

Effects of Supercritical CO₂ on Quantity and Quality of Extracted Oil from Myctophidae Fish and Comparison It with the Wet Pressing as a Commercial Method

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Abstract

The aim of this study was investigation the effects of different methods (wet pressing: WP and supercritical fluid extraction: SFE) of oil extraction from lantern fish (Myctophidae) in terms of quantity and quality. Results showed there was no significant difference in oil production rate ($p > 0.05$), but there was a significant difference between detected volatile components (alkanes, aldehydes, amines and acetic acid) ($p < 0.05$). Some of Neutral lipids (Wax Esters (WE), Triacylglycerol (TAG) and Free Fatty Acids (FFA)) showed significant difference ($p < 0.05$) and Cholesterol (CHOL) levels did not show significant differences between treatments ($p < 0.05$). Also, fatty acid profiles showed the level of DHA in SFE treatment was significantly higher than WP ($p < 0.05$). The acidity value (AV) did not show a significant difference ($p < 0.05$). Also, the amount of heavy metals (Mercury (Hg), arsenic (As), cadmium (Cd) and lead (Pb)) in extracted oils by SFE was lower than the other method ($p < 0.05$). Therefore, the results of this study indicate the potential of lantern fish for extraction of fish oil in human consumption and the SFE method for extracting it.

Introduction

Myctophids (Family Myctophidae) are the most abundant and diverse mesopelagic fishes in most of the world's oceans (Saunders *et al.*, 2019), including the Southern Ocean, where they comprise around 35 species in 12 genera and an estimated biomass that may substantially exceed 70–200 million tonnes (Mt) (Irigoiien *et al.*, 2014). Myctophids have a brownish gray flesh (Shaviklo and Rafipour, 2013) and the size of them is 1-5 cm and they weigh about 1-6 grams (Shaviklo and Moradi, 2019). Myctophidae are cheap fish and in most countries due to not being consumed directly by humans as food, the major part of them are transferred to fish meal and fish oil factories. However, more valuable compounds can be extracted from these fish and create high added value. These compounds are

fishmeal, fish oil, fish silage, mince, Surimi, fish protein isolate/ hydrolysate (FPI/FPH), lubricating oil, cosmetics, wax, collagen, gelatin, some enzymes, silage, bioactive compounds, pigments, lecithin, leather and fish oil, and PUFAs (Poly Unsaturated Fatty Acids) especially omega 3 (Välimaa *et al.*, 2019; Shaviklo, 2020). Studies show that Myctophids have high levels of fatty acids, especially omega-3, due to the amount and composition of fatty acids in this family of fish, can be a good source for use in the food industry and the production of fortified drugs (Bahri *et al.*, 2015; Shaviklo, 2020).

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 literatures (Hosseini *et al.*, 2021). The importance of

using omega-3 has been emphasized, especially in studies conducted since 2000. Extensive research has shown the positive effects of omega-3 fatty acids on cardiovascular disease, especially atherosclerosis (Hu *et al.*, 2019), rheumatism (Handelsman & Shapiro, 2017), severe inflammation such as asthma (Stoodley *et al.*, 2020), psoriasis (Balic *et al.*, 2020), mental illness (Natacci *et al.*, 2018), prevention of several types of cancer (Black, 2017; Freitas & Campos, 2019), intestinal diseases (Song *et al.*, 2019), prevention of oxidative stress diseases such as non-alcoholic fatty liver disease (NAFLD) (Yan *et al.*, 2018) and Alzheimer's disease (AD) (Belkouch *et al.*, 2016). Therefore, omega-3 can be considered as a very important marine origin product and lantern fish can be a great source of that.

The production of high quality fish oil as source of omega-3 depends to raw material and suitable extraction procedure. Several conventional and modern separation techniques can be applied for isolation of fish oil extract from fish tissues. Obtained fish oil consists of saturated, monounsaturated and polyunsaturated fatty acids, the latest of which is the focal point of this work (Kuvendziev *et al.*, 2018). So far, various methods such as enzymatic extraction (Lee *et al.*, 2017), alkali hydrolysis (Li *et al.*, 2018), microwave-assisted (Li *et al.*, 2018), solvent extraction (Ciriminna *et al.*, 2019), cold pressing (Fouda, 2020), etc. have been tested for oil extraction from various fish tissues.

