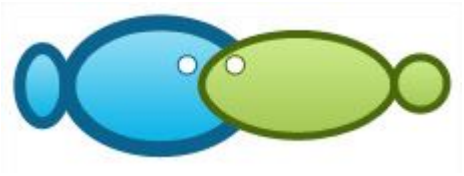

Extraction of oil from tuna by-product by supercritical fluid extraction (SFE) and comparison with wet reduction method

Mohammad M. Taati, Bahareh Shabanpour, Mehdi Ojagh

Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. Corresponding author: B. Shabanpour, bshabanpour@yahoo.com

Abstract. Fish oil is one of the most valuable marine products that has been used as unrefined for animal feed, poultry and aquaculture, and can be used by human due to existence of the Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). In this study, oil extraction was studied with two methods of supercritical fluid (SFE) and wet press (WP) from by-products of canned tuna factory from two aspects of yield and product quality, focusing on human consumption. For this purpose, the ratio of the obtained oil from the raw material as well as the lipid peroxidation indices were evaluated. The product yield rate did not show significant different in either of the two methods. Some volatile compounds such as aldehydes were observed only in the WP method, and the number of found alkanes in this method was higher than that of SFE. In contrast, citric acid as non-lipid organic acid was found only in SFE treatment, and dimethyl amine (DMA) was found to be the main cause of the specific smell of fish in each of the two treatments. Therefore, the results of this study indicate the potential of tuna by-products for extraction of fish oil for human consumption and the SFE method for extracting it.
Key Words: tuna by-products, lipid peroxidation, volatile compounds, DHA, supercritical carbon dioxide.

Introduction. The fish industry is a wide sector that includes several production processes such as filleting, curing, salting, smoking, canning, etc. Nowadays, it is estimated that more than 70% of the total fish captures are processed, generating a large amount of solid wastes and by-products, which often represent more than 50% of the total fish weight (Shahidi 2006). On the other hand, production of high quality fish oil has acquired a great importance since it is considered one of the main natural sources of omega-3 polyunsaturated fatty acids (PUFAs), which benefits in human health have been extensively reported in the literature (Chow 2000).

The importance of using omega-3 has been emphasized especially in studies from 2000 (Rubio-Rodriguez et al 2010). A great deal of research has shown the positive effects of omega-3 fatty acids on cardiovascular diseases, particularly atherosclerosis (Siscovick et al 2017), rheumatoid arthritis (rheumatism) (Stancík et al 2006; Handelsman & Shapiro 2017), severe inflammations like asthma (Reisman et al 2007), psoriasis (chronic autoimmune disease) (Zulfakar et al 2007; Guida et al 2014), psychological diseases (Ross et al 2007; Song & Zhao 2007), prevention of several types of cancers (Chen et al 2007; Calviello et al 2007; Fabian et al 2015; Black 2017), intestinal diseases (Turner et al 2007), prevention of fatty liver disease (Chen et al 2015) and reduction of Alzheimer's disease (Belkouch et al 2016) and so on. Therefore, omega-3 can be considered as a very important oral and herbal drug.

Production of omega-3 rich fish oils has become a good opportunity for valorizing fish by-products and increasing the competitiveness of the fish industry. In the last years, by-products from different types of fishes, such as tuna (Chantachum et al 2000), herring (Aidos et al 2003), salmon (Wu & Bechtel 2008), or walleye pollock (Wu & Bechtel 2009), have been proposed as raw materials for fish oil production. However, the production of high quality fish oil as source of omega-3 involves, not only searching for an omega-3 rich raw material, but also developing a suitable extraction procedure. The most common method used for fish oil production is wet reduction, which involves three

