



Quality Evaluation of Oil Recovered from By-products of Bigeye Tuna Using Supercritical Carbon Dioxide Extraction

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Abstract

Supercritical carbon dioxide (SE) and Soxhlet extractions with hexane (HE) were used to obtain oil from skins, scales and bones of bigeye tuna (*Thunnus obesus*). SE was done at a previously optimized conditions of 40° C and a pressure of 25 MPa with a gas-flow rate of 10 kg/h. By SE, 85.6, 83.2 and 87.7% of oil was recovered (extractability) from skins, scales and bones, respectively, considering the HE extraction to be the total oil. The oils obtained with SE and HE were evaluated for their physicochemical properties such as color, viscosity, acid value, peroxide value, free fatty acid value, fatty acid compositions and heavy metal content. The oils contained 27.7-31.5% polyunsaturated fatty acids including 24.7-28.3% eicosapentaenoic and docosahexaenoic acid. The extraction method did not significantly affect fatty acid composition ($P>0.05$) except for EPA and DHA. SE significantly ($P\leq 0.05$) reduced the heavy metal content of the oil. The color and viscosity were better by SE than HE. The acid, peroxide and free fatty acid values were also lower by SE than HE, suggesting that SE may be a potential commercial way to get a high qualified oil.

Keywords: Tuna, *Thunnus obesus*, Supercritical extraction, Carbon dioxide.

Introduction

Fish has long been regarded as a valuable source of high-quality food in the human diet due to presence of long-chain polyunsaturated fatty acids (PUFA), especially omega 3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids have various health benefits and are used in the prevention and treatment of coronary heart disease, blood platelet aggregation, hypertension, abnormal cholesterol levels, diabetics, arthritis, mental illness, autoimmune disorders and cancer (von Schacky, 2003; Kim & Mendis, 2005). It is recommended to increase the consumption of omega-3 fatty acids to maintain an omega-6/omega-3 ratio between 5:1 and 10:1 in the diet (FAO/WHO, 1994). This ratio is not maintained in most diets except those which are characterized by high consumption of fishes. The western diets are rich in saturated fatty acids and the ratio of omega-6/omega-3 is much higher than recommended (Fernández & Juan, 2000). Extracted fish oil could be a good source of omega-3 fatty acids, which can be used to supplement the diet.

Fish processing industries generate large amounts of wastes or by-products, as much as 70% of the original materials, in the form of skins, scales, bones, viscera, gills, dark muscles and heads

(Guerard, Guimas, & Binet, 2002). These waste by-products have traditionally been discarded as waste or used as low-value by-products like animal feed, fertilizers, etc. The discarded materials often create troublesome health, environmental and waste disposal problems. Proper utilization of these fishery by-products is important to make the processing industries sustainable as it also increases the overall value of the catch, reduces the cost of processing waste disposal or treatment, and ultimately lowers environmental pollution.

Several methods have been applied for extracting fish oils from whole fish or fish by-products. The Soxhlet extraction method involves the use of large amount of hazardous solvent and requires a lot of energy. Besides, fish oils are oxidized with the relatively high temperature and long time needed. Another common method is wet reduction, which involves three basic steps: cooking at high temperatures (95-100° C), pressing and centrifuging (Food and Agricultural Organization (FAO), 1986). This process can produce high volumes of crude fish oil although subsequent refining steps are required to make the oil edible.

Recently, supercritical fluid extraction has been widely employed as an alternative to organic solvent extractions including fish oil (Rubio-Rodriguez *et al.*,

2008; Jung, Kang, & Chun, 2012). This method allows extracting oil in an oxygen free situation with moderated temperatures and short extraction times, which reduces oxidation. Carbon dioxide (CO₂) is probably the most widely used supercritical fluid because of its critical temperature (31.1° C), which makes it an ideal solvent for extracting heat sensitive materials. It is also an inert gas, which is non-toxic, non-flammable and available at low cost. Another advantages of supercritical CO₂ (SC-CO₂) is that it can selectively extract low polar lipid compounds, avoiding the co-extraction of polar impurities such as some inorganic derivatives with heavy metals (Rubio-Rodriguez *et al.*, 2012). Furthermore, after SC-CO₂ extraction of oil, the de-oiled portion of the by-products could be used as a raw material for the extraction of collagen, which may facilitate another value addition.

Tuna are an economically important and widely distributed fish species, with an estimated harvest of 7.7 million metric tonnes globally in 2014 (FAO, 2016). Big eye tuna is usually processed for sashimi or canned. Both formats use only the white meat, resulting in an abundance of by-products or wastes (Guerard *et al.*, 2002; Herpandi, Rosma, & Wan Nadiyah, 2011). No published research was found that evaluated big eye tuna by-product oils extracted by SC-CO₂. Therefore, the objective of this study was to evaluate the quality of tuna fish oils extracted from the skins, scales and bones of this fish using SC-CO₂ compared with the oil extracted by hexane extraction method.

Materials and Methods

Preparation of Raw Materials

Big eye tuna (*T. obesus* Lowe, 1839) with an average size of 35±5 kg were harvested from the Pacific Ocean by Dongwon Fisheries Co., Ltd., Busan, Korea using long lines. The ungutted fish were stored at -60° C in ultra-cold storage immediately after catching and then brought to the processing plant within 6-8 wk. The skins with scales and bones with some attached meat, produced as by-products in the processing plant, were transported to the laboratory in an insulated box within one h. The samples were washed thoroughly with cold distilled water (4° C) and freeze dried for 72 h using an EYELA FDU-2100 freeze-drier (Rikakikai Co., Ltd., Tokyo, Japan). After drying, skins, scales and bones were separated manually and ground using an electric blender (Hanil, HMF-3260S, 2000 mL, Seoul, Korea) at maximum speed, screened using 1 mm sieve (No 18, Chung Gye Sang Gong Sa, Seoul, Korea), and then stored at -20° C until used for the extraction of oil for a maximum of three months. Before freeze-drying, a portion of skins, scales and bones were also separated manually, air-dried for 24 h, ground and the proximate

composition, moisture, protein and ash content, was determined according to the methods of the Association of Official Analytical Chemists methods 925.04, 981.10, and 938.08, respectively (AOAC, 1995). Lipid content was measured by conventional Soxhlet extraction method using hexane as stated below.