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 (Santativongchai *et al.*, 2020). 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.

On the other hand, in the last years, supercritical fluid extraction (SFE) has become an attractive technology for obtaining high quality fish oil from different species (Rubio-Rodriguez *et al.*, 2012). Mostly, using supercritical carbon dioxide (scCO₂) due to its unique properties as solvent. Like other SCFs, scCO₂ presents liquid-like density and gas-like viscosity, diffusivity, and compressibility, which provide good and tunable solvent power as well as good mass transport properties (Melgosa *et al.*, 2021). In addition, scCO₂ is safe (nontoxic, and non-flammable) and presents accessible critical conditions (TC=31.1°C, pC=7.38 Mpa (Angus *et al.*, 1976). These mild critical conditions and the oxygen displacement make scCO₂ the solvent of choice for processing of thermolabile and easily-oxidizable compounds, such as omega-3 PUFAs, with lower risk of degradation (Melgosa *et al.*, 2021). Furthermore, the tunability of the supercritical carbon dioxide (SC-CO₂) 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.*, 2012).

Several studies have taken advantage of the benefits of scCO₂ as a green solvent in degumming and bleaching (Čmolík & Pokorný, 2000), deacidification (Lai *et al.*, 2008) and reduction the amount of heavy elements (Hajeb *et al.*, 2015) of different oils. Supercritical refining can be applied to fish oil in order to remove coextracted impurities such as FFAs, oxidation products, and endogenous volatile compounds. So, scCO₂ can be effective on different components levels of extracted oil from fish.

The aim of present study was investigation the effects of two oil extraction methods (wet pressing and supercritical fluid extraction) from lantern fish (at a laboratory scale) on yield, level of some chemical compounds and quality indices.

Material and Method

Fish Sampling and Processing

Approximately 40 kg of the fresh whole lantern fish (3-6 cm) were obtained from the local market (Chabahar, Sistan & Balochestan Province, Iran). The lantern fish were transported on ice (ratio of ice to fish: 3:1) within 60 minutes of landing. The fish were then frozen at -20°C to minimize the effects of biochemical changes during transportation from port to the laboratory (located in Tehran, Iran). The fish with ice pack were transported to the laboratory within 12 hours after landing. The whole lantern fish were grinded (by CFS model meat grinder, Netherlands) and were frozen under -20°C until used.

Analysis of Raw Materials Composition

The raw materials were homogenized and their nutrients including water (moisture), protein, fat and ash were measured according to AOAC (2005) standard methods. The amount of moisture (g/100 g of raw material), protein (%), fat (%) and ash (%) were analyzed by the Oven, Kjeldahl, Soxhlet and Electric furnace respectively. Atomic absorption spectrometry (AAS) was used to evaluate the toxic metals Hg, As, Cd and Pb (µg/g) in raw materials according to APAH (2005) standards.

Oil Extraction Methods

Wet Pressing (or Wet Reduction)

In wet pressing method, grined lantern fish were previously thawed at room temperature (20-22°C) during 8 h, 1 L water was added to 200 g lantern fish and was cooked for 20 minutes. After cooking the tissues were pressed, then, water co-extracted together with the oil was removed by centrifuging (Centrikon T-124, Kontron Instruments).

SFE Method: Extraction Equipment and Procedure

In this extraction method, to determine the optimal treatment in terms of production level, first some treatments were defined. This treatment was performed based on different fluid pressure (P) and temperatures (T). Each of the mentioned treatments had 3 replications. For this purpose, 1000 g of the raw samples for each replicate were placed in the freeze-dryer for 72 hours (to reduce the humidity of the samples below 20%) and then were transferred into the extraction chamber of the SFE. Extraction conditions were considered as: carbon dioxide solvent, pressures of 30, 35 and 40 MPa, temperatures of 50, 55 and 60°C, constant solvent flow rate of 1±15 g/min and dissolution time 3 hours. After performing these treatments (9 treatments in total), the group with the highest yield was selected for qualitative analysis and comparison with WP treatment. The 9 experimental treatments of the oil extraction section by SFE are mentioned in Table 1.