basic steps: cooking at high temperatures (85-95°C), pressing and centrifuging (FAO 2014). This process permits obtaining high volumes of crude fish oil, although subsequent refining steps are required in order to make the crude fish oil suitable for edible purposes. Other processes, such as enzymatic reaction with proteases, have been studied for obtaining crude oil from fish by-products (Linder et al 2005). In the last years, supercritical fluid extraction (SFE) has become an attractive technology for obtaining high quality fish oil from some by-products (Letisse et al 2006; Rubio-Rodriguez et al 2012), not only because it uses moderate temperatures and provides an oxygen free media, which aim to reduce the omega-3 oxidation during the extraction process, but also because it allows extracting selectively low polar lipid compounds, avoiding the co-extraction of polar impurities such as some inorganic derivatives with heavy metals. Furthermore, the tunability of the supercritical carbon dioxide (SC-CO_2) regarding density, and therefore solvation power, by changing temperature and/or pressure, makes fish oil de-acidification possible, alternatively to conventional physical and chemical fish oil refining (Kawashima et al 2006). In SFE process raw materials should be freeze-dried in order to reduce their moisture to values below 20% (Rubio-Rodriguez et al 2008).

The aim of this work was to compare two extraction processes (wet reduction and supercritical fluid extraction) to obtain oil from tuna by-products, at a laboratory scale, taking into account, not only the extraction yield, but also the volatile components of oil.

Material and Method

Fish sampling and processing. Approximately 30 kg of tuna by-products was obtained from Sahel Seyd Konarak (canned tuna factory), Chabahar, Iran in January 2016. The by-products were transported on ice within 45 minutes of landing. The fish was then frozen at -30ºC to minimize the effects of biochemical changes during transportation from factory to the laboratory (located in Tehran). The fish was transported to the laboratory within 15 hours after sampling. The whole by-products were grinded and were stored under -20ºC until used.

Oil extraction methods.

Wet reduction (wet pressing - WP). In wet reduction method, grinded fish by-products were previously thawed at room temperature during 12 h, 1 L water was added to 200 g by-products and then cooked for 20 minutes. After cooking the tissues were pressured, then, water co-extracted together with the oil was removed by centrifuging (Centrikon T-124, Kontron Instruments).

SFE method: extraction equipment and procedure. Before putting samples in extraction chamber of SFE set, raw and grinded materials were placed in freeze-dryer to reduce their moisture. The supercritical CO_2 extraction experiments were carried out in a bench scale SFE plant whose P&I diagram has been presented in Figure 1. The usual elements of an SFE plant with solvent recycling were installed, i.e. pump, extractor (2 L), separator (1 L), heating and cooling systems, pressure dampers, rupture disks for safety and instruments for measurement and control of the process parameters. The equipment was designed to perform the extraction stage of SFE under the following conditions: temperature, $T \geq 40$°C, pressure, $p \geq 25$ MPa, solvent flow, $F \geq 10$ kg CO_2 h^{-1} and 3 h for extraction time. In an SFE experiment, approximately 100 g of freeze-dried tuna by-product were placed in the extractor that was later ressurized up to the extraction pressure, p, with carbon dioxide (liquid $CO_2 \geq 99.9\%$). Then, the solvent was circulated at the desired extraction temperature, T, with a certain solvent flow, F, and during a specific time, t. The solvent was continuously recycled to the extractor after removing the solute in the separator where the solvent power of CO_2 was reduced by reducing pressure down to approximately 5 MPa and keeping temperature lower than 40°C. The separation temperature was kept around 40±2°C.

