositions of fractionated (Fraction Type-B) tuna head oil using SC-CO_2 (values are mean ± standard deviation).

Fatty acids (%)	Control	F-1	F-2	F-3
C14:0	5.31 ± 0.70^{b}	12.71 ± 0.24^{a}	4.28 ± 0.11^{b}	0.54 ± 0.03^{c}
C15:0	1.55 ± 0.41^{b}	3.21 ± 0.07^{a}	1.84 ± 0.05^{b}	0.26 ± 0.07^{c}
C16:0	29.06 ± 1.24^{b}	30.72 ± 0.88^{a}	28.51 ± 0.27^{b}	4.39 ± 0.11^{c}
C17:0	1.14 ± 0.38^{c}	1.56 ± 0.11^{bc}	1.62 ± 0.16^{ab}	1.87 ± 0.06^{a}
C18:0	8.37 ± 1.13^{a}	4.58 ± 0.09^{c}	6.47 ± 0.08^{b}	7.15 ± 0.13^{ab}
C22:0	1.13 ± 0.07^{ab}	0.44 ± 0.06^{c}	0.89 ± 0.05^{bc}	1.37 ± 0.10^{a}
16:1n7	5.12 ± 0.33^{ab}	5.72 ± 0.21^{a}	4.87 ± 0.13^{bc}	4.51 ± 0.18^{c}
17:1n7	2.36 ± 0.52^{b}	1.74 ± 0.04^{c}	2.16 ± 0.07^{bc}	3.06 ± 0.08^{a}
18:1n9	13.42 ± 0.97^{c}	13.36 ± 0.16^{c}	14.46 ± 0.39^{bc}	16.38 ± 0.56^{a}
20:1n9	1.07 ± 0.04^{bc}	0.61 ± 0.11^{c}	1.97 ± 0.06^{ab}	2.63 ± 0.11^{a}
22:1n9	0.69 ± 0.10^{c}	0.74 ± 0.12^{c}	1.52 ± 0.17^{b}	2.45 ± 0.16^{a}
18:2n6	1.17 ± 0.03^{b}	2.92 ± 0.15^{a}	2.84 ± 0.12^{a}	2.57 ± 0.17^{a}
18:3n3	0.51 ± 0.07^{c}	2.46 ± 0.10^{b}	2.08 ± 0.16^{b}	4.28 ± 0.12^{a}
20:4n6	2.58 ± 0.11^{c}	2.58 ± 0.03^{c}	3.26 ± 0.07^{b}	5.82 ± 0.08^{a}
20:5n3	1.38 ± 0.08^{d}	2.58 ± 0.09^{c}	4.65 ± 0.13^{b}	9.17 ± 0.13^{a}
22:5n3	0.73 ± 0.06^{d}	1.08 ± 0.15^{c}	1.82 ± 0.12^{bc}	3.26 ± 0.05^{a}
22:6n3	18.90 ± 0.93^{b}	5.73 ± 0.11^{d}	12.49 ± 0.16^{c}	26.13 ± 0.22^{a}
Σ SFA	46.56 ± 1.27^{b}	53.22 ± 0.97^{a}	43.61 ± 0.92^{c}	15.58 ± 0.41^{d}
ΣMUFA	22.66 ± 1.03^{c}	22.17 ± 0.43^{c}	24.98 ± 0.35^{b}	29.03 ± 0.32^{a}
ΣPUFA	25.27 ± 0.92^{c}	17.35 ± 0.26^{d}	27.14 ± 0.27^{b}	51.23 ± 0.35^{a}
Others FAs	5.51 ± 0.09^{b}	7.26 ± 0.14^{a}	4.27 ± 0.12^{c}	4.16 ± 0.17^{c}

*Different superscript letters in the same row indicate significant difference ($p < 0.05$).

Fatty acid constituents were simultaneously extracted and collected into six steps (F1 to F6) at Fractionation Type-C (Table 4). It can be clearly seen that the highest amount of total SFA was eluted in the earlier fractions F1 and F2 (Fractionation Type A, Table 2) compared to the later fractions (Fractionation Type B, Table 3). The total SFA was found to decrease rapidly from F3 up to F6 (Table 4); whereas the elution of total MUFA increased gradually from F1 up to F6 and was always found to be higher at the later fractions. Similar trends were also found in fractionated PUFA, where the fatty acid constituents were found to be drastically higher up to the last fraction (F6). Although the SFA was unable to be discarded fully at any fraction, F4, F5, and F6 could be regarded

Table 4. Fatty acid compositions of fractionated (Fraction Type-C) tuna head oil using SC-CO_2 (values are mean ± standard deviation).