SC-CO₂ Extraction (SE)

A laboratory scale supercritical fluid extraction (SFE) system, designed and assembled by Food Science and Technology Department in Pukyong National University was used in this study. Forty g of freeze-dried ground skins or 100 g of scales or bones powders were loaded into a 200 mL stainless steel extraction vessel. A thin layer of cotton was placed at the bottom and top of the sample in the vessel before closing. A high pressure pump (Milroyal, Milton Roy, Ivyland, PA, USA) was used to pump CO₂ into the extraction vessel at the desired pressure. The CO₂ pressure was controlled by a back pressure regulator (BPR). The extraction temperature was maintained by connecting water baths to the extraction vessel and the separator. A gas flow meter (Shinagawa, DC-1, Tokyo, Japan) was used to measure the CO₂ consumed during the extraction process. The extraction was carried out under a previously determined set of optimal extraction conditions (pressure 25 MPa, temperature 40° C, flow rate 10 kg CO₂/h) (Rubio-Rodriguez *et al.*, 2008, 2012). The extracted oil was collected from the separating vessel and stored at -20° C in brown glass bottle with nitrogen gas (99.99% purity, Daesung Industrial Co., Ltd., Seoul, Korea) until analysis, a maximum of two months. The total oil yield and extractability were expressed as a percentage as defined below:

$$\text{Yield (\%)} = \frac{\text{Oil extracted (g)}}{\text{Weight of sample (g)}} \times 100$$

$$\text{Extractability (\%)} = \frac{\text{Total yield (g) by SCCO}_2 \text{ extraction}}{\text{Total yield (g) by Soxhlet extraction}} \times 100$$

Soxhlet Extraction by Hexane (HE)

Oil was extracted using the method of Ferdosh *et al.* (2015) with slight modifications. About 5 g of freeze-dried ground skins (or 15 g of bones or scales) were put into a thimble and extracted with 100 mL n-hexane (97.8% purity, SK Chemicals, Gyeonggi-do, Korea) in a Soxhlet apparatus (64825 Supelco, Sigma Aldrich, St. Louis, MO, USA). The flask was heated at 65° C using a heating mantles (Wisd, Daihan Scientific Co., Ltd., Gangwon-do, Korea) for 16 h. The extracted oil was evaporated under vacuum at 50° C using an Eyela N-1100 rotary evaporator (Tokyo, Japan) and then placed in an oven at 40° C for 1 h before being transferred into a desiccator before

reweighing.

Evaluation Of Oil Quality

Color

Color of oil samples was measured using a Lovibond RT Series, Portable Reflectance spectrophotometer (Amesbury, UK). The instrument was calibrated with a black and a white tile. The oil samples were in a 1 cm path-length glass cuvette and L^* , a^* and b^* indicating lightness/brightness, redness/greenness and yellowness/blueness, respectively, were recorded by the instrument's software. Total difference in color (ΔE^*) was calculated using the following equation (CIE, 1976).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Where, ΔL^* , Δa^* and Δb^* are the differences between the corresponding color parameter of the sample and that of the white standard ($L^* = 93.83$, $a^* = -1.06$, $b^* = -0.15$).

Viscosity

Viscosity of oils was measured using a viscometer (Brookfield DVII+Pro EXTRA, Middleboro, MA, USA), with a small sample adapter, which permits the use of only 10 mL of oil in each analysis. The spindle LV2 (62) at 100 rpm was used. Temperature was controlled using a water bath at 25° C with a precision of $\pm 2^\circ$ C. The torque range was between 15 to 25%.

Oil Stability Analysis

Acid values (AV), peroxide values (POV), free fatty acid (FFA) values and *p*-anisidine values (PAV) were determined according to the American Oil Chemists' Society (AOCS) official methods Cd 3d-63, Cd 8-53, Ca 5a-40 and Cd 18-90, respectively (AOCS, 2006). Total oxidation value (TOTOX) was calculated as twice the peroxide value plus the *p*-anisidine value (Deepika *et al.*, 2014).

Fatty Acid Composition Analysis

Gas chromatography (GC) analysis was done to determine the fatty acid composition of tuna by-products oils using the method Haque, Asaduzzaman, and Chun (2014). The analysis was carried out using a 6890 Agilent (Agilent Technologies, Wilmington, NC, USA) gas chromatograph with a fused silica capillary column (100 m length x 0.25 mm internal diameter, 0.2 μ m of film) (Supelco, Bellefonte, PA, USA). Fatty acid methyl esters were prepared according to official methods and recommended practices of the AOCS Ce 2-62 (AOCS, 2006). Oven temperature was programmed to start with a constant

temperature of 130° C for 3 min, then increased to 240° C at a rate of 4° C/min and then held at 240° C for 10 min. The temperature of both injector and detector was 250° C. Fatty acid methyl esters were identified by comparing the retention time with a standard fatty acid methyl ester mixture (Supelco). Individual fatty acid with known concentration in the standard gave a response for the area of that fatty acid. Then, the response for that particular area (under each peak) assuming a linear response to the amount of that fatty acid was used for the determination of unknown fatty acid and expressed as mg/100 mg of oil (%).

Determination of Metal Content

Heavy metals content was determined in raw materials and oil samples by using an ICP-OES optima 2000DV (Perkin Elmer, Shelton, CT, USA) equipped with winLab32 software. The instrument's operating conditions were followed as described by Kumaravel and Alagusundaram (2014) with slight modifications. Solid powders and oil samples were prepared by acid digestion using the method of Agemian, Sturtevant, and Austen (1980) and Rubio-Rodriguez *et al.* (2012), respectively, with some modifications. For skins, scales and bones, 0.9 g of powder was put into Teflon tubes (T16-64-789, LKLABKOREA Inc., Seoul, Korea), followed by addition of 9 mL of 67% sulfuric acid (purity 95%, CAS 7664-93-9, Junsei Chemical Co., Ltd., Tokyo, Japan) and 65% nitric acid (purity 95%, CAS 7697-37-2, Junsei Chemical Co., Ltd.). For oil samples, 0.9 g oil was mixed with 18 mL of 65% nitric acid in the Teflon tubes. In addition, for the analysis of Hg, 8 mL of 5 ppm AuCl₃ solution (379948, Sigma Aldrich) was added to stabilize the Hg (Briscoe, 2015). The mixtures were heated on a graphite block (YKM-36, YLK, Hunan, China) at 200° C for approximately 12-18 h until digestion was completed. The sample was allowed to cool and transferred into a 100 mL volumetric flask. The sample was brought to the mark with 2% sulfuric acid for ICP-OES analysis. The heavy metals were detected at 253, 193, 220, 206, 238, 327, 228 nm for Hg, As, Pb, Zn, Fe, Cu and Cd, respectively. The metals were quantified using a calibration curve for each metal. Each calibration curve was obtained using three standard concentration of each standard solution in the range 0.1-1 ppm for Hg, As, Pb, Cd and 1 -10 ppm for Cu, Zn, Fe. All standard solutions were made of pure metal dissolved in 2% nitric acid except As and Pb which were made of As₂O₃ and Pb(NO₃)₂, respectively. All standards used for this test were from Sigma Aldrich (Cat. no. 43149, 68921, 18562, 01969, 36379, 28941 and 41318 for Fe, Cu, Zn, As, Cd, Hg and Pb, respectively). A reagent blank solution was run in parallel and its value subtracted from the sample results.