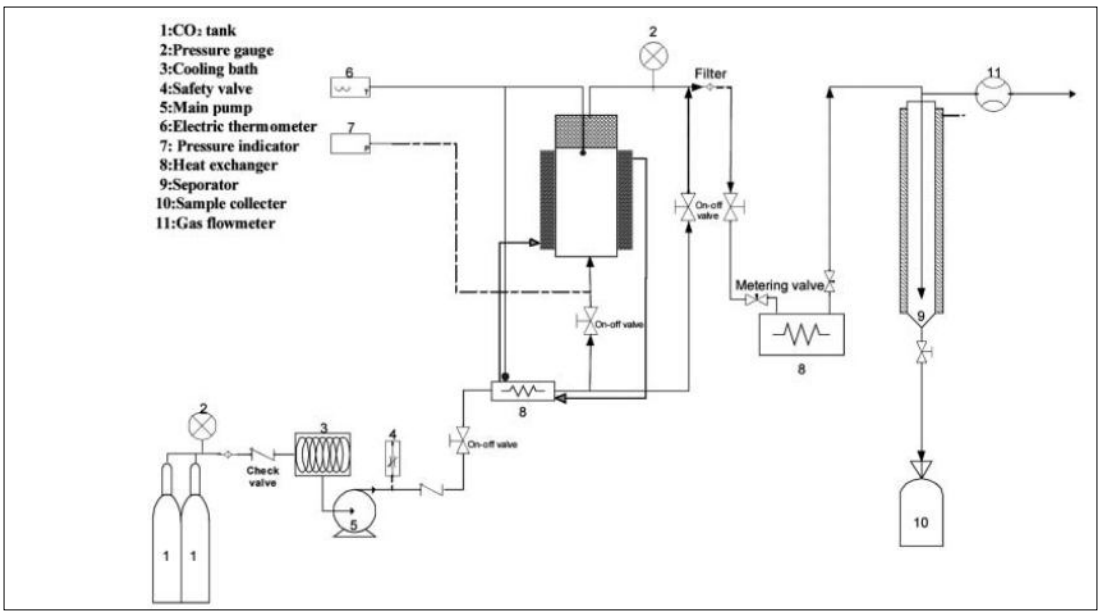

Figure 1. Flow diagram of the SC-CO_2 extraction.

Determination of yield. Yield was expressed as a percentage of oil separated from by-products tuna. Yield was calculated as follows (Chantachum et al 2000):

$$\%Yield = \frac{\text{Wt: of crude oil} \times 100}{\text{Wt: of tuna by-products} \times 100}$$

Volatile component analysis. Volatile compounds were analyzed by GC–MS after Solid Phase Dynamic Extraction (SPDE) sampling. The SPDE device (Chromtech, Idstein, Germany) was equipped with a needle coated with a nonpolar 50 lm film of polydimethylsiloxane with 10% embedded activated carbon phase (PDMS/AC). Samples were incubated for 1 min at 70°C; and after equilibration, extraction was performed (50 aspiration cycles, extraction speed 40 µL s^{-1}). Gas chromatography analyses were carried out with a 6890N Series GC System coupled to a 5973i mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The SPDE needle was injected and thermally desorbed at 250°C. Compounds were separated on a HP5 capillary column (50 m length ×0.32 mm I.D.), fused silica capillary column coated with a 1.05 lm film thickness (Quadrex Corporation, New Haven, USA). The temperature of the column was increased at a rate of 3°C min^{-1} from 40 to 240°C.

Results. The amount of obtained oil by WP and SFE methods is showed in Figure 2. Yield in WP method was higher than SFE process, but this difference was not significant ($p > 0.05$).

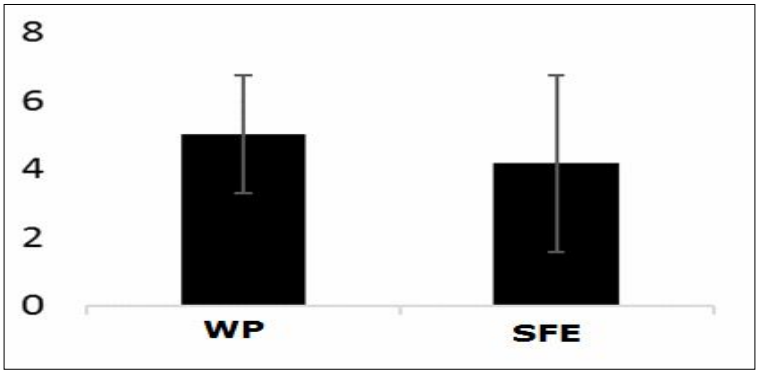

Figure 4. Percentage of yield of obtained oils from tuna by-products by WP and SFE methods.