Quality Analysis of Extracted Oils

Determination of Yield

Yield was expressed as a percentage of oil separated from lantern fish. Yield was calculated as follows:

$$\%Yield = \frac{Wt: of crude oil \times 100}{Wt: of lantern fish}$$

Chemical Analysis of Extracted Oils

Volatile Component Analysis

Volatile components are the main cause of unpleasant odor in fish oil. Therefore, in order to investigate their levels in different oil extraction methods and to find a suitable extraction method, the volatile compounds were measured:

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 μm 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). 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 μm film thickness. Quadrex Corporation, New Haven, USA). The temperature of the column was increased at a rate of 3°C/min from 40 to 240°C.

Neutral Lipids Analysis

The measured neutral lipids in this study were as follows:

Wax esters (WE), Triacylglycerols (TAG), Free Fatty Acids (FFA) and Cholesterol (CHOL). These component were determined according to described method by Rubio-Rodriguez *et al*, (2012). The total amount of neutral lipids was analyzed by liquid chromatography in a HPLC system (Shimadzu, Model Nexera Quaternary) that was equipped with an auto-injector. The separations were done at ambient temperature in a column (Lichrospher Diol 5 mm, 4 × 250 mm) and the detection was performed in an evaporative light scattering detector (Shimadzu Nexera Quaternary series) at 45°C and 3.5 bar. The mobile phase consisted of a mixture of solvents: (A) hexane/acetic acid (99.5/0.5 by volume) and (B) hexane/1-propanol/acetic acid/water (85/14.4/0.5/0.1 by volume). The solvent gradient used was as follow: first, solvent A was flowing for 1 min, after that, solvent B was added in three steps, up to 10% in 9 min, to 44% in 12 min and to 100% in 8 min. Finally, the stationary phase was rinsed with solvent A during 5 min. Total solvent flow rate was kept constant at 1 mL/min all along the analysis. Calibration was carried out using standards of palmitil palmitate (99%), tripalmitin (>99%), dipalmitin (99%), monopalmitin (99%) and palmitic acid (99%) in hexane.

Table 1. Treatments of oil extraction from lantern fish by SFE process

Treatments	Temperature (°C)	Pressures (MPa)	solvent flow rate (g/min)	Dissolving duration
Treatment 1	50	30	15	3
Treatment 2	55	30	15	3
Treatment 3	60	30	15	3
Treatment 4	50	35	15	3
Treatment 5	55	35	15	3
Treatment 6	60	35	15	3
Treatment 7	50	40	15	3
Treatment 8	55	40	15	3
Treatment 9	60	40	15	3

Determination of Fatty Acid Profile

The fatty acid profile was determined according to the AOAC official method 991.39 by GC (Gas Chromatography). In this method, after methylation of fish oil and formation of Fatty Acid Methyl Esters (FAMES), the FAMES were analyzed by a GC (Shimadzu, Tokyo, Japan) equipped with a flame ionization detector and a fused-silica capillary column (PEG-20M, 30 m × 0.32 mm × 0.5 μm). The temperatures of the injection port and detector were set at 250°C. For 5 minutes the column was initially held at 80°C, then temperature reached to 175°C at the rate of 15°C/min, and held at this temperature for 5 min and programming to 215°C at the rate of 5°C/min and finally kept at this temperature for 30 min. Identification of GC peaks was finally achieved by comparing their retention times with those of the corresponding standards.

Determination of Acidity Value (AV)