Statistical Analysis

Values are presented as the mean \pm standard deviation of triplicate determinations. Statistical analysis was carried out using one-way analysis of variance (ANOVA) using SPSS software (version 18.0 software, SPSS Inc., Chicago, IL, USA). Significant differences between means were determined using Duncan's Multiple Range test and $P \leq 0.05$ was regarded as significant.

Results

Proximate Composition of Tuna by-Products

Proximate composition of tuna skins, scales and bones is shown in Table 1. Skins contained the

highest amount of lipid (25.9%), followed by scales (23.0%) and bones (14.5%) on a dry weight basis. A significant amount of ash was found in scales ($26.1 \pm 0.3\%$) and bones ($26.3 \pm 0.3\%$) compared to skins ($4.54 \pm 0.2\%$). Protein content was higher in the skins than scales and bones. Scales contained the lowest amount of moisture ($2.8 \pm 0.1\%$) in the freeze-dried samples.

Extraction of oil

Total oil was extracted from skins, scales and bones of tuna using both SE and HE (Figure 1). Significantly lower ($P \leq 0.05$) amounts of oil were extracted from bones by both of the extraction methods. The amount of oil extracted by HE was higher than that of SE ($P \leq 0.05$) for all samples. SC-

Table 1. Proximate composition of Bigeye tuna by-products

Body part	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Moisture (%) in freeze-dried sample
Skin	62.3 ± 0.9	25.1 ± 0.3	9.74 ± 0.1 ($25.9^* \pm 0.9$)	1.56 ± 0.1	4.54 ± 0.2
Scale	40.6 ± 0.4	19.0 ± 0.3	13.7 ± 0.1 ($23.0^* \pm 0.7$)	26.1 ± 0.3	2.80 ± 0.1
Bone	45.3 ± 0.5	21.0 ± 0.2	7.92 ± 0.1 ($14.5^* \pm 0.9$)	26.3 ± 0.3	4.49 ± 0.2

Values are expressed as mean \pm standard deviation from triplicate determination. *Dry weight basis

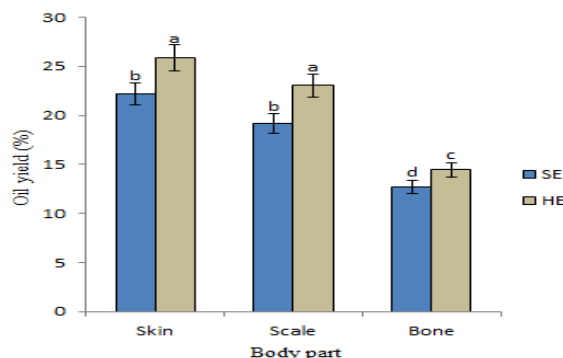


Figure 1. Yield of oil in skins, scales and bones extracted by SE and HE. Means \pm SD (n = 3). Different small letters in each column bar indicate significant differences ($P \leq 0.05$).

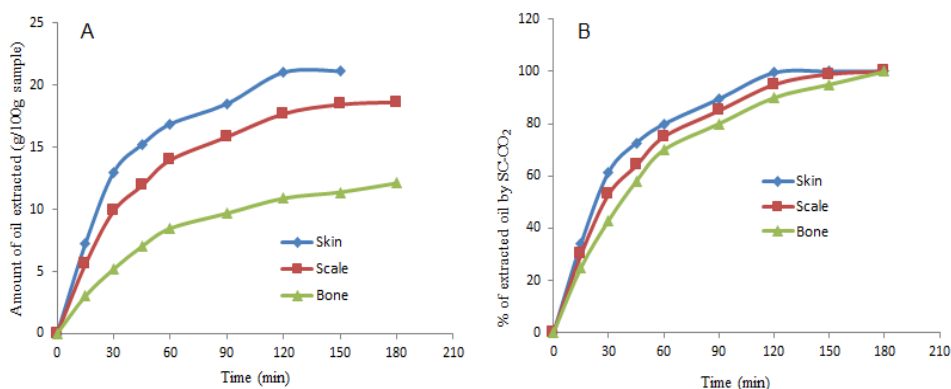


Figure 2. SC-CO₂ extraction curves (A) obtained by weighing the oil extracted with time (B) progression (%) of oil extraction with time.

CO₂ oil extraction patterns are shown in Figure 2. About 50% of the oil was extracted within the first 30 min. Then, the rate of extraction decreased down with time and became constant after 180 min (Fig 2B).

Color

The color of different oil samples was significantly different ($P \leq 0.05$) as shown in Table 2. All SC-CO₂ extracted oils (SEO) showed higher L*-value (lightness) than those of hexane extracted oils (HEO), where a*-values (redness) were higher in HEO. Among all the samples, the SEO of skins showed the lowest ΔE_n (67.6).

Viscosity

All SEO showed significantly ($P \leq 0.05$) lower viscosity than HEO. The viscosity ranges from 41.4 to 53.6 cP (Centipoise) for by-products' oil of tuna (Table 2).

Oil Stability Analysis

Acid value (AV), peroxide value (PV), free fatty acid value (FFA), *p*-Anisidine value (PAV) and TOTOX values of SEO were significantly lower ($P \leq 0.05$) than that of HEO (Table 2). AV for SEO of skins, scales and bones were 5.1 ± 0.2 , 4.2 ± 0.2 and 4.0 ± 0.1 mg KOH/g, respectively, while they were 7.4 ± 0.2 , 5.7 ± 0.2 and 6.5 ± 0.2 mg KOH/g, respectively, for HEO. The highest AV was seen for HEO of skins and the lowest value was seen for SEO of bones. PV and FFA had a narrow range - 2.3 to 3.7 (meq/kg) and 0.7 to 1.7%, respectively. TOTOX values for SEO of skins, scales and bones were 9.9 ± 0.2 , 9.0 ± 0.2 and 7.8 ± 0.2 , respectively, while they were 13.6 ± 0.3 , 13.6 ± 0.3 and 14.0 ± 0.3 , respectively, for HEO.

Fatty Acid Composition

Fatty acid composition of the fish oils is shown

in Table 3. A total of 23 fatty acids were identified in the oil of tuna by-products. Mono-unsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were most abundant in all samples, followed by saturated fatty acids (SFA). Significant differences ($P \leq 0.05$) of SFA and MUFA were found with different respective body parts irrespective of the extraction methods. Total SFA was the highest in scales (31.5-32.9%), followed by bones (27.2-27.9%) and skins (24.1-25.8%). On the other hand, total MUFA and PUFA were highest in scales. Total EPA and DHA content were also at the highest level in scale oil. Although, the fatty acids composition generally did not vary based on the extraction methods, the SEO contained significantly higher amounts of EPA+DHA in skins, scales and bones samples. The dominant fatty acids in all the oils were DHA, followed by oleic acid and palmitic acid. The tuna by-products contained a considerable amount of EPA+DHA (24.7-28.3%), showing the highest amount in scales (28.3%). DHA is the major constituent of the PUFAs in tuna by-products oil, contributing about 74% of PUFAs, where EPA contributes only about 14%. The n-6/n-3 ratio was quite low in the oil (0.08 to 0.18). The SEO showed lower values than HEO.