Results of volatile component analysis

Alkanes. As shown in Table 1, alkanes (one of most important volatile component that form in oil and lipid oxidation) like Decane, Undecane, Dodecane, Tridecane, Pentadecane, 2,6,10,14-Tetramethyl-pentadecane were detected in both methods, and 3-Methyl-Decane was not detected in both of them. Also, 2-Methyl-Decane and Cyclohexadecane were observed only in SFE method. So, it can be argued that there was not a complete similarity in the production of alkenes in the obtained oils by WP and SFE.

Table 1

The detected alkanes in oil obtained from tuna by-products by WP and SFE methods

Component	*WP*	*SFE*
Decane	√	√
2-Methyl-Decane	-	√
3-Methyl-Decane	-	-
Undecane	√	√
Dodecane	√	√
Tridecane	√	√
Pentadecane	√	√
Cyclohexadecane	-	√
2,6,10,14-Tetramethyl-pentadecan	√	√

Aldehydes, acids and amines. Investigating the existence or nonexistence of aldehydes in the present study showed that in extracted fish oils by WP (Table 2), Hexanal, Nonanal and Heptanal Waxy (from aldehydes) were found and in the oils obtained by the SFE method they were not found. Also, dimethyl amin (from amines) was found in extracted oil samples with both methods. On the other hand, Acetic acid existed only in samples from the SFE method.

Table 2

The detected aldehydes, acids and amines in oil obtained from tuna by-products by WP and SFE

Component	*WP*	*SFE*
Heptanal Waxy	√	-
Hexanal	√	-
Nonanal	√	-
Acetic acid	-	√
Dimethyl amin	√	√

Discussion. Fish oil production is important from two perspectives of human and animal consumption. The presence of this food in livestock feed, and in particular aquatic, is important for supplying the energy of the feed as well as essential fatty acids and is also considered in human consumption for providing essential fatty acids such as EPA and DHA. Therefore, the production of fish oil for two aims, yield and the quality of the product, was studied.

Yield. The results of oil yield coefficient in WP and SFE showed that this value was higher in WP method, although this difference was not significant. The reason for this can be attributed to the mechanical pressure in WP method. The results of oil yield coefficient in WP and SFE showed that this value was higher in WP method, although this difference was not significant. The reason for this can be attributed to the mechanical pressure that was introduced in the WP method, which results in more oil being extracted from the cooked mass.

Also, the higher temperature in the WP method than the SFE can be considered as another factor in this. Heat contributes to the denaturation of the protein matrixes of the tissue that the oil strongly bonds to them. Following this process, solids and liquids can be removed mechanically. Also, heat causes the breaking of globules and cells of the oil, resulting in the release and fluidity of the oil, which can increase the efficiency (Chantachum et al 2000). The results of oil extraction from fish by-products by four methods of WP, SFE, enzymatic extraction and cold extraction showed that the efficiency of the SFE method and WP was higher than the other methods. In this study, there was no significant difference between the efficacy of the WP and SFE methods, which is in agreement with the results of the study of Rubio-Rodriguez et al (2012).

Alkanes. One of the quantitative indices of lipid peroxidation, which is produced by the peroxidation of polyunsaturated fatty acids, is alkanes. Alkanes are the result of omega-6 and omega-3 deficiencies in oil, which can be considered as an appropriate indicator for lipid peroxidation (Burk & Ludden 1989).

Oxygen level is one of the important factors that increase or decrease the formation of alkanes in lipid peroxidation. The results of Kostrucha & Kappus (1986) showed that there is an inverse relationship between the formation of alkanes and the oxygen level, as the amount of alkanes increases with the decreasing of the oxygen content. In the present study, in the SFE method, due to the presence of carbon dioxide as a fluid and vacuum conditions in the system, oxygen was not present in the SFE set, and thus, during the extraction process, the amount of alkanes was probably increased. So that, the presence of alkanes such as 2-Methyl-Decane and Cyclohexadecane was observed only in this method.

