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Concentrations of omega-3 fatty acids from rainbow sardine fish oil by various methods

Homayooni, B., *Sahari, M. A. and Barzegar, M.

Department of Food Technology, Faculty of Agriculture, Tarbiat Modares University, P.O. Box 14115-336, Tehran, Iran

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Abstract

Fractionation processes of fish oil fatty acid for the production of long-chain polyunsaturated fatty acids (PUFA) of omega-3 series have become very important because of the beneficial properties of these substances to human health, and, as a functional food. To obtain high yield of extraction and concentration of omega-3, rainbow sardine fish (*Dussumieria acuta*) oil were studied by: supercritical fluid extraction (T = 40, 50, 60ºC and 150, 250, 350 bar), urea complexation (T = 1, -5, -10ºC), and low-temperature crystallization with ethanol solvent (T = 10, 0, -5ºC). The omega-3 concentration yield and fatty acid compositions of concentrated oils were compared. The results of supercritical fluid extraction (SFE) method showed that the highest decrease in saturated (SFA) and monounsaturated (MUFA) fatty acids were obtained at 50 - 60ºC and 350 bars. Results revealed that the highest reduction of SFA and MUFA, and the highest increase in PUFA (omega-3), which resulted in the highest omega-3 yield (47.53%), was obtained at -10ºC in urea complexation method ($p < 0.05$). The higher PUFA concentrations in low-temperature crystallization method with ethanol solvent were obtained at -5ºC, with EPA and DHA purities rising up to 17.74 and 25.51%, respectively.

Introduction

Recent studies have clearly shown the importance of polyunsaturated fatty acids (PUFAs) and its nutritional value for human health (Kinsella, 1986). Fish is one of the richest sources of dietary supply of these fatty acids. Polyunsaturated fatty acids (especially the *n3* and *n6* PUFAs) have been found as the essential fatty acids with curative and/or preventive effects on cardiovascular disease, cancers, and neurodevelopment status in infants (Conner, 1997). Experimental data indicate that consumption of fish oil containing PUFA prevents and/or cures arterial hypertension (Millar and Waal-Manning, 1992), colon and prostate cancer (Marchioli, 2001; Marchioli, 2002), inflammatory diseases (Belluzi *et al*., 1993) and disorders of the immune system (Levine and Labuza, 1990). Besides, ecosapentaenoic acid (EPA C20:5*n3*) and docosahexaenoic acid (DHA C22:6*n3*), found only in fish and sea foods, play a vital role in development and functioning of the nervous system (brain), photoreception (vision), and the reproductive system (Alasalvar *et al.*, 2002; Skonberg and Perkins, 2002; Sidhu, 2003).

Fish (mackerel, salmon, mako shark, sardines, tuna, capelin …) is one of the richest and cheapest sources of dietary supply of ω-3 fatty acids, including EPA and DHA (Pettinello *et al*., 2000; Domingo, 2007; Guil-Guerrero *et al*., 2007). It has been reported that PUFA concentrates devoid of more saturated fatty acids are preferred to marine oils. They, at the lowest possible amount, supply the daily intake of total lipid (Haagsma *et al.*, 1982). Therefore, consumption of appropriate amounts of ω-3 fatty acids needs to be considered. In production of highly concentrated ω-3 components, separation of essential fatty acids is difficult. Marine oils are complex mixtures of fatty acids with varying chain lengths and instauration degrees. Therefore, commercial production of marine oil concentrates with high percentages of EPA and DHA is now a main concern for researchers in this area (Shahidi and Wanasundara, 1998). The available methods to produce PUFA concentrates include urea complexation, supercritical fluid extraction, low-temperature crystallization, molecule distillation, and lipase concentration. Urea complexation is the simplest and most efficient technique for obtaining ω-3 PUFAs concentrates in the form of free fatty acids. For elimination of fatty acids, urea complexation is a good established technique. In this method, alkaline hydrolysis of the oil triacylglycerol (TAG; using alcoholic KOH or NaOH) are performed and then these free fatty acids are mixed with an ethanolic solution of urea for complex formation. By cooling and filtering, these urea complexes may subsequently be easily removed during crystallization. Finally, enriched ω-3 PUFAs as liquid or non-urea complexed fraction (NUCF) is obtained (Wanasundara and

*Corresponding author.
Email: *sahari@modares.ac.ir*
Tel: +98 21 48292328; Fax: +98 21 48292200

Shahidi, 1999).