Heavy Metal Content in Raw Materials and Oil

A total of seven heavy metals were studied in this experiment (Table 4). Zn and Fe were detected in all the raw materials and their respective oils. Although As was detected in all the raw materials, it was only detected in the HEO of skins. Pb and Hg were not detected in any raw materials and oils. Cu and Cd were only detected in skins at trace amounts.

Discussion

The proximate composition of big eye tuna was similar to other types of tuna that had been studied by Karunarathna and Attygalle (2010), who studied the proximate composition of various body parts of five

Table 2. Evaluation of oil quality

Parameters	Skins		Scales		Bones	
	SEO	HEO	SEO	HEO	SEO	HEO
AV (mg KOH/g)	5.1 ± 0.2^c	7.4 ± 0.2^a	4.2 ± 0.2^d	5.7 ± 0.2^c	4.0 ± 0.1^d	6.5 ± 0.2^b
PV (meq/kg)	2.8 ± 0.1^c	3.6 ± 0.2^a	2.6 ± 0.1^c	3.5 ± 0.2^b	2.3 ± 0.1^d	3.7 ± 0.1^a
FFA (%)	1.4 ± 0.1^b	1.7 ± 0.1^a	0.7 ± 0.03^d	0.8 ± 0.04^c	0.7 ± 0.04^d	0.8 ± 0.05^c
PAV	4.2 ± 0.1^b	6.3 ± 0.2^a	3.8 ± 0.1^c	6.5 ± 0.2^a	3.2 ± 0.1^c	6.6 ± 0.2^a
TOTOX value	9.9 ± 0.2^c	13.6 ± 0.3^b	9.0 ± 0.2^{cd}	13.6 ± 0.3^b	7.8 ± 0.2^d	14.0 ± 0.3^a
L	28.9 ± 0.2^a	21.8 ± 0.1^d	26.5 ± 0.1^b	19.2 ± 0.4^e	25.3 ± 0.1^c	12.2 ± 0.1^f
a*	-1.93 ± 0.1^d	$+3.13 \pm 0.1^b$	-2.98 ± 0.1^e	$+1.05 \pm 0.01^c$	-3.39 ± 0.1^f	$+8.44 \pm 0.1^a$
b*	18.6 ± 0.1^a	12.8 ± 0.1^c	8.91 ± 0.1^f	12.1 ± 0.3^d	10.7 ± 0.1^e	14.0 ± 0.3^b
ΔE^*	67.6 ± 0.5^f	73.3 ± 0.2^c	67.9 ± 0.4^e	75.6 ± 0.6^b	69.4 ± 0.4^d	83.4 ± 0.5^a
Viscosity (cP)	41 ± 1^c	48 ± 1^b	43 ± 1^c	52 ± 1^a	42 ± 1^c	54 ± 1^a

Values are expressed as mean±standard deviation from triplicate determination. Different superscript lowercase letters within columns indicates statistical significant difference ($P \leq 0.05$). SEO, Supercritical carbon dioxide extracted oil; HEO, Hexane extracted oil.

Table 3. Fatty acid composition of bigeye tuna by-products

Fatty acids (%)	Skins		Scales		Bones	
	SEO	HEO	SEO	HEO	SEO	HEO
Myristic Acid (14:0)	2.1	2.3	2.3	3.1	2.3	2.1
Tridecanoic acid (13:0)	ND	ND	3.3	0.6	1.6	ND
Pentadecanoic acid (15:0)	0.8	0.7	0.9	1.2	0.6	1.0
Palmitic acid (16:0)	15.4	14.3	18.0	16.3	15.9	15.3
Stearic acid (18:0)	3.9	3.5	3.9	5.1	5.1	4.8
Arachidic acid (20:0)	0.7	0.6	0.7	0.8	0.5	0.7
Behenic acid (22:0)	ND	ND	0.6	0.7	ND	0.7
Tricosanoic acid (23:0)	2.9	2.7	3.1	3.1	1.9	2.6
∑ SFA	25.8	24.1	32.9	31.5	27.9	27.2
Palmitoleic acid (16:1)	4.9	4.3	4.9	5.1	3.5	3.9
Cis-10 Heptadecanoic acid (17:1)	1.2	1.0	1.3	1.3	0.7	1.1
Elaidic Acid (18:1n-9t)	0.7	2.4	2.3	0.75	2.8	1.5
Oleic Acid(18:1n-9c)	20.9	19.4	20.2	19.7	19.5	18.1
Eicosenoic acid (20:1n-9)	1.9	1.7	2.1	2.2	1.9	2.3
Erucic acid (22:1n-9)	ND	ND	ND	0.6	ND	0.6
Nervonic acid (24:1n-9)	1.0	0.8	0.8	1.2	0.9	1.0
∑ MUFA	30.6	29.6	31.6	30.8	29.3	28.5
Linoleic acid (18:2n-6c)	1.5	1.5	1.3	1.5	1.5	1.5
Alpha-Linolenic acid (18:3n-3)	ND	ND	ND	0.5	ND	0.7
Gamma-Linolenic acid (18:3n-6)	ND	ND	0.6	0.7	0.8	0.6
Eicosadienoic acid (20:2n-6)	0.8	0.8	0.7	1.3	0.9	1.1
Eicosatrienoic acid (20:3n-6)	ND	ND	ND	ND	0.9	0.6
Docosadienoic acid (C22:2n-6)	ND	ND	0.6	0.7	ND	0.7
EPA(20:5n-3)	4.2	3.6	4.8	4.5	5.1	4.7
DHA (22:6n-3)	23.6	21.8	23.5	21.5	21.6	20.0
∑ PUFA	30.1	27.7	31.5	30.7	30.8	29.9
∑ EPA+DHA	27.8	25.4	28.3	26.0	26.7	24.7
∑ FA	86.5	81.4	96.0	93.0	88.0	85.6
n-6/n-3 ratio	0.08±0.02	0.09±0.0	0.11±0.01	0.16±0.02	0.15±0.01	0.18±0.02

Values are expressed as mean from triplicate determination. SFA, saturated fatty acids; MUFA, mono unsaturated fatty acids; PUFA, Poly-unsaturated fatty acids; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acids; FA, fatty acid; SEO, supercritical extracted oil; HEO, hexane extracted oil; ND, not detected.