Aldehydes, acids and amines. Aldehydes, organic acids and amines are the most important volatile compounds, which the fishy odor and taste of fish oil are strongly dependent on their presence. Some aldehydes, such as hexanal or nannal, are produced by the process of auto-oxidation (self-oxidation) of lipids. Therefore, their presence in fish oil is basically affected by extraction methods and parameters involved (especially temperature, ambient oxygen, light, and metals) (Rubio-Rodriguez et al 2012). In contrast, some other volatile compounds are formed during the storage of fish and due to bacterial and enzymatic activities on proteins, amino acids and carbohydrates. Thus, the presence of these in oil can be attributed to the freshness of the raw material (Table 3). For example, trimethylamine amide oxide can be formed by the action of bacteria such as *Shewanella putrefaciens*, dimethylamine oxide due to enzymatic activity during storage and acetic acid through the anaerobic degradation of amino acids (Huss 1995).

Table 3

Volatile compounds produced by different fish degradation processes (adapted from Huss 1995)

Process	*Substrate*	*Compounds produced*
Bacterial degradation	Trimethylamine oxide	Trimethylamine
	Cysteine	H_2S
	Methionine	CH_3SH, $(CH_3)2S$
	Carbohydrates and lactate	Acetate, CO_2, H_2O
	Inosine	Hypoxanthine
	Glycine, serine, leucine	Esters, ketones, aldehydes
	Urea	NH_3
Enzymatic action	Trimethylamine oxide	Dimethylamine
Auto oxidation process	Lipids	Aldehydes
		Ketones
		Alcohols
		Short-chain organic acids
		Alkanes
Anaerobic (spoilers)	Amino acids	NH3, acetic acid, butyric acid, propionic acid

In the present study, heptanal, hexanal and nonanal aldehydes were only detected in the oil obtained by the WP method. This is probably due to the low content of atmospheric oxygen in the SFE process and the gentle temperature during extraction, which reduces the possibility of oxidative oxidation (as the main factor for the formation of aldehydes) (Roh et al 2006). On the other hand, acetic acid was identified only in oil obtained by SFE. The reason for this may be attributed to the process of anaerobic decomposition of amino acids and formation of acetic acid. As previously mentioned, there is no atmospheric oxygen during extraction process with supercritical fluid and this can affect on anaerobic decomposition. In the case of dimethylamine (a factor creating a specific smell of fish), this substance probably has not been removed in the high temperature in the WP method and has also been added to the oil.

In the extractor of the SFE, due to its high pressure and constant flow of carbon dioxide, DMA has been separated from the material and partly absorbed in extracted oil (Chun et al 2014), hence dimethylamine was found in the obtained samples from each of the two methods. The results of identification and isolation of volatile compounds in tuna oil showed that volatile compound levels (including alkanes, alkenes, aldehydes, alcohols and ketones) in concentrated oil by SFE was significantly lower than the initial oil sample (crude oil) (Roh et al 2006).

Conclusions. In the present study, the quantitative comparison and yield of oil extracted from tuna by-products by WP and SFE methods revealed the suitability of each of the two methods for oil extraction in terms of quantity. In general, oil extraction methods, in addition to quantity, can be effective in the formation of compounds from oxidation of fats and various types of contaminants. Since the SFE extraction process takes place in vacuum and free atmospheric oxygen, and the samples have low moisture content, SFE can be used as an effective way to prevent lipid oxidation (especially in oils with high levels of TAG and PUFA). In the present study, extracted oils by SFE method, in terms of existence and type of compounds and indicators of lipid oxidation showed better conditions and quality, which can be considered as the special advantages of this method. Therefore, according to the results, it can be stated in the first that tuna by-product has a suitable potential for extracting oil with a human consumption approach. Also, considering the specifics of the SFE process in oil extraction, it can be considered a suitable method for extracting fish oil.