Supercritical extraction technology uses carbon dioxide as the solvent, and allows the design of environment-friendly processes, the processing of biological materials (carbon dioxide has a near-ambient critical temperature), and the possibility of obtaining products free of solvent residuals (Rajaei *et al*., 2005). Supercritical carbon dioxide (SC-CO_2) can be suitably modified by adjusting the system pressure for more solubility and selectivity of PUFAs. Supercritical fluid extraction (SFE) of PUFAs in both batch and continuous processes has been established. This selectivity occurs only on the basis of the number of carbons in the chain, not on the number of double bonds (Pettinello *et al.*, 2000).

Crystallization in low-temperature was originally developed to separate certain TAG, fatty acids, esters, and other lipids, which are highly soluble at temperatures above 0ºC in organic solvents, and they retain their solubility at temperatures down to -80ºC. Type and instauration degree of fatty acids changes the melting point and thus separation of fatty acid mixtures becomes possible. Long-chain saturated fatty acids (with higher melting points) crystallize at low temperatures and then PUFAs separate as liquid form. This process can be carried out in presence or absence of a special solvent.

Over the last few years, numerous applications of the methods have been reported in the literature for concentration of ω-3-PUFAs from fish oil (Haagsma *et al*., 1982; Shahidi and Wanasundara, 1998; Udaya *et al*., 1999; Wanasundara and Shahidi, 1999; Pettinello *et al*., 2000; Guil-Guerrero *et al.*, 2007; Rubio-Rodriguez *et al*., 2008). In the current study, we evaluated the fractionation of TAGs from sardine (*Dussumieria acuta*) oil using the SC-CO_2, as a function of the temperature and pressure and a function of temperature for the low-temperature crystallization method. Furthermore, urea complexation was used in combination with chemical hydrolysis as a function of the temperature to compare the results obtained from these methods. The fatty acid composition of SC-CO_2 extract and the other methods were measured, with the aim of obtaining fractions rich in ω-3 fatty acids, particularly the EPA and DHA.

Materials and Methods

Fish, sampling and processing

Approximately 30 kg of sardine (*Dussumieria acuta*) specimen was caught by long line near the West of the Qeshm Island (Ram Chaah) in December 2009. The length and weight of the specimen was 10 - 12 cm and 10.04 g, respectively. The fish was 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 sea to the laboratory (located in Tehran). The fish was transported to the laboratory within 15 hours after capture. The fish was stored under -18ºC until used. All analyses were carried out at the Department of Food Science and Technology; Tarbiat Modares University (Tehran). The chemicals used in the study were of the analytical grade. Fish oil was extracted from the homogenized white muscle using the Bligh and Dyer's (1959) method.

Fatty acid composition

Fatty acid methyl esters prepared by placing of oil (0.1 g) in a screw cap vial and 5 ml of methanolic NaOH (0.5 N) and hexane (1 ml) were added. The vial was then heated in boiling water bath for 10 min. After this time, the vial was removed from boiling water and allowed to cool to near room temperature. Then, 2.175 ml of methanolic boron trifluoride (BF_3) was added, as catalyst, and the vial was heated in boiling water bath. After 3 min, the vials were cooled and saturated sodium chloride solution (1 ml) and hexane (1 ml) were added to the vial. The cap was placed on the vial and the contents shaken thoroughly. The vial was allowed stand for 5 min and 0.2 µl of the top hexane layer containing the methyl esters was removed and injected to the gas chromatograph (GC; Unicam 4600, UK) target and then was analyzed using the conditions described below (Metcalf *et al.*, 1966).