Table 4. Heavy metals content in fish oils extracted by SE and HE

Heavy metal (mg/kg)	Skins			Scales			Bones		
	Raw*	SEO	HEO	Raw*	SEO	HEO	Raw*	SEO	HEO
Arsenic (As)	4.6±0.0	ND	4.7±0.1	2.4±0.04	ND	ND	2.2±0.04	ND	ND
Lead (Pb)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cadmium (Cd)	0.1±0.01	ND	ND	ND	ND	ND	ND	ND	ND
Mercury (Hg)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Copper (Cu)	1.0±0.1	0.4±0.4	1.1±0.4	ND	0.3±0.2	0.9±0.02	ND	0.05±0.02	0.2±0.1
Zinc (Zn)	128±3	1.4±0.2	7.6±0.4	79.8±1	1.9±0.0	3.2±1	80±0.2	2.3±1	2.7±2
Iron (Fe)	25±1	8.2±2	15.7±1	14.5±0.3	0.9±0.2	11.2±1	26.8±2	0.7±0.5	3.4±1

Values are expressed as mean±standard deviation from triplicate determination. SEO: Supercritical CO₂ extracted oil; HEO: Hexane extracted oil; ND: Not detected. *Freeze-dried sample.

different tuna although their study did not include big eye tuna. However, proximate composition of fish may vary with species, size, sex, season and geographic harvest area (Selmi & Sadok, 2010). The yield of extracted oil may vary between traditional solvent extraction methods and SE. Ferdosh *et al.*

(2015) extracted 26.4% and 24.8% oil using HE and SE, respectively, from the skins of tuna (*Euthynnus affinis*), which was similar to the current results. Fish oil (11.7% from wet weight) was produced commercially from anchovy (Ceyhan & Emir, 2015). In this study, SE was done under the single condition

previously optimized by Rubio-Rodriguez *et al.* (2008). The extractability with SE was 85.6, 83.2 and 87.7% for skins, scales and bones, respectively. This result is a little higher than that of Haque *et al.* (2014), in which they reported extractability as 78.0% for mackerel muscle when they compared HE and SE. The higher extractability might be attributed to optimum conditions used SE and/or nature of sample. The yield using SE has been reported to depend on temperature, pressure, type of sample, size of sample and moisture content in the sample (Rubio-Rodriguez *et al.*, 2008; 2012). The higher extraction yield using HE might be due to extraction at high temperature for a longer period of time, which facilitates having some protein and other compounds in the extracted oil (Ferdosh *et al.*, 2015).

In SC-CO₂ oil extraction patterns (Fig 2), oil extraction rate was higher at initial stage that was decreased gradually with the time elapse. A similar pattern for SE extraction curves was reported by Rubio-Rodriguez *et al.* (2008). At the beginning of the extraction, a higher level of accessible oil reduces the internal mass transfer resistance, thus increases the rate of extraction yield. After the extraction of most accessible oil, the rate of extraction slows due to the higher internal mass transfer resistance (Rubio-Rodriguez *et al.*, 2008; 2012). The skins were most readily extracted using SE, followed by scales and bones (Fig 2A). This was probably due to of the different internal mass transfer resistances of the different samples.

Oil color is a parameter that can be an indication of purity and is also important to consumers. The lowest value of ΔE_n (67.6) was found in the SEO of skins, which coincided with the highest lightness (L^* -value). The darker color of HEO indicated the existence of more impurities and possibly oxidative products. It also costs more to refine darker crude oil to achieve an acceptable light-colored product (Noriega-Rodriguez *et al.*, 2009; Suseno, Tajul, Nadiyah, & Noor, 2012).

Viscosity of oil can be affected by the impurities, which include free fatty acids, proteins, pigments, moisture and volatile flavors (Wiedermann, 1981). Higher viscosity generally indicates a lower purity of fish oil. The viscosity of oil can be affected by impurities in the oil, and the oil's density, melting point, degree of unsaturation and temperature (Zahir, Saeed, Hameed, & Yousuf, 2014; Suseno, Yang, Nadiyah, Abdullah, & Saraswati, 2015). The viscosity of sardine oil obtained from fish meal was reported as 51.7 cP at 25° C (Suseno *et al.*, 2015). Farag and Basuny (2009) reported that the viscosity of sunflower oil decreased after using different absorbents during refining.

AV is the indicator of free fatty acids in the oil. Results showed that the acid values of the extracted oil ranged from 3.9 to 7.4 mg KOH/g, which was within the acceptable limit of 7-8 mg KOH/g (Deepika *et al.*, 2014). However, all SEO showed a

significantly lower value ($P \leq 0.05$) than that of HEO. The lower acid value of SEO might be due to extraction at a lower temperature for a shorter time. Wrolstad, Durst, and Lee (2005) reported that the acid value of fish oil depends on oil composition, extraction conditions, sample preparation and freshness of raw materials. Low AV indicates the freshness and suitability of edible oil while the higher value is associated with the rancidity caused by hydrolysis of ester bonds and oxidation of double bonds (Das, Chakraborty, Das, Bhattacharjee, & Das, 2016).

The PV is an indicator of the oxidation state of the lipids by measuring hydroperoxides as a primary oxidative product. The acceptable limit of peroxide value set by the Global Organization for EPA and DHA (GOED) and FAO for human consumption is ≤ 5 meq/Kg (FAO/WHO, 2013). The results from the present study showed that the peroxide value for all sample was within the recommended level. However, the SEO showed a lower value of peroxide, again indicating less primary oxidation. Several factors such as fatty acid composition, the concentration of oxygen, the presence of light and antioxidants influence the formation of hydroperoxides and degradation into secondary oxidation products (Sullivan & Budge, 2010; Ritter, 2012). The hydroperoxides can readily be converted into aldehyde, ketones, esters, acids, alcohols and short-chain fatty acids with heating and the presence of metals. Chantachum, Benjakul, and Sriwirat (2000) found that oil extracted with higher heat treatments showed higher peroxide values. Deepika *et al.* (2014) reported that the iron-containing protein denatures during heat treatment and releases Fe, which induces lipid oxidation.

The FFA content is an important quality parameter for oils, indicating the degree of hydrolysis. The lower FFA content indicates higher quality and lower oxidation. However, the acceptable limit of FFA in an edible crude fish oil is suggested as 2–5% FFA (Young, 1985). The FFA in all the samples studied was within the acceptable limit, which indicated that no significant lipid degradation occurred with either extraction method. The lower FFA in SEO might be attributed to the lower extraction temperature for a shorter period of time. Low FFA content in oil (0.6–1.2%) was seen by Aryee and Simpson (2009) when they extracted salmon oil by using different solvents for different periods of time.