Acknowledgements. The authors of this article announce their gratitude and appreciation to the Sahel seyd Konarak (Tohfeh) and Omics companies for the financial and spiritual support of the project.

References

Aidos I., Kreb N., Boonman M., Luten J. B., Boom R. M., Van Der Padt A., 2003 Influence of production process parameters on fish oil quality in a pilot plant. Journal of Food Science 68:581-586.

Belkouch M., Hachem M., Elgot A., Lo Van A., Picq M., Guichardant M., Lagarde M., Bernoud-Hubac N., 2016 The pleiotropic effects of omega-3 docosahexaenoic acid on the hallmarks of Alzheimer's disease. Journal of Nutritional Biochemistry 38:1-11.

Black H., 2017 Omega-3 fatty acids and cancer prevention. Journal of Integrative Oncology 6:e112.

Burk R. F., Ludden T. M., 1989 Exhaled alkanes as indices of *in vivo* lipid peroxidation. Biochemical Pharmacology 38(7):1029-1032.

Calviello G., Serini S., Piccioni E., 2007 n-3 polyunsaturated fatty acids and the prevention of colorectal cancer: molecular mechanisms involved. Current Medicinal Chemistry 14(29):3059-3069.

Chantachum S., Benjakul S., Sriwirat N., 2000 Separation and quality of fish oil from precooked and non-precooked tuna heads. Food Chemistry 69:289-294.

Chen Y. Q., Edwards I. J., Kridel S. J., Thornburg T., Berquin I. M., 2007 Dietary fat-gene interactions in cancer. Cancer Metastasis Reviews 26:535-551.
Chen Y., Xu C., Yan T., Yu C., Li Y., 2015 ω-3 fatty acids reverse lipotoxity through induction of autophagy in nonalcoholic fatty liver disease. Nutrition 31:1423-1429.
Chow C. K., 2000 Fatty acids in foods and their health implications. Second edition (revised and expanded). Marcel Dekker Inc., New York-Basel, pp. 48-53.
Chun B. S., Roh H. S., Kang K. Y., Kwon M. J., Weber A., Wilkinson G., 2014 Identification and removal of off flavors from sardine oil with supercritical fluid extraction. Food Processing and Technology 5:331-337.
Fabian C. J., Kimler B. F., Hursting S. D., 2015 Omega-3 fatty acids for breast cancer prevention and survivorship. Breast Cancer Res 17:62-77.
FAO, 2014 The production of fish meal and oil. FAO Fisheries Technical Paper, No. 142, Rome, Italy, 63 pp.
Guida B., Napoleone A., Trio R., Nastasi A., Balato N., Laccetti R., Cataldi M., 2014 Energy-restricted, n-3 polyunsaturated fatty acids-rich diet improves the clinical response to immuno-modulating drugs in obese patients with plaque-type psoriasis: a randomized control clinical trial. Clinical Nutrition 33:399-405.
Handelsman Y., Shapiro M. D., 2017 Triglycerides, atherosclerosis, and cardiovascular outcome studies: focus on omega-3 fatty acids. Endocrine Practice 23(1):100-112.
Huss H. H., 1995 Quality and quality changes in fresh fish. FAO, Fisheries and Aquaculture Department, Rome, Italy, pp. 89-103.
Kawashima A., Iwakiri R., Honda K., 2006 Experimental study on the removal of dioxins and coplanar polychlorinated biphenils (PCBs) from fish oil. Journal of Agricultural and Food Chemistry 54:10294-10299.
Kostrucha J., Kappus H., 1986 Inverse relationship of ethane or n-pentane and malondialdehyde formed during lipid peroxidation in rat liver microsomes with different oxygen concentrations. Biochimica et Biophysica Acta 879:120-125.
Letisse M., Rozieres M., Hiol A., Sergent M., Comeau L., 2006 Enrichment of EPA and DHA from sardine by supercritical fluid extraction without organic modifier. I. Optimization of extraction conditions. Journal of Supercritical Fluids 38(1):27-36.
Linder M., Fanni J., Parmentier M., 2005 Proteolytic extraction of salmon oil and PUFA concentration by lipases. Marine Biotechnology 7(1):70-76.
Reisman J., Schachter H. M., Dales R. E., Tran K., Kourad K., Barnes D., Sampson M., Morrison A., Gaboury I., Blackman J., 2007 Treating asthma with omega-3 fatty acids: where is the evidence? A systematic review. BMC Complementary and Alternative Medicine 6:26-34.
Roh H. S., Park J. Y., Park S. Y., Chun B. S., 2006 Isolation of off-flavors and odors from tuna fish oil using supercritical carbon dioxide. Biotechnology and Bioprocess Engineering 11:496-502.
Ross B. M., Seguin J., Sieswerda L. E., 2007 Omega-3 fatty acids as treatments for mental illness: which disorder and which fatty acid? Lipids in Health and Disease 6:21.
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*) byproducts: study of the influence of process parameters on the extraction yield and oil quality. Journal of Supercritical Fluids 47:215-226.
Rubio-Rodríguez N., Beltrán S., Jaime I., de Diego S. M., Sanz M. T., Carballido J. R., 2010 Production of omega-3 polyunsaturated fatty acid concentrates: a review. Innovative Food Science and Emerging Technologies 11:1-12.
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 109:238-248.
Shahidi F., 2006 Maximixing the value of marine by-products. CRC Press, Boca Raton, USA, pp. 103-125.