Gas liquid chromatography (Unicom model 4600) was used to determine the fatty acid profile of sardine oil (% of total fatty acids); using a fused-silica capillary column (BPX70; SGE, Melbourne, Australia) with 30 m × 0.25 mm × 0.22 m film thickness, and a split injector (1.2 L injections) at 250ºC and FID at 300ºC. Helium was used as the carrier gas (pressure of 20 Psi). The temperature of the column was 190ºC. The peaks were identified according to their retention times using fatty acids methyl esters standards. All samples were run in triplicate. An internal standard method was used to calculate the fatty acid composition (the internal standard was C15:0).

SC-CO_2 fractionation

A Suprex NPS/225 system (Pittsburg, KS, USA) operated in the supercritical fluid extraction (SFE) mode was used for the extraction. In this study, extractions were accomplished with 3 ml extraction vessel. Nine extractions were carried out at constant static time of 20 min, temperatures of 40, 50 and 60 ºC,

Table 1. The factors designed for SC-CO_2 fractionation of fish oil

Run number	Pressure (bar)	Temperature (ºC)	Extraction yield (%)[a]
1	150	40	22.60 ± 5.50
2	250	40	39.85 ± 6.52
3	350	40	42.75 ± 4.20
4	150	50	18.25 ± 3.18
5	250	50	29.56 ± 0.29
6	350	50	35.07 ± 1.05
7	150	60	27.44 ± 1.42
8	250	60	34.63 ± 8.55
9	350	60	44.81 ± 0.47

[a]Mean ± standard division (n = 3)

pressure of 150, 250, and 350 atm, and dynamic time of 40 min (Letisse *et al*., 2006; Gironi and Maschietti, 2006; Perretti *et al*., 2007; Correa *et al*., 2008). Table 1 shows the SFE experimental conditions for fish oil fractionations. A Duraflow manual variable restrictor (Suprex, USA) was used in the SFE system to collect the extracted oil. The supercritical carbon dioxide flow rate through the Duraflow restrictor was approximately 0.3 ml/min (compressed). Exactly 0.5 g (0.1 mg) of lyophilized and powdered sardine fish was weighed out, and after mixing with an appropriate amount of glass beads, was placed in the extraction vessel (3 ml). The extraction was then performed with SC-CO_2 under the nine conditions mentioned in Table 1. The extracted oil was collected in 3 ml of ethanol in 5-ml volumetric flask. In order to increase the collection efficiency, the volumetric flask was placed in an ice bath during the extraction time.

Urea complexation

The mixture of fatty acids was obtained from sardine oil by the saponification and acidification method, as described in previous study (Udaya *et al.*, 1999). Separation of DHA and EPA from the hydrolyzed fatty acids mixture of sardine oil was carried out by urea-fatty acid adducts formation according to the following procedure: free fatty acids (10 g) were mixed with urea (10%, w/v) in ethanol 95%, and heated at 60 - 70ºC with stirring until the whole mixture turned into a clear homogeneous solution. Urea to fatty acids ratio changed by using different amounts of urea (up to 3 times). Initially, the urea-fatty acid adduct was allowed to crystallize at room temperature but other temperature (-10, -5, 1ºC) were used later for further crystallization. The crystals formed (urea-fatty acid adducts are also referred to as the urea complexing fraction) were separated from the liquid (non-urea complexing fraction) by filtration under suction using a Buchner funnel lined with a thin layer of glass wool. The filtrate was diluted with an equal volume of water and acidified to pH 2 - 3 with H_2SO_4 6M. An equal volume of hexane was subsequently added and the mixture was stirred thoroughly and transferred to a separator funnel. The hexane layer, containing liberated fatty acids, was separated from the aqueous layer containing urea. The hexane layer was washed with distilled water to remove any remaining urea and then dried over anhydrous sodium sulphate and the solvent was removed using a rotary evaporator.