Fatty acids (%)	Control	F-1	F-2	F-3	F-4	F-5	F-6
C14:0	5.31 ± 0.70^{d}	15.34 ± 0.13^{a}	9.24 ± 0.10^{b}	6.25 ± 0.08^{c}	1.21 ± 0.11^{e}	0.47 ± 0.07^{f}	0.32 ± 0.06^{f}
C15:0	1.55 ± 0.41^{d}	5.06 ± 0.08^{a}	3.11 ± 0.05^{b}	2.03 ± 0.05^{c}	1.17 ± 0.08^{de}	0.61 ± 0.06^{ef}	0.46 ± 0.05^{f}
C16:0	29.06 ± 1.24^{c}	36.17 ± 0.11^{a}	32.42 ± 0.18^{b}	28.16 ± 0.09^{c}	17.62 ± 0.56^{d}	4.26 ± 0.04^{e}	1.15 ± 0.10^{f}
C17:0	1.14 ± 0.38^{d}	1.13 ± 0.03^{d}	2.51 ± 0.05^{bc}	2.64 ± 0.07^{bc}	3.13 ± 0.16^{a}	3.01 ± 0.10^{ab}	2.18 ± 0.03^{c}
C18:0	8.37 ± 1.13^{a}	3.29 ± 0.05^{e}	5.83 ± 0.14^{d}	6.02 ± 0.11^{cd}	6.36 ± 0.14^{c}	7.42 ± 0.17^{b}	7.55 ± 0.13^{b}
C22:0	1.13 ± 0.07^{bc}	0.27 ± 0.07^{e}	0.52 ± 0.03^{d}	0.67 ± 0.07^{cd}	0.96 ± 0.08^{cd}	1.47 ± 0.05^{b}	1.95 ± 0.08^{a}
16:1n7	5.12 ± 0.33^{bc}	6.62 ± 0.14^{a}	5.37 ± 0.16^{b}	5.24 ± 0.06^{b}	5.26 ± 0.12^{b}	4.84 ± 0.08^{cd}	4.23 ± 0.05^{d}
17:1n7	2.36 ± 0.52^{cd}	1.06 ± 0.06^{e}	1.95 ± 0.07^{d}	2.36 ± 0.10^{cd}	3.09 ± 0.07^{bc}	3.54 ± 0.13^{b}	4.28 ± 0.11^{a}
18:1n9	13.42 ± 0.97^{d}	14.36 ± 0.13^{c}	15.49 ± 0.22^{b}	15.35 ± 0.11^{b}	18.82 ± 0.09^{a}	18.74 ± 0.16^{a}	18.62 ± 0.37^{a}
20:1n9	1.07 ± 0.04^{de}	0.73 ± 0.04^{e}	1.21 ± 0.08^{cd}	1.37 ± 0.07^{bc}	1.75 ± 0.13^{b}	2.92 ± 0.07^{a}	3.21 ± 0.16^{a}
22:1n9	0.69 ± 0.10^{de}	0.45 ± 0.07^{e}	0.68 ± 0.06^{de}	1.16 ± 0.08^{c}	1.42 ± 0.06^{c}	2.68 ± 0.09^{b}	3.25 ± 0.05^{a}
18:2n6	1.17 ± 0.03^{f}	4.87 ± 0.11^{a}	3.92 ± 0.10^{bc}	3.61 ± 0.13^{c}	3.57 ± 0.05^{cd}	3.44 ± 0.06^{d}	2.25 ± 0.14^{e}
18:3n3	0.51 ± 0.07^{d}	2.74 ± 0.13^{bc}	2.85 ± 0.05^{ab}	2.97 ± 0.05^{a}	2.82 ± 0.27^{ab}	2.55 ± 0.12^{c}	2.57 ± 0.06^{bc}
20:4n6	2.58 ± 0.11^{d}	1.33 ± 0.04^{e}	2.69 ± 0.11^{cd}	2.88 ± 0.14^{cd}	3.16 ± 0.08^{bc}	3.7 ± 0.16^{a}	3.24 ± 0.08^{b}
20:5n3	1.38 ± 0.08^{e}	0.68 ± 0.07^{f}	1.64 ± 0.06^{e}	2.49 ± 0.05^{d}	3.13 ± 0.16^{c}	5.86 ± 0.08^{ab}	6.12 ± 0.03^{a}
22:5n3	0.73 ± 0.06^{f}	0.55 ± 0.10^{f}	1.03 ± 0.04^{e}	2.51 ± 0.09^{d}	4.68 ± 0.13^{c}	6.17 ± 0.14^{b}	6.54 ± 0.07^{a}
22:6n3	18.9 ± 0.93^{c}	0.37 ± 0.08^{g}	4.87 ± 0.09^{f}	9.53 ± 0.11^{e}	17.17 ± 0.25^{d}	24.14 ± 0.22^{b}	28.21 ± 0.13^{a}
ΣSFA	46.56 ± 1.27^{c}	61.26 ± 1.28^{a}	53.63 ± 0.63^{b}	45.77 ± 0.14^{c}	30.45 ± 0.48^{d}	17.24 ± 0.53^{e}	13.61 ± 0.29^{f}
ΣMUFA	22.66 ± 1.03^{f}	23.22 ± 0.37^{e}	24.70 ± 0.28^{de}	25.48 ± 0.36^{d}	30.34 ± 35^{c}	32.72 ± 0.13^{b}	33.59 ± 0.18^{a}
ΣPUFA	25.27 ± 0.92^{d}	10.54 ± 0.15^{g}	17.00 ± 0.18^{f}	23.99 ± 0.72^{e}	34.53 ± 0.28^{c}	45.86 ± 0.19^{b}	48.93 ± 0.20^{a}
Others FAs	5.51 ± 0.09^{a}	4.98 ± 0.12^{ab}	4.67 ± 0.11^{b}	4.76 ± 0.08^{b}	4.68 ± 0.14^{b}	4.18 ± 0.07^{cd}	3.87 ± 0.08^{d}