Secondary oxidation products are determined by PAV. However, all samples meet the standard of PAV (≤ 20) for oil set by the GOED and FAO for human consumption (FAO/WHO, 2013). The higher PAV of HEO might again be attributed to harsher extraction conditions. Similar results were also reported by Lee, Uddin, and Chun (2008) for mackerel viscera.

The TOTOX is a parameter used to determine the content of various primary and secondary

oxidative products such as hydroperoxides, aldehydes and ketones, which are produced by the degradation of polyunsaturated fatty acids under pro-oxidant conditions including high temperatures, oxygen, light and metal compounds. The acceptable limit of TOTOX value for all samples in the study was far below (7.8-14.0) the allowable limit (≤ 26) for human consumption set by FAO (1995).

The fatty acid composition showed significant differences ($P \leq 0.05$) for SFA, MUFA and PUFA with different body parts in the study by Karunarathna and Attygalle (2010). SEO contained significantly ($P \leq 0.05$) higher amount of EPA+DHA than HEO in the skins, scales and bones in this study. This results was in agreement with other studies (Rubio-Rodriguez *et al.*, 2008; Amiguet *et al.*, 2012; Lee, Asaduzzaman, Yun, Yun, & Chun, 2012). The dominant fatty acids in tuna by-products oil were DHA, followed by oleic acid and palmitic acid, which was almost the same as the results of other studies (Chantachum *et al.*, 2000; Deepika *et al.*, 2014). The tuna by-products contained considerable amount of EPA+DHA, 28.3% in scales, 27.8% in skins and 26.7% in bones. Ferdosh *et al.* (2015) reported 17.9-19.0% EPA+DHA in skins oil of three kinds of tuna. The difference might be due to the type of tuna, location of harvesting area and differences in extraction conditions. EPA+DHA content in skins oil of tuna was higher than that of mackerel (26.4%), salmon (17.3%), golden snapper (26.0%), threadfin breams (26.8%) (Osman, Jaswir, Khaza'ai, & Hashim, 2007; Sahena *et al.*, 2010; Deepika *et al.*, 2014). DHA is the main PUFA in tuna by-products, contributing about 74% of the PUFA. Other studies support these findings (Saito *et al.*, 2005; Ferdosh *et al.*, 2015).

Nowadays, there is an increasing concern about the n-6/n-3 ratio since the amount of n-6 fatty acid in the western diet has increased by several folds rapidly (Simopoulos, 2008). An n-6/n-3 ratio above 4 has been reported to lead to various harmful effects on human health including cardiovascular disease, and being pro inflammation, cancer, and obesity. (Karunarathna & Attygalle, 2010; William *et al.*, 2011; Simopoulos, 2016). On the other hand, a lower n-6/n-3 ratio has positive effect to prevent those diseases (Simopoulos, 2008; Mansara, Deshpande, Vaidya, & Kaul-Ghanekar, 2015). Results from the present study indicate that all the extracted oils had a very good n-6/n-3 ratio, with SEO having a lower ratio than HEO.

Heavy metals are regarded as the most serious pollutants in the ocean and have a tendency to accumulate in different organs of fish (Zhao *et al.*, 2012). Among the heavy metals, As, Pb, Cd and Hg are considered most harmful when consume above their toxicity levels. Heavy metals were lower in SEO than HEO (Table 4). Results indicated that SE significantly reduced the heavy metal content in oil

compared to HE due to its higher selectivity for non-polar compounds. This result was in agreement with Rubio-Rodriguez *et al.* (2012). As, which is one of the heavy metals of most concern due to its detrimental effect on public health, was found beyond its acceptable limit (0.1 ppm) in HEO of skins (FAO/WHO, 1995); however, it was not detected in the SEO of skins, or the SEO/HEO of scales and bones. The conditions for SC-CO₂ extraction and type of samples have been reported to have an influence on heavy metal content in oil (Hajeb *et al.*, 2014). Cu, Zn and Fe are considered as essential minerals for human, but these minerals might have toxic effect at higher doses (Albretsen, 2006; Singh, Gautam, Mishra, & Gupta, 2011). Besides the toxic effect on health, Fe and Cu act as pro-oxidants that catalyzes fat oxidation, Cu being 10X more active than Fe (FAO, 1986). A further problem with Fe is that when S is also present, a darkening of the oil color frequently occurs during deodorization. In this study, Fe and Cu content was less in SEO than HEO. Therefore, SE can produce a safe edible fish oil from skins, scales and bones in terms of heavy metal content and being less prone to oxidation.

The result presented in this study show that SE provided a better quality oil in terms of its physical properties such as color and viscosity, and oxidative stability such as AV, PV, FFV, PAV, TOTOX values and fatty acid compositions. The SE also reduced the heavy metal content of the oil. Therefore, SE can be applied to extract oil from fish by-products to increase the value of tuna wastes.

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References

- Agemian, H., Sturtevant, D.P., & Austen, K.D. (1980). Simultaneous Acid Extraction of Six Trace Metals from Fish Tissue by Hot-block Digestion and Determination by Atomic Absorption Spectrometry. *Analyst*, 105(1247), 125-130. <http://dx.doi.org/10.1039/AN9800500125>
- Amiguet, V.T., Kramp, K.L., Mao, J., McRae, C., Goulah, A., Kimpe, L.E., Blais, J.M., & Arnason, J.T. (2012). Supercritical Carbon Dioxide Extraction of Polyunsaturated Fatty Acids from Northern Shrimp (*Pandalus borealis* Kreyer) Processing By-products. *Food Chemistry*, 130(4), 853-858. <http://dx.doi.org/10.1016/j.foodchem.2011.07.098>
- AOAC. (1995). Official Methods of Analysis of Association of Official Analytical Chemists. Washington, DC, USA. Association of Official Analytical Chemists, 1141 pp.
- AOCS. 2006. Official Methods and Recommended Practices of the American Oil Chemists' Society,