Low temperature crystallization

In a typical experiment, 5 g of sardine oil was treated with 150 ml ethanol 99.9% as a solvent. The process of low temperature crystallization was carried out under a mild production with reduction of crystallization temperature slowly (within 1 - 6 h). Thereafter, the solution was cooled at different tested temperatures (-5, 0, and 10ºC) for 24 h and then, a liquid and solid phase (LP and SP, respectively) were obtained. The LP obtained was separated, while the remaining SP was filtered through sintered glass filters (100 µm pore size) and thoroughly washed with absolute ethanol. The resulting filtrate was then added to the LP and this liquor was concentrated in a vacuum rotary evaporator at 40 - 50ºC.

Statistical analysis

Data were presented as mean ± standard division (SD) of three replicates and were subjected to analysis of variance (ANOVA). Significant means were compared by one-way procedure tests and at $\alpha = 0.05$ level (n = 3).

Results and Discussion

Fatty acid composition

The fatty acid composition of sardine oil is summarized in Table 2 (sardine fat content = 6.89 ± 0.67). To have a over view on the results, fatty acids were grouped as SFAs (14:0, 16:0, 17:0, 18:0), MUFAs (16:1, 17:1, 18:1ω-9) and PUFAs (18:2ω-6, 18:3ω-3, 20:4ω-6, 20:5ω-3, 22:6ω-3).

Fish oil contains high level of highly unsaturated fatty acids (SFA < PUFA + MUFA), uniquely high levels of ω-3 fatty acids, including EPA and DHA. The fatty acid profile of fish oil varies quite considerably between and within different species.

In sardine oil, EPA and DHA compose 15.3% and 17.4% of total fatty acid content, respectively. This profile was used as a standard to determine differences in PUFA percentages among various concentrates obtained by the procedures employed. Palmitic acid (C16:0) was the major fatty acid among the SFAs. Also, C16:0 was the major fatty acid among all other fatty acids in sardine fish oil. The same results was obtained for *Channa* spp. Distribution of fatty acids in sardine oil were seen as PUFA > SFA > MUFA. It is in agreement with findings on cod liver and salmon (Robio-Rodriguez *et al*., 2008), which were shown to have PUFA higher than SFA. PUFA composition

Table 2. Fatty acid composition (of total fatty acids) of sardine fish oil

Fatty acids	g 100 g^{-1} from total lipid fraction[a]
C 14:0 (Myristic acid)	11.25 ± 0.75
C 16:0 (Palmetic acid)	24.36 ± 0.44
C 16:1 (Palmitoleic acid)	9.21 ± 0.70
C 17:0 (Margaric acid)	1.06 ± 0.50
C17:1 (*Cis*10-heptadanoic acid)	0.57 ± 0.11
C18:0 (Stearic acid)	1.66 ± 0.17
C 18:1 (Oleic acid)	5.12 ± 0.47
C 18:2 -6 (Linoleic acid)	7.07 ± 0.02
C 18:3 ω-3 (Linolenic acid)	1.67 ± 0.18
C 20:4 (Arachidonic acid)	2.15 ± 0.29
C 20:5 ω-3 (EPA)	15.39 ± 0.73
C 22:6 ω-3 (DHA)	17.45 ± 2.09
Other	2.95 ± 0.24
Σ SFA	38.33 ± 0.86
Σ MUFA	14.91 ± 0.34
Σ PUFA	43.74 ± 1.45
PUFA/SFA	1.13 ± 0.06
Σ -3	34.52 ± 1.17
Σ -6	9.22 ± 0.27
ω-3/ -6	3.74 ± 0.02
EPA+DHA/C16	1.34 ± 0.08

[a]Means ± standard division (n = 3)

may vary among fish species. The amount of PUFA in fishes is affected generally by diet. It is noteworthy that the highest quantity of PUFA in sardine fish oil was associated with ω-3 components. EPA and DHA were major in total ω-3PUFAs in sardine fish oil. The lowest amount of ω-3 fatty acids were found in linolenic acid (C18:3 ω-3). Distribution of PUFA in sardine oil was DHA > EPA > C18:2. The ω-3: ω-6 ratios have been suggested as a useful indicator for comparing relative nutritional value of fish oils. It has been suggested that a ratio of 1:1 to 1:5 would constitute a healthy human diet (Zuraini *et al*., 2006; Nazemroaya *et al*., 2011). This study shows that marine fish is richer in ω-3 than ω-6 PUFAs (ω-3: ω-6 = 3.74). The ratio reveals that marine fish is a good source of PUFA. In sardine fish oil, the ratio of PUFA/SFA was 1.13.