*Different superscript letters in the same row indicate significant difference ($p < 0.05$).

as PUFA predominant fractions, where the DHA were also found to be higher than any other fractions. The highest amount of PUFA (48.93%), especially omega-3 DHA (28.21%), was eluted at the last fraction (F6). Thus, F4 to F6 are considered as PUFA-rich fractions, where DHA, omega-3, and omega-6 fatty acids were predominant. The nonidentified fatty acids, defined as other fatty acids in this study, were also found, though they are in ignorable amounts (about 5 to 7% only) at all types of fractionation process such as fractionation.

In Fractionation Type-A (Table 2), about 8.7 and 25.2% DHA, 14.7 and 39.8% omega-3, and 5.7 and 7.0% omega-6 fatty acids were found at F1 and F2, respectively. Similarly, the DHA content of 5.7, 12.5, and 26.1%; omega-3 fatty acids of 11.8, 21.0, and 42.8%; and omega-6 fatty acids of 5.5, 6.1, and 8.3% were found at F1, F2, and F3, respectively, at Fractionation Type-B (Table 3). Whereas, in Fraction Type-C (Table 4), less amount of DHA was eluted at F1 (0.4%), F2 (4.9%), and F3 (9.5%) and drastically increased at F4 (17.2%), F5 (24.1%), and F6 (28.2%). Similarly, 4.3, 10.4, 17.5, 27.8, 38.7, and 43.4% of omega-3 fatty acids; and 6.2, 6.6, 6.5, 6.7, 7.1, and 5.5% of omega-6 fatty acids were eluted at F1, F2, F3, F4, F5, and F6, respectively. Therefore, F4 to F6 could be defined as the precise fraction of DHA, omega-3, and omega-6 PUFA. A much smaller amount of EPA and arachidonic acid (AA) existed in the head of tuna. At supercritical state, liquid CO_2 penetrated the sample matrix and dissolved more readily soluble fatty acid portion of the oil which was eluted during subsequent extraction steps. In this manner, six fractions were collected separately, among which the first three fractions contained mainly the SFA fish oil, and the following three fractions were predominant in PUFA, especially EPA and DHA.