Siscovick D. S.,, Barringer T. A., Fretts A. M., Wu J. H., Lichtenstein A. H., Costello R. B., Kris-Etherton P. M., Jacobson T. A., Engler M. B., Alger H. M., Appel L. J., Mozaffarian D., 2017 Omega-3 polyunsaturated fatty acid (fish oil) supplementation and the prevention of clinical cardiovascular disease: a science advisory from the American Heart Association. Circulation 135(15):867-884.

Song C., Zhao S., 2007 Omega-3 fatty acid eicosapentaenoic acid. A new treatment for psychiatric and neurodegenerative diseases: a review of clinical investigations. Expert Opinion on Investigational Drugs 16(10):1627-1638.

Stancík R., Rovenský J., Stančíková M., 2006 Diet in the rheumatoid arthritis and the omega-3 fatty acids. Rheumatologia 20:159-166.

Turner D., Zlotkin S. H., Shah P. S., Griffiths A. M., 2007 Omega 3 fatty acids (fish oil) for maintenance of remission in Crohn's disease. Cochrane Database of Systematic Reviews 2:CD006320.

Wu T. H., Bechtel P. J., 2008 Salmon by-product storage and oil extraction. Food Chemistry 111:868-871.

Wu T. H., Bechtel P. J., 2009 Quality of crude oil extracted from aging walleye pollock (*Theragra chalcogramma*) by-products. Journal of American Oil Chemists' Society 86:903-908.

Zulfakar M. H., Edwards M., Heard C. M., 2007 Is there a role for topically delivered eicosapentaenoic acid in the treatment of psoriasis? European Journal of Dermatology 17:284-291.

Received: 26 September 2017. Accepted: 28 November 2017. Published online: 07 December 2017.
Authors:
Mohammad Mehdi Taati, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Golestan, Gorgan, Shahid Beheshti Avenue, Postal code: 49138-15739, e-mail: taati.mehdi64@gmail.com.
Bahareh Shabanpour, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Golestan, Gorgan, Shahid Beheshti Avenue, Postal code: 49138-15739, e-mail: bshabanpour@yahoo.com
Mehdi Ojagh, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Golestan, Gorgan, Shahid Beheshti Avenue, Postal code: 49138-15739, e-mail: Ojagh@gau.ac.ir

How to cite this article:
Taati M. M., Shabanpour B., Ojagh M., 2017 Extraction of oil from tuna by-product by supercritical fluid extraction (SFE) and comparison with wet reduction method. AACL Bioflux 10(6):1546-1553.