SC-CO_2 fractionation

Since various parameters can potentially affect the extraction process, optimization of experimental conditions is a critical step in the development of the SFE method. In fact, the fluid pressures and temperatures and the extraction time are generally considered to be the most important factors. Table 2 shows different conditions of SFE for extracting the fish oil and fractionating it.

In Table 3, the fatty acid contents of the oils extracted under different operational conditions of SFC are shown. Under these operational conditions, the extraction rate of SFA and MUFA is higher than PUFAs, where the major fatty acids (about 70% of total oil) in the extracted oil were SFAs and MUFAs.

In the current study, the parameters were selected according to the parameters of preliminary experiments and interactions among variables; as shown in Table 1, the highest amount of extraction yield was observed at 350 bars and temperature of

Table 3. Composition (% of total fatty acids) a of oil obtained by SFE[b]

Run number	SFAs (%)	MUFAs (%)	PUFAs (%)
1	56.16 ± 11.05	19.49 ± 0.48 ^{cde}	1.32 ± 0.19 ^{cd}
2	60.13 ± 6.72	19.85 ± 0.72 ^{cde}	1.06 ± 0.01 ^{d}
3	67.88 ± 0.84	20.87 ± 0.51 ^{bcd}	1.25 ± 0.12 ^{cd}
4	64.36 ± 4.19	21.65 ± 1.43 ^{b}	1.07 ± 0.05 ^{d}
5	66.16 ± 0.01	21.64 ± 0.11 ^{b}	1.57 ± 0.04 ^{c}
6	70.22 ± 2.71	21.26 ± 0.39 ^{bc}	1.57 ± 0.22 ^{c}
7	66.05 ± 2.88	18.89 ± 0.06 ^{e}	2.72 ± 0.61 ^{a}
8	67.62 ± 1.2	19.49 ± 0.04 ^{de}	2.15 ± 0.13 ^{b}
9	65.94 ± 0.84	23.20 ± 0.62 ^{a}	2.95 ± 0.47 ^{a}

[a]Mean ± standard division (n = 3)
[b]Means in a column with identical letters are not significantly different (P < 0.05)

60ºC. At lower pressures, the solubility of oil was affected by the vapor pressure of the oil; apparently, CO_2 acts as an ideal gas that does not have any special solvent characteristics in this case. However, at higher pressures, the solubility of the oil increased due to the increased density of CO_2. As the density increases, the distance between molecules decreases and the interaction between the oil and CO_2 increases, leading to greater oil solubility in CO_2 (De Castro *et al*., 1994).

As shown in Table 3, the highest amount of SFA and MUFA in extracted oil are obtained at 50 - 60ºC and 350 bar, which indicates that the solubility of fish oil has been increased as pressure and temperature increased. Higher levels of temperature and pressure significantly increased the yield of extraction. This behavior has been verified in the literature (Rajaei *et al*., 2005). In agreement with the results shown in Table 3, the major proportion of extracted oil is constituted short-chain fatty acids, which tend to migrate into the CO_2 phase. In other words, the oil that remained in the equilibrium cell after extraction contained high concentration of PUFAs, including EPA and DHA as the major components. Thus, it can be considered as a fractionation process to enrich fish oil.

Urea complexation

The fatty acid composition of sardine oil and PUFA concentrates obtained by urea inclusion are presented in Table 4. DHA and EPA were the major fatty acids, presented in concentrates after urea complexation. Some results (Haagsma *et al*., 1982; Udaya *et al*., 1999) have reported that in urea complexation which carried out for cod liver, DHA is the main portion in the non-urea complexing fraction for menhaden and seal blubber oils. However, in this fraction, content of EPA was less than DHA. In the mixture of fatty acids, the EPA content is lower than DHA, and thus EPA has a greater tendency to form a urea adduct than DHA. The highest amounts of DHA and EPA were found at temperatures of -10ºC and 1ºC, respectively, where DHA was enriched from 17.45 to 29.61% and EPA from 15.39 to 19.76%.