- Champaign, Illinois, USA, American Oil Chemists' Society.
- CIE, 1976. International commission on illumination, Colorimetry (Report no. 15). Bureau Central de la CIE, Paris, France.
- Albretsen, J. (2006). The Toxicity of Iron, An Essential Element. *Veterina Medicine*, 82–90.
- Aryee, A.N.A., & Simpson, B.K. (2009). Comparative Studies on the Yield and Quality of Solvent-extracted Oil from Salmon Skin. *Journal of Food Engineering*, 92(3), 353-358. <http://dx.doi.org/10.1016/j.jfoodeng.2008.12.011>
- Briscoe, M. (2015). Determination of Heavy Metals in Food by Inductively Coupled Plasma–Mass Spectrometry: First Action 2015.01. *Journal of AOAC International*, 98(4), 1113-1120. <http://dx.doi.org/10.5740/jaoac.int.2015.01>
- Ceyhan, V., & Emir, M. (2015). Structural and Economic Analysis of Turkish Fishmeal and Fish Oil Industry. *Turkish Journal of Fisheries and Aquatic Sciences*, 15, 841-850. http://dx.doi.org/10.4194/1303-2712-v15_4_07
- Chantachum, S., Benjakul, S., & Sriwirat, N. (2000). Separation and Quality of Fish Oil from Precooked and Non-precooked Tuna Heads. *Food Chemistry*, 69(3), 289-294. [http://dx.doi.org/10.1016/S0308-8146\(99\)00266-6](http://dx.doi.org/10.1016/S0308-8146(99)00266-6)
- Das, J., Chakraborty, D., Das, S., Bhattacharjee, S.C., & Das, P.K. (2016). Physicochemical Parameters and Heavy Metal Content in Soybean Oil from Bangladesh. *Pakistan Journal of Nutrition*, 15(6), 565-571.
- Deepika, D., Vegneshwaran, V.R., Julia, P., Sukhinder, K.C., Sheila, T., Heather, M., & Wade, M. (2014). Investigation on Oil Extraction Methods and Its Influence on Omega-3 Content from Cultured Salmon. *Journal of Food Processing and Technology*, 5(12), 401-413. <http://dx.doi.org/10.4172/2157-7110.1000401>
- FAO. (1986). The production of fishmeal and oil. FAO Fisheries technical paper-142. Retrieved from <http://www.fao.org/docrep/003/X6899E/X6899E00.H TM>.
- FAO. (2016). The state of world fisheries and aquaculture 2016. Contributing to food security and nutrition for all. Rome, Italy, 200 pp.
- FAO/WHO. (1994). Fats and oils in human nutrition. Reports of a joint expert consultation, Rome, Italy. Retrieved from <http://www.fao.org/docrep/V4700E/V4700E00.htm>
- FAO/WHO. (1995). General standard for contaminants and toxins in food and feed (Codex standard 193-1995; Revised in 2015). Rome, Italy, Codex Alimentarius, 44 pp.
- FAO/WHO. (2013). Joint FAO/WHO food standards program codex committee on fats and oils. Proposed draft standard for fish oils. Twenty third session, Langkawi, Malaysia. Retrieved from ftp://ftp.fao.org/codex/meetings/ccfo/ccfo23/fo23_03e.pdf.
- Farag, R.S., & Basuny, A.M.M. (2009). Improvement in the Quality of Used Sunflower Oil by Organic and Inorganic Adsorbents. *International Journal of Food Science and Technology*, 44(9), 1802-1808. <http://dx.doi.org/10.1111/j.1365-2621.2009.02002.x>
- Ferdosh, S., Sarker, Z.I., Norulaini, N., Oliveira, A., Yunus, K., Chowdury, A.J., Akanda, J., & Omar, M. (2015). Quality of Tuna Fish Oils Extracted from Processing the By-products of Their Species of Neritic Tuna using Supercritical Carbon Dioxide. *Journal of Food Processing and Preservation*, 39(4), 432-441. <http://dx.doi.org/10.1111/jfpp.12248>
- Fernández, P.M., & Juan, S. (2000). Fatty Acid Composition of Commercial Spanish Fast Food and Snack Food. *Journal of Food Composition and Analysis*, 13 (3), 275-281. <http://dx.doi.org/10.1006/jfca.2000.0893>
- Guerard, F., Guimas, L., & Binet, A. (2002). Production of Tuna Waste Hydrolysates by a Commercial Neutral Protease Preparation. *Journal of Molecular Catalysis B: Enzymatic*, 19-20, 489–498. [http://dx.doi.org/10.1016/S1381-1177\(02\)00203-5](http://dx.doi.org/10.1016/S1381-1177(02)00203-5)
- Hajeb, P., Jinap, S., Shakibazadeh, S., Afsah-Hejri, L., Mohebbi, G.H., & Zaidul, I.S.M. (2014). Optimisation of the Supercritical Extraction of Toxic Elements in Fish Oil. *Food Additives and Contaminants*, 31(10), 1712-1722. <http://dx.doi.org/10.1080/19440049.2014.942707>
- Haque, A.S.M.T., Asaduzzaman, A.K.M., & Chun, B.S. (2014). Fatty Acid Composition and Stability of Extracted Mackerel Muscle Oil and Oil-polyethylene Glycol Particles Formed by Gas Saturated Solution Process. *Fisheries and Aquatic Sciences*, 17(1), 67-73. <http://dx.doi.org/10.5657/FAS.2014.0067>
- Herpandi, N.H., Rosma, A., & Wan Nadiah, W.A. (2011). The Tuna Fishing Industry: A New Outlook on Fish Protein Hydrolysates. *Comprehensive Reviews in Food Science and Food Safety*, 10(4), 195-207. <http://dx.doi.org/10.1111/j.1541-4337.2011.00155.x>
- Jung, G.W., Kang, H.M., & Chun, B.S. (2012). Characterization of Wheat Bran Oil Obtained by Supercritical Carbon Dioxide and Hexane Extraction. *Journal of Industrial and Engineering Chemistry*, 18(1), 360-363. <http://dx.doi.org/10.1016/j.jiec.2011.11.094>
- Karunaratna, K.A.A.U., & Attyagalle, M.V.E. (2010). Nutritional Evaluation in Five Species of Tuna. *Vidyodaya Journal of Sciences*, 15, 7-16. <hdl.handle.net/123456789/1012>
- Kim, S.K., & Mendis, E. (2005). Bioactive Compounds from Marine Processing Byproducts—A review. *Food Research International*, 39(4): 383-393. <http://dx.doi.org/10.1016/j.foodres.2005.10.010>
- Kumaravel, S., & Alagusundaram, K. (2014). Determination of Mineral Content in Indian Spices by ICP-OES. *Oriental Journal of Chemistry*, 30(2), 631-636. <http://dx.doi.org/10.13005/ojc/300231>
- Lee, M.K., Uddin, M.S., & Chun, B.S. (2008). Off-flavors Removal and Storage Improvement of Mackerel Viscera by Supercritical Carbon Dioxide Extraction. *Journal of Environmental Biology*, 29(4), 591-597.
- Lee, J.H., Asaduzzaman, A.K.M., Yun, J.H., Yun, J.H., & Chun, B.S. (2012). Characterization of the Yellow Croaker *Larimichthys polyactis* Muscle Oil Extracted with Supercritical Carbon Dioxide and an Organic Solvent. *Fisheries and Aquatic Sciences*, 15(4), 275-281. <http://dx.doi.org/10.5657/FAS.2012.0275>
- Mansara, P.P., Deshpande, R.A., Vaidya, M.M., & Kaul-Ghanekar, R. (2015). Differential Ratios of Omega Fatty Acids (AA/EPA+ DHA) Modulate Growth, Lipid Peroxidation and Expression of Tumor Regulatory MARBPs in Breast Cancer Cell Lines

