DEVELOPMENT OF A SUPERCRITICAL FLUID EXTRACTION METHOD FOR DETERMINATION OF TOTAL FAT CONTENT IN FISH PRODUCTS

Favati F.* [1], Galgano F.[1], Di Lauro R.[1], Pietrafesa A.[1], and Lo Voi A.[2]
[1]Dept. of Biology DBAF, Univ. of Basilicata, C.da Macchia Romana - 85100 Potenza – Italy
[2]S.S.I.C.A., Via Nazionale 121/123 - 84012 Angri - Italy
Favati@unibas.it fax +33-0971-205503

A supercritical fluid extraction method was developed for the assessment of the fat content in fish products. Various fish species (bass, salmon, eel, cod, tuna) having a fat content ranging from 0.2 to 25%, were utilized in the study as fresh or commercially frozen products. The fat recovery efficiency of Soxhlet, Bligh and Dyer and supercritical CO_2 extraction methods was compared and the optimized working parameters with supercritical CO_2 were 35 MPa, 60 °C, and use of EtOH (5% mol/mol) as modifier.

INTRODUCTION

Fish oils contain high levels of polyunsaturated fatty acids and because of the importance of these moieties for human health, of major interest is the accurate determination of total fat content in fish tissues, as well as the identification and quantitative assessment of the various fatty acids. Triacylglycerols represent the majority of lipids in fish tissues, and the removal of these compounds from the animal matrix is usually achieved using conventional analytical procedures such as Soxhlet extraction; however, a significant amount of phospholipids is also present and the complete recovery of this fraction can be achieved with acid hydrolysis extraction techniques or with methods based on chloroform-methanol extraction, such as the Bligh and Dyer [1] or the Folch, Less and Sloane-Stanley methods [2]. While acid hydrolysis is effective for total fat determination, it can cause chemical degradation of the extracts that, as a result, are not more suitable for fatty acid profiling of the matrix. Conversely, the methods based on chloroform-methanol extraction allow complete recovery of the various lipids with no major chemical damages of the different moieties. However, all these procedures are time-consuming, require the use of hazardous organic solvents, and often necessitate highly skilled technicians to obtain repeatable results.

Supercritical fluid extraction (SFE) has been utilized as an analytical tool for total fat determination in food [3-6], with several advantages, such as less time required, no or little use of organic solvents and no chemical damages of the recovered compounds, allowing to obtain a lipid fraction suitable for fatty acid profiling. In this study, a SFE method using supercritical carbon dioxide (SC-CO_2) and ethanol as modifier, was developed to assess the total fat content in fish products having a fat content ranging from 0.2 to 25%.

MATERIALS AND METHODS

Fish samples. The experiments were conducted using several fish species, fresh or frozen, having a quite different moisture and fat content, ranging from 56 to 86% and from 0.2 to 25%, respectively (Table 1). Among the fresh samples, European eel (*Anguilla anguilla*),

European sea bass (*Dicentrarchus labrax*), Atlantic salmon (*Salmo salar*) and albacore tuna (*Thunnus alalunga*) were used for the extractions, while two different commercial brand of frozen Atlantic cod (*Gadus morhua*) were also tested. A local fish farm provided samples of European sea bass of four different sizes, 150 ± 25 g, 210 ± 25 g, 340 ± 25 and 475 ± 25 g, respectively. All the fresh fishes, when needed, were cleaned and the extractions performed on the edible flesh; from the European eel two samples were prepared removing partially or totally the skin.

	Fish sample									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Moisture (%)	72.3	69.4	67.1	63.9	63.3	57.4	56.0	86.0	85.4	70.5
Fat (%)	8.4	9.4	11.8	16.4	16.5	22.5	25.0	0.2	0.2	0.3

Table 1. Moisture and fat content of the various fish samples: 1-European sea bass (150 ± 25 g); 2-European sea bass (210 ± 25 g); 3-European sea bass (340 ± 25 g); 4-European sea bass (475 ± 25 g); 5-Atlantic salmon; 6-European eel partially skinned; 7-European eel completely skinned; 8-Atlantic cod brand "A"; 9-Atlantic cod bran "B"; 10-albacore tuna. Fat content assessed by Soxhlet extraction.

Extractions. Supercritical fluid extractions were performed with the home built apparatus shown in Figure 1, using a 192 cm^3 extraction vessel (316 SS tube, 800 mm x 17.5 mm i.d.). The unit was equipped with two gas boosters (AG-30 and AGT-62/152, Haskel Corp., Burbank, CA), the first allowing to reach a working pressure of 30 MPa, the second a pressure of 138 MPa. The pressurized CO_2 flowed through an heating coil (HC) prior entering the extraction vessel, and the solute laden stream leaving the extractor flowed to the separator through a micrometering valve (MV), utilized to adjust the flow rate at the desired value. The addition of a modifier was possible through the use of a HPLC pump (mod. 1050 Hewlett-Packard, Rome, Italy) having a maximum working pressure of 40 MPa. The solvent was added to the supercritical fluid immediately prior entering the extraction vessel, and the use of EtOH as modifier was also tested at 2.5, 5.0 and 10.0 mole % levels when working at 35 MPa. As extraction conditions the following combinations of pressures and temperatures were tested: 35 MPa/60°C, 50 MPa/60°C, 50 MPa/80°C, 70 MPa/60°C. The CO_2 flow rate was set to 18 g/min, and the total amount of solvent that went through the bed was 980 g, according to the operating conditions, until no significant amounts of extracted material could be collected. The experiments were conducted on a constant quantity of finely comminuted fish sample (45 g) "as is" or added with sand or with Hydromatrix (Varian, Torino, Italy) in the ratio 70/30, 50/50 and 40/60. A plug of glass wool was put at both ends of the extractor and after each test the lines were washed with diethyl ether; afterward the extract and the washing solution were pooled, added with anhydrous sodium sulfate, filtered and the solvent removed at 50°C under reduced pressure using a Rotavapor (Büchi, Milan, Italy).

The determination of the total fat content of the samples was also performed for comparison using the method proposed by Bligh and Dyer [1] and by Soxhlet extraction for 6 h, using petroleum ether as the solvent.

Analyses. Moisture content of the matrix was determined according to standard methods [7]; the amount of extractable lipids was determined gravimetrically, after the extraction step, on the basis of the weight of the original samples. Fatty acid composition of the extracts was

assessed by gas chromatographic (GC) analysis of the fatty acid methyl esters (FAMEs) using a Varian 3400 GC (Varian, Torino, Italy) equipped with a flame ionization detector. FAMEs