The acidity value was measured according to the standard methods proposed in AOAC (Perrin, 1996). For this purpose, 1 gram of fish oil was dissolved in 50 ml of ethanol-ether solution. After dissolving oil in solution and adding phenolphthalein reagent (1%), it was titrated

with 0.1 N sodium hydroxide until the pink color remained in the reaction media for 30 seconds. Then the acidity rate was calculated according to the following equation:

$$AV \text{ (mg/g)} = \frac{V \times C \times 56.1}{m}$$

Measurement of Toxic Elements

In order to evaluate the amount of toxic elements in extracted oil by two different methods, the amount of Hg, As, Cd and Pb metals was determined according to Hajeb et al., (2015) method. In this method, 1 gram samples of fish oil were diluted with 5 ml of HN03 (65%), and the mixture was digested at 40 to 90°C for 3 hours. The digested samples were cooled and subsequently diluted to 40 ml using ultra-pure water. The metals in the filtered samples (filtration by Whatman's GD/XP syringe filters) were analyzed using inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7800 Quadrupole). The blank samples were analyzed in the same process, and the concentrations were determined using standard solutions prepared in the same acid matrix. Multi elemental standard solutions with a concentration of 1000 mg/L of different elements were

Table 2. Nutrient compositions analysis of raw materials

Nutrient	Unit	Amount
Moisture	%	74.6±2.52
Protein	%	15.7±2.23
Fat	%	5.7±1.06
Ash	%	3.9±1.78
Hg	μg/g	29.6±0.6
As	μg/g	14.1±6.7
Cd	μg/g	1.8±1.04
Pb	μg/g	12.05±6.94

Table 3. The detected alkanes in obtained oil from lantern fish by WP and SFE

Component	WP	SFE
Decane	✓	✓
2-Methyl-Decane	-	✓
3-Methyl-Decane	-	-
Undecane	✓	✓
Dodecane	✓	✓
Tridecane	-	✓
Pentadecane	✓	✓
Cyclohexadecane	-	✓
2,6,10,14-Tetramethyl-pentadecane	✓	✓

Table 4. The detected aldehydes, acids and amines in obtained oil from tuna by-products by WP

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

used. Also, Internal standard solution was 40 µg/L Rhodium (Rh), Indium (In), and Thulium (Tm).

Calculation of the concentration is generally done automatically by the software of the ICP/MS instrument. The following steps are performed for each element: The count rates are corrected according to the correction functions chosen, the count rates are measured in the zero member, and calibration and test solutions are normalized on the count rates of the internal standard. The calibration function is then calculated. By the use of the count rates, the calibration function and the dilution factor of the concentrations of the elements are calculated. The content, C, as mass fraction, of the element to be determined in mg/kg (µg/g) of sample is calculated using the following equation:

$$C = \frac{a \times V \times F}{m \times 1000}$$

Where a was the content (mg/L) of the element in the test solution, V was the volume (mL) of the digestion solution after being made up, F was the dilution factor of the test solution, and m was the initial sample mass (g).

Results and Discussion

Results of the Nutrient Compositions Analysis of Raw Materials

The amount of nutrient compositions and heavy metals levels of the raw materials (lantern fish) are mentioned in Table 2.

Yield

As mentioned in the materials and methods, in order to select the best SFE treatment, 9 treatments were defined based on different pressure and temperature and optimal treatment was selected in terms of the yield. As shown in Figure 1, the amount of oil extracted by different treatments of SFE method did not show a significant difference (p<0.05). However, the yield in P40T60 (Pressure 40 MPa, Temperature 60°C) treatment was higher than other treatments. Therefore, the extracted oils in P40T60 treatment (pressure 40 MPa, temperature 60°C, CO₂ flow rate of 15 g/min and dissolution time 3 hours) were selected for comparison with WP method and qualitative analysis.