Table 4. The fatty acid composition[a,b] (g 100 g^{-1} from total lipid fraction) of sardine oil and PUFA concentrates obtained by urea complexation

Fatty acids	Sardine oil	PUFA concentrates		
		1ºC	-5ºC	-10ºC
C 14:0	11.25 ± 0.75 [a]	6.80 ± 0.29 [b]	7.87 ± 0.43 [b]	7.78 ± 0.12 [b]
C 16:0	24.36 ± 0.44 [a]	13.13 ± 0.81 [c]	14.83 ± 0.37 [b]	10.32 ± 0.07 [d]
C 16:1	9.21 ± 0.70	9.15 ± 0.34	9.32 ± 0.44	9.83 ± 0.18
C 17:0	1.06 ± 0.50	0.49 ± 0.26	0.35 ± 0.19	0.47 ± 0.03
C17:1	0.57 ± 0.11	0.71 ± 0.05	0.69 ± 0.16	0.66 ± 0.01
C18:0	1.66 ± 0.17 [ab]	1.47 ± 0.15 [b]	1.71 ± 0.10 [ab]	1.91 ± 0.07 [a]
C 18:1	5.12 ± 0.47 [a]	2.36 ± 0.81 [b]	3.11 ± 0.30 [b]	1.54 ± 0.02 [c]
C 18:2 ω-6	7.07 ±0.02 [b]	9.02 ± 0.73 [a]	8.45 ± 0.08 [a]	8.82 ± 0.07 [a]
C 18:3 ω-3	1.67 ± 0.18	2.00 ± 0.32	1.76 ± 0.02	1.85 ± 0.02
C 20:4 ω-6	2.15 ± 0.29 [c]	3.54 ± 0.02 [b]	3.56 ± 0.04 [ab]	3.97 ± 0.07 [a]
C 20:5 ω-3	15.39 ± 0.73 [c]	19.76 ±0.06 [a]	18.36 ± 0.37 [b]	19.47 ± 0.01 [ab]
C 22:6 ω-3	17.45 ± 2.09 [b]	28.55 ±0.78 [a]	27.59 ± 0.94 [a]	29.61 ± 0.52 [a]
Other	2.95 ± 0.24	3.10 ±0.98	2.31 ± 0.03	3.68 ± 0.03

[a]Mean standard division (n = 3)
[b]Means in a row with identical letters are not significantly different (P < 0.05)

Fractionation results showed a total reduction in SFAs and MUFAs. The highest reduction was observed at the -15ºC. Complete removal of saturated fatty acids by urea complexation may be impossible since some of the saturated fatty acids do not complex with urea during crystallization. Moreover, a significant total increase was found in PUFA. The PUFA content increased 1.45 fold from the original sardine oil. In urea complexation reaction, urea-to-fatty acid and crystallization temperature had an obvious effect on experiment results. Commonly, enrichment of PUFA in concentrate and overall recovery varied inversely with increasing this ratio and decreasing temperature. To achieve the maximum content of DHA and EPA in the concentrate with a reasonable recovery, these experimental variables should be controlled carefully.