However, the significant differences were found in SFA, MUFA, and PUFA (omega-3, omega-6, and others) in the fractional and cumulative yield. These findings are in agreement with Markom et al. (2001) who reported the solubility of crude palm oil in SC-CO_2 low for the triglycerides which are rich in SFA and have low molecular weight fatty acid constituents such as C12:0, C14:0, and C16:0. The authors also reported the first elution of low molecular weight and saturated fatty acid constituents followed by the higher and mainly unsaturated fatty acids—i.e., C18:1, C18:3, and C20:0 (Markom et al., 2001). Zaidul et al. (2006) applied SC-CO_2 to fractionate short and medium (C8 to

C14) and longer chain (C16–C18:2) fatty acid constituents in terms of triglycerides of palm kernel oil. The authors reported simultaneous extraction and fractionation up to the fourth fractions (F1 to F4). The shorter chain C8, C10, and C12 fatty acid constituents were found to be eluted in the first fraction (F1); whereas, the longer chain 16, C18:0, C18:1, and C18:2 fatty acid constituents were completely eluted at the fourth fraction (F4). The trends were also similar to our previous study where the fatty acids were extracted from the different parts of Indian mackerel separately using various SC-CO_2 techniques (Sahena et al., 2010b).

Osman et al. (2007) extracted total oil from Indian mackerel of Malaysian waters and reported 16.1, 19.3, 4.2, 36.5, 13.8, and 38.6% of EPA, DHA, AA, PUFA (omega-3), and PUFA (omega-6), respectively. The trends were similar to the findings of this study. Osman et al. (2001) also reported 33.4, 20.0, and 35.3% of omega-3 PUFA, omega-6 PUFA, and other PUFA, respectively, which was much greater than those of the SFAs (7.71%) and the MUFA (3.25%). Zuta et al. (2003) also determined the fatty acid compositions of Indian mackerel processing skin and reported a total of 24.9, 39.2, and 22.6% SFA, MUFA, and PUFA, respectively.

The lipid and fatty acid content of highly migratory tuna by-products was analyzed by Saito et al. (2005), and the lipid content of tuna species was generally found to be high with DHA constituents when water temperature was colder. The fatty acids composition in the viscera of migratory tuna species were DHA dominant unsaturated fatty acids comprising of 20% or more of the total fatty acids in the tissue lipids of the tuna by-products (Saito et al., 1999). The authors also reported changes in DHA level of triglycerides in *T. tongol* by-products, such as orbital 19.8 to 26.2%, liver 9.0 to 17.3%, and pyloric cecum 20.4 to 25.9% due to the changing of seawater temperature from 28 to 22°C, respectively (Saito et al., 2005). Tropical little tuna (*Euthynnus affinis*) head was found to have very low content of EPA (1.48%) and DHA (15.7%; Khoddami et al., 2012). Similar results were also found in non-precooked skipjack tuna heads oil processed by wet-reduction method, where EPA and DHA content were 0.1 and 18.8%, respectively (Chantachum et al., 2000). These results are in agreement with our study. The variation in fatty acid compositions in our study was probably due to differences in the raw material used and high seawater temperature due to the tropical climate in Malaysia, which has a lower content of EPA and DHA than the cold water tuna. However, the fatty acid profile of seawater fishes varied due to their prey lipids, temperature of seawater, breeding season, age, geographical location, and type of species (Saito et al., 2005; Çelik et al., 2005; Haliloğlu et al., 2003). For example, a marine planktonic diet results in low levels of omega-6 PUFA, of which EPA and DHA are the predominant fatty acids (Justi et al., 2003). Thus, marine fish are distinguished by having higher concentrations of omega-3 PUFA than the omega-6 PUFA since their diet consists largely of plankton (Sahena et al., 2010b).

Conclusion

Application of ethanol as a cosolvent of SC-CO_2 is highly effective to recover PUFA, especially DHA, omega-3, and omega-6 from fish by-products such as tuna head. Simultaneous extraction and fractionation of fish oil into several steps (i.e., six fractions) were more effective than either Fractions 2 or 3 separating PUFA rich fish oil. The later fractions, such as Fractions 4 to 6, were predominant in PUFA containing DHA, omega-3, and omega-6 fatty acid constituents; the last fraction (F6) was the richest PUFA fraction, with the highest amount of DHA, omega-3, and omega-6 fatty acids. Therefore, Fractions 4 to 6 could be regarded as DHA, omega-3, and omega-6 PUFA-rich fractions regardless of the existence of small amounts of either SFA or MUFA along with an ignorable amount of nonidentified fatty acid constituents. Further analyses are needed to study the physico-chemical and biochemical properties of these fractionated PUFA to claim precise fish oil products that might contain purified DHA, omega-3, and omega-6 fatty acid constituents.

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