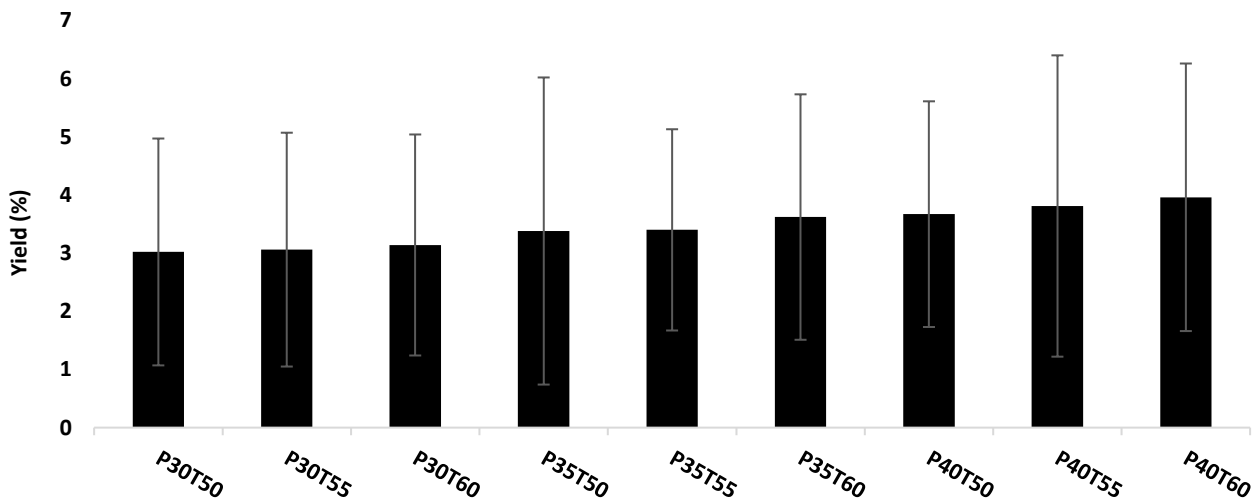


Figure 1. The yield of extracted oil from lantern fish in different treatments of SFE method.

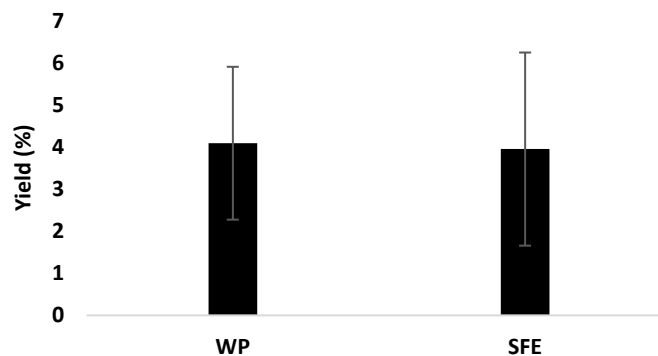


Figure 2. Yield of obtained oils from lantern fish by WP and SFE methods.

The amount of obtained oil by WP and SFE methods has been showed in figure 2. Yield in WP method was higher than SFE process, but this different was not significant ($p > 0.05$).

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. So, the yield of extracted oils are so important and that is affected by the extraction methods. 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 the higher yield in WP than SFE can be attributed to the mechanical pressure in 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 fat globules and cells, resulting in the release and fluidity of the oil, which can increase the efficiency (Chantachum *et al.*, 2000).

On the other hand, regarding the lower rate of oil extraction by SFE method compared to WP method, it can be said that in WP method, high temperature and mechanical pressure cause more separation of extracellular and intracellular fats than SFE and in the

result is a higher yield. In other words, high pressure and temperature had higher efficiency for oil extraction than supercritical CO₂. Therefore, about comparing the oil production yield by two extraction methods can be said that the difference in oil extraction rate was due to the role of extraction methods. The results of oil extraction from fish by-products by 4 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 (cold and enzyme extraction). 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 present study (Rubio-Rodriguez *et al.*, 2012).

Results of Volatile Component Analysis

Alkanes

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

Table 5. The amount of neutral lipids measured in oil extracted from fish lanterns by WP and SFE methods (Mean±SD)

Components (%)	WP	SFE
Wax esters (WE)	1.79±0.6	1.49±0.91
Triacylglycerols (TAG)	93.02±4.81	92.78±2.74
Free Fatty Acids (FFA)	3.69±1.12	2.87±0.83
Cholesterol (CHOL)	1.78±0.98	2.37±0.94

Table 6. Profile of fatty acids extracted from lantern fish by WP and SFE methods (mean±SD)