Low temperature crystallization

Table 5 shows the fatty acids profiles of the solutions cooled at different temperatures (10, 0, and -5ºC). The basis of the concentration process employed here was solubility differences among fatty acids dependent on the degree of unsaturation. When a solution of saponified fatty acid is cooled, SFA and MUFAs crystallize before PUFAs. In all tested temperatures, with respect to crude oil, SFA and PUFAs significantly decreased and increased, respectively ($P < 0.05$), but MUFA purities did not change. The highest decrease of SFA and MUFA contents were observed at -5ºC, taking into account that with respect to crude oil a significant decrease in C16:0 purity in the concentrate was obtained at -5ºC. Higher PUFA concentrations were obtained at -5ºC, with EPA and DHA purities rising up to 17.74 and 25.51%, respectively. At higher temperatures, concentration efficiency decreased. The results

Table 5. The fatty acid composition[a,b] (g 100 g^{-1} from total lipid fraction) of sardine oil and PUFA concentrates obtained by low temperature crystallization

Fatty acids	Sardine oil	PUFA concentrates		
		10ºC	0ºC	-5ºC
C 14:0	11.25 ± 0.75 [a]	9.18 ± 0.41 [b]	9.39 ± 0.23 [b]	7.26 ± 0.09 [c]
C 16:0	24.36 ± 0.44 [a]	22.78 ± 0.43 [b]	23.42 ± 0.15 [ab]	14.10 ± 0.94 [c]
C 16:1	9.21 ± 0.70	8.34 ± 0.50	8.31 ± 0.08	8.93 ±0.11
C 17:0	1.06 ± 0.50 [a]	1.10 ± 0.26 [a]	ND	ND
C17:1	0.57 ± 0.11 [b]	ND*	ND	0.77 ± 0.02 [a]
C18:0	1.66 ± 0.17	1.45 ± 0.15	1.44 ± 0.04	1.59 ± 0.06
C 18:1	5.12 ± 0.47 [b]	5.94 ± 0.06 [a]	5.64 ± 0.14 [ab]	3.15 ± 0.11 [c]
C 18:2 ω-6	7.07 ±0.02 [c]	9.08 ± 0.57 [a]	7.66 ± 0.03 [bc]	8.14 ± 0.05 [b]
C 18:3 ω-3	1.67 ± 0.18 [bc]	2.02 ± 0.20 [ab]	1.59 ± 0.24 [c]	2.21 ± 0.10 [a]
C 20:4 ω-6	2.15 ± 0.29 [c]	2.97± 0.24 [b]	2.88 ± 0.03 [b]	8.87 ± 0.10 [a]
C 20:5 ω-3	15.39 ± 0.73 [b]	15.15 ± 0.16 [b]	15.94 ± 0.44 [b]	17.74 ± 0.57 [a]
C 22:6 ω-3	17.45 ± 2.09 [c]	20.53 ± 0.06 [b]	21.99 ± 0.5 [b]	25.51 ± 0.6 [a]
Other	2.95 ± 0.24 [a]	1.35 ± 0.27 [b]	1.66 ± 0.07	1.62 ± 0.04 [b]

[a]Mean standard division (n = 3)
[b]Means in a row with identical letters are not significantly different (P < 0.05).
*Non detected

indicated that the amount of ω-3 PUFAs in non-crystallized portion increased as the temperature of the process decreased.

Conclusion

In the current study, we examined three different methods ω-3 PUFAs extraction and concentration processes for sardine oil. Under the studied conditions, supercritical CO_2 was slightly effective in fractionating sardine oil in the triacylglycerol form. It seems that, fractionation process can be difficult for the complex structure of the fish oil and its triacylglycerol diversity. Based on the findings of the current study, although the concentrated oil of ω-3PUFAs needs one more extraction process, a reasonable efficiency can be achieved for ω-3PUFAs concentration. The method of low temperature crystallization was tested, and can be considered as an alternative to the urea inclusion method for obtaining PUFAs concentrates for the food industry. The methods have comparable efficiencies in PUFAs concentration, but the former one has additional advantage of using safer and more bio-compatible processes. However, in enrichment of the fish oil, urea complexation method as a chemical process was more effective than the physical method of low temperature crystallization. Considering that 1:3 of the total fatty acids of the studied fish oil are ω-3, it seems that the fatty acids of interest would be present in almost all triglyceride molecules. Therefore, its fractionation is difficult. The results suggest that concentrates can be commercialized to produce dietary supplements to balance between the ω-3/ω-6 PUFAs and concentrate of EPA and/or DHA produced at higher purities in the food or pharmaceutical industry.

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