- MCF7 and MDA-MB-231. *PLoS one*, 10(9), e0136542. <http://dx.doi.org/10.1371/journal.pone.0136542>
- Noriega-Rodriguez, J.A., Ortega-Garcia, J., Angulo-Guerrero, O., Garcia, H.S., Medina-Juarez, L.A., & Gamez-Meza, N. (2009). Oil Production from Sardine (*Sardinops sagax caerulea*). *CyTA- Journal of Food*, 7(3), 173-179. <http://dx.doi.org/10.1080/19476330903010243>
- Osman, F., Jaswir, I., Khaza'ai, H., & Hashim, R. (2007). Fatty Acid Profiles of Finfish in Langkawi Island, Malaysia. *Journal of Oleo Science*, 56(3), 107-113. <http://dx.doi.org/10.5650/jos.56.107>
- Ritter, J.S. (2012). *Chemical measures of fish oil quality: Oxidation products and sensory evaluation* (PhD Thesis). Dalhousie University, Halifax, Nova Scotia, Canada.
- Rubio-Rodriguez, N., de Diego, S.M., Beltran, S., Jaime, I., Sanz, M.T., & Rovira, J. (2008). Supercritical Fluid Extraction of the Omega-3 Rich Oil Contained in Hake (*Merluccius capensis*-*Merluccius paradoxus*) By-products: Study of the Influence of Process Parameters on the Extraction Yield and Oil Quality. *Journal of Supercritical Fluid*, 47(2), 215-226. <http://dx.doi.org/10.1016/j.supflu.2008.07.007>
- Rubio-Rodriguez, N., de Diego, S.M., Beltran, S., Jaime, I., Sanz, M.T., & Rovira, J. (2012). Supercritical Fluid Extraction of Fish Oil from Fish By-products: A Comparison with Other Extraction Methods. *Journal of Food Engineering*, 109(2), 238-248. <http://dx.doi.org/10.1016/j.jfoodeng.2011.10.011>
- Sahena, F., Zaidul, I.S.M., Jinap, S., Yazid, A.M., Khatib, A., & Norulaini, N.A.N. (2010). Fatty Acid Compositions of Fish Oil Extracted from Different Parts of Indian Mackerel (*Rastrelligere kanagurta*) Using Various Techniques of Supercritical CO₂ Extraction. *Food Chemistry*, 120(3), 879-885. <http://dx.doi.org/10.1016/j.foodchem.2009.10.055>
- Saito, H., Seike, Y., Ioka, H., Osako, K., Tanaka, M., Takashima, A., Keriko, J.M., Kose, S., & Rodriguez-Souza, J.C. (2005). High Docosahexaenoic Acid Levels in Both Neutral and Polar Lipids of a Highly Migratory Fish: *Thunnus tonggol* (Bleeker). *Lipids*, 40(9), 941-953. <http://dx.doi.org/10.1007/s11745-005-1455-4>
- Selmi, S., & Sadok, S. (2010). The Influence of Season on Lipid Content and Fatty Acids Profile of *Euthynnus alletteratus* Fillets and By-products. *Journal of Muscle Foods*, 21(2), 365-378. <http://dx.doi.org/10.1111/j.1745-4573.2009.00188.x>
- Simopoulos, A.P. (2008). The Importance of the Omega-6/Omega-3 Fatty Acid Ratio in Cardiovascular Disease and Other Chronic Diseases. *Experimental Biology and Medicine*, 233(6), 674-688. <http://dx.doi.org/10.3181/0711-MR-311>
- Simopoulos, A.P. (2016). An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk of Obesity. *Nutrients*, 8(3), 128. <http://dx.doi.org/10.3390/nu8030128>
- Singh, R., Gautam, N., Mishra, A., & Gupta, R. (2011). Heavy Metals and Living Systems: An Overview. *Indian Journal of Pharmacology*, 43(3), 246-253. <http://dx.doi.org/10.4103/0253-7613.81505>
- Sullivan, J.C., & Budge, S.M. (2010). Monitoring Fish Oil Volatiles to Assess the Quality of Fish Oil. *Lipid Technology*, 22(10), 230-232. <http://dx.doi.org/10.1002/lite.201000054>
- Suseno, S.H., Tajul, A.Y., Nadiyah, W.A., & Noor, A.F. (2012). Improved of Color Properties on Sardine *llalemuru* Oil during Adsorbent Refining using Magnesol xl. *International Food Research Journal*, 19(4), 1383-1386.
- Suseno S.H., Yang T.A., Nadiyah, W., Abdullah, W., & Saraswati. (2015). Physicochemical Characteristics and Quality Parameters of Alkali-refined Lemuru Oil from Banyuwangi, Indonesia. *Pakistan Journal of Nutrition*, 14(2), 107-111.
- Von Schacky, C. (2003). The Role of Omega-3 Fatty Acids in Cardiovascular Disease. *Current Atherosclerosis Report*, 5(2), 139-145. <http://dx.doi.org/10.1007/s11883-003-0086-y>
- Wiedermann, L.H. (1981). Degumming, Refining and Bleaching Soybean Oil. *Journal of the American Oil Chemists' Society*, 58(3), 159-166. <http://dx.doi.org/10.1007/BF02582328>
- William, C.D., Whitley, B.M., Hoyo, C., Grant, D.J., Iraggi, J.D., Newman, K.A., ... Freedland, S.J. (2011). A High Ration of Dietary n-3/n-6 Polyunsaturated Fatty Acids is Associated with Increased Risk of Prostate Cancer. *Nutrition Research*, 31(1), 1-8. <http://dx.doi.org/10.1016/j.nutres.2013.07.004>
- Wrolstad, R.E., Durst, R.W., & Lee, J. (2005). Tracking Color and Pigment Changes in Anthocyanin Products. *Trends in Food Science and Technology*, 16(9), 423-428. <http://dx.doi.org/10.1016/j.tifs.2005.03.019>
- Young, F.V.K. (1985). The Refining and Hydrogenation of Fish Oil. England, International Association of Fish Meal Manufacturers, 27pp.
- Zahir, E., Saeed, R., Hameed, M.A., & Yousuf, A. (2014). Study of Physicochemical Properties of Edible Oil and Evaluation of Frying Oil Quality by Fourier Transform-Infrared (FT-IR) Spectroscopy. *Arabian Journal of Chemistry*. <http://dx.doi.org/doi:10.1016/j.arabjc.2014.05.025>
- Zhao, S., Feng, C., Quan, W., Chen, X., Niu, J., & Shen, Z. (2012). Role of Living Environments in the Accumulation Characteristics of Heavy Metals in Fishes and Crabs in the Yangtze River Estuary, China. *Marine Pollution bulletin*, 64(6), 1163-1171. <http://dx.doi.org/10.1016/j.marpolbul.2012.03.023>