Fatty acids (%)	SFE	WP
C12:0	0.05±0.02	0.07±0.02
C14:0	1.98±1.23	2.11±1.06
C15:0	0.81±0.42	0.73±0.2
C16:0	14.36±6.28	15.74±5.01
C16:1	6.02±4.71	5.3±3.26
C17:0	0.78±0.16	0.83±0.09
C18:0	4.89±1.42	5.43±1.68
C18:1	18.07±6.57	21.47±5.68
C18:2	0.8±0.2	0.9±0.3
C18:3 n6	1.07±0.2	1.43±0.05
C18:3 n3	0.7±0.3	0.8±0.2
C20:0	1.42±0.67	1.18±0.1
C20:1	0.46±0.19	0.41±0.06
C20:4 n3	1.38±0.9	1.74±0.6
C20:4 n6	0.6±0.3	0.71±0.2
C20:5 n3 (EPA)	7.1±2.05	6.48±2.67
C22:0	0.65±0.29	0.44±0.3
C22:5 n3	2.09±0.6	1.7±0.84
C22:6 n3 (DHA)	10.2±2.18 ^a	7.1±2.06 ^b
EPA+DHA	17.3 ^a	13.58 ^b

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 Cohen (1982) showed that there is an inverse relationship between the formation of alkanes and the oxygen level, as the amount of alkanes increases with decreasing 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, in such a way that most of the evaluated alkanes were observed in this method (SFE). Also, temperature is one of the important factors that increase or decrease the formation of alkanes in the oxidation of lipids. In the present study, in WP method, the rate of alkanes formation has been reduced compared to the SFE method, that is probably due to the higher temperature than 70°C during the cooking process. So that, the presence of alkanes such as 2-Methyl-Decane and Cyclohexadecane was observed only in this method. Taati et al (2017) reported that the number of found alkanes in supercritical fluid method was higher than wet pressing method in fish oil extracted from by-products of canned tuna factory, that is agreement with the results of the present study.

Aldehydes, Acids and Amines

Investigating the existence or nonexistence of aldehydes in the present study showed that in extracted fish oils by WP (Table 4), 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 was exist only in samples from the SFE method.

Aldehydes, organic acids and amines are the most important volatile compounds, which the fishy odor and taste of fish oil strongly dependent on their presence. Some aldehydes, such as hexanal or nonanal, 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 (specially, temperature, ambient oxygen, light, and metals) (Miyashita *et al.*, 2018). 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. For example, trimethylamine amide oxide (TMA) 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).

In the present study, heptanal, hexanal and nonanal aldehydes were only detected in the obtained oil 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 auto-oxidation (as the main factor for the formation of aldehydes) (Roh *et al.*, 2006). However, in the oils obtained by SFE method, none of the targeted aldehydes were identified. This may be due to the lack of oxygen in the processes as well as the not so high temperature during extraction, which reduces the possibility of auto-oxidation (Rubio-Rodriguez *et al.*, 2012).

On the other hand, acetic acid was identified only in obtained oil 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),

About dimethylamine (fishy odor agent) detected in extracted oils by both WP and SFE methods can be said, due to the long time of the extraction process in the WP method, the effect of enzymes on trimethylamine oxide (TMAO) and DMA formation was probably longer and this substance probably has not been removed in the high temperature in the WP method and has also been added to the oil. In SFE method, in the extractor of the SFE, due to high pressure and constant flow of carbon dioxide, DMA has been separated from the material and partly absorbed in extracted oil, hence dimethylamine was found in the obtained samples from SFE method, too. The results of the identification and isolation of volatile compounds in tuna oil showed that the levels of volatile compounds (including alkanes, alkenes, alkynes, aldehydes, alcohols and ketones) in oils Concentrated with SFE was significantly lower than the sample of primary oil (crude oil) (Roh *et al.*, 2006).

In the study of comparing two methods WP and SFE for extraction of fish oil from tuna by-products, 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 both treatments (Taati *et al.*, 2017).

Results of Neutral Lipids Measurement

As can be seen in Table 5 the levels of wax esters in the two treatments WP and SFE did not show a significant difference ($p < 0.05$). Also, the amount of triacylglycerols (TAG) and free fatty acids (FFA) did not show significant difference between the two groups ($p < 0.05$). On the other hand, no significant difference in cholesterol was recorded in the two experimental treatments ($p < 0.05$).

Low levels of wax esters and high levels of triacylglycerols can indicate low levels of intracellular lipids and high levels of extracellular lipids bound to proteins in raw materials. Due to the weak bond of extracellular lipids with protein matrixes, the rate of oil extraction increases and consequently the amount of triacylglycerols increases. High levels of triacylglycerols in the WP and SFE methods may be due to high temperatures and physical pressure in the WP method and the dissolution of lipids in the supercritical carbon dioxide of the SFE method, which causes the release of oil from proteins bound to them. Relatively good yield in these methods in comparison with the amount of oil in the raw materials can also indicate the weak bonds between lipids and protein matrices in lantern fish. Also, high levels of triacylglycerols may indicate high levels of PUFAs in the oil.

On the other hand, hydrolysis of triacylglycerols produces free fatty acids. Therefore, with increasing levels of triacylglycerols and PUFAs, the production of free fatty acids also increases (Pacheco *et al.*, 2014). In the present study, the amount of triacylglycerols in the oils obtained by WP and SFE methods was high and consequently the amount of free fatty acids was also observed to be high. Study of the effect of cooking time and temperature of tuna by-products in WP method on the quantitative and qualitative parameters of the extracted oil showed that with increasing the amount of triacylglycerols, the levels of free fatty acids also increased, which is consistent with the results of the our study. Also, the levels of triacylglycerols and free fatty acids in the treatment at 95°C for 30 minutes were significantly higher than other treatments (Chantachum *et al.*, 2000). In another study, the results of qualitative analysis of oils extracted from different parts of rainbow trout by supercritical carbon dioxide showed that treatments containing higher triacylglycerols also had more free fatty acids (Fiori *et al.*, 2012). Chai *et al* (2012)

reported 26% of the fat content of *Benthoosema pterotum* (one species of Myctophidae) was phospholipids. Also, the amount of triglycerides, cholesterol and free fatty acids were 44.3%, 14.3% and 12.1%, respectively. The amount of wax esters was less than 1% in that study.

Result of Fatty Acid Analysis in Extracted Oil Samples

The results of the analysis of fatty acids in extracted fish oil by WP and SFE methods showed (Table 6) that there was a significant difference between DHA levels in SEF treatment and WP treatment, so that the amount of docosahexanoic Acid (DHA) in extracted oils by SFE (10.2%) was significantly higher than the amount of this fatty acid in WP treatment (p<0.05). Also, the amount of EPA (7.1%) in the extracted oils by SFE method was higher than WP treatment, but this difference was not significant (p<0.05).

The amount of DHA in SFE treatment was significantly higher than the WP treatment. This indicates that the supercritical fluid (carbon dioxide) used in this study in terms of temperature conditions (60°C), pressure (40 MPa), fluid flow rate (15 g of carbon dioxide per minute) and the solution time (3 hours) for the dissolution of DHA fatty acid was more appropriate and closer to its dissolution conditions. In the present study, the fluid conditions used to extract the oil were more selective than DHA. Hence, the amount of DHA in SFE treatment was higher than other method (WP) of oil extraction from lantern fish. Chai *et al* (2012) reported that the amount of saturated fatty acids in *Benthoosema pterotum* was 40.3%, and the levels of monounsaturated fatty acids, omega-6 and omega-3 fatty acids were 23.1%, 4.4% and 30.1% respectively that the amount of detected omega 3 fatty acids in that research was higher than the present study.

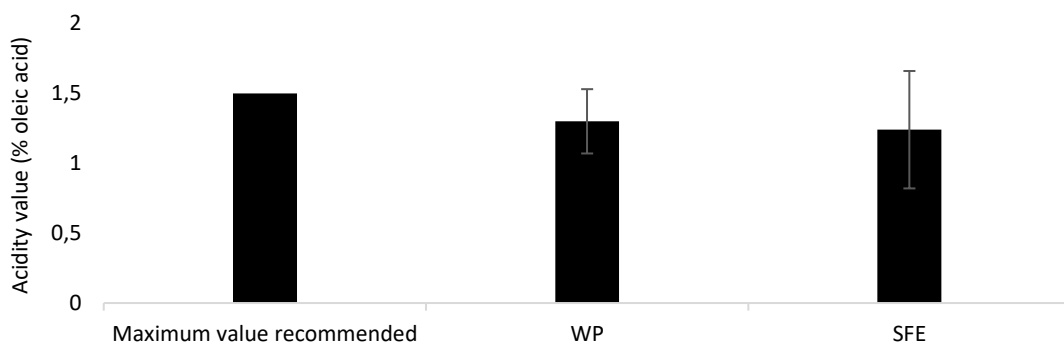


Figure 3. Acidity value (% oleic acid) of oil extracted from lantern fish by WP and SFE methods.

Table 7. The amount of heavy metals in oil extracted from lantern fish by WP and SFE methods

Heavy metal	WP (µg/g)	SFE (µg/g)
Mercury (Hg)	11±3.09 ^a	4±2.61 ^d
Arsenic (As)	4.23±2.81 ^a	1.76±0.65 ^c
Cadmium (Cd)	0.41±0.12 ^a	Trace ^b
Lead (Pb)	3.59±2.03 ^a	1.02±0.68 ^c

Result of Acidity Value

There was no significant difference between the acidity value (Figure 3) of WP and SFE treatments ($p < 0.05$). On the other hand, the acidity value of both treatments had a significant difference and less than the maximum recommended acidity value by Rubio-Rodriguez *et al* (2012) for fish oil ($p < 0.05$).

Oil acidity is one of the important parameters in oil quality, which is affected by the amount of free fatty acids and other non-fatty acids such as acetic acid. This indicates that the acidity of the oil can vary due to factors such as oil compositions and extraction methods. In general, oils containing higher amounts of triacylglycerol and PUFA have higher levels of free fatty acids which can reduce acidity. In our study, the amount of triacylglycerols and free fatty acids in the output oils of both WP and SFE methods was high. In contrast, acetic acid was observed only in samples obtained by SFE method. Therefore, it can be stated that the effect of free fatty acids on acidity was probably greater than non-fatty acids in the present study. On the other hand, the acidity value in WP and SFE treatments were significantly lower than the maximum acidity value recommended for the fish oil. The obtained data and the fact that the acidity value is not higher than the recommended level in both treatments can indicate the proper storage of raw materials and the desirability of the methods used for oil extraction.

Results of study by Hao *et al.*, (2015) on different methods of oil extraction from sturgeon showed that the acidity of oils obtained by supercritical fluid was significantly lower than WP methods, which is not consistent with the results of the present study. This discrepancy with the results of the present study may be due to use of modifiers in the Hao *et al.*, (2015) study.

Result of Heavy Metals Level in the Extracted Oil Samples

As shown in Table 7 there was a significant difference between the levels of heavy metals Hg, As, Cd and Pb between the experimental groups. The amount of Hg and As measured in the extracted oils by SFE method was significantly lower than the extracted oils by WP method ($p < 0.05$).

Mercury (Hg), arsenic (As), cadmium (Cd) and lead (Pb) are heavy and toxic metals that can accumulate in fish tissue (especially the liver). In the present study the amount of these metals in oil samples obtained by WP method was higher than SFE. The reason for the higher levels of these metals in WP treatment could be due to the pressing of the whole cooked mass and the release of water, oil and small solid particles from it. These factors cause heavy metals to leave the tissue and enter the oil.

On the other hand, the amount of heavy metals measured in the extracted oils by SFE method was low. In general, metals such as lead, arsenic, cadmium,

nickel, mercury, etc. are often attached to the polar compounds in the tissue (Hajeb *et al.*, 2015), and since oils are non-polar in nature, less heavy metals are attached to them. Therefore, due to the solubility of non-polar compounds (such as fatty acids) in CO_2 and the insolubility of polar compounds (such as heavy metals) in this fluid, it can be said that these factors probably caused lower levels of heavy metals in SFE treatment.

Conclusions

In the present study, the quantitative comparison and yield of extracted oil from lantern fish by WP and SFE methods showed 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 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 lantern fish 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 high quality fish oil.

Ethical Statement

Not applicable

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Author Contribution

Mehdi Taati Kelet (MTK): Methodology; Investigation; Analysis; Original Draft. Amir Homayoun Keyhan (AHK): Conceptualization; Analysis; Investigation; Supervision.

Mohammad Nobakht (MN): Investigation; Writing - review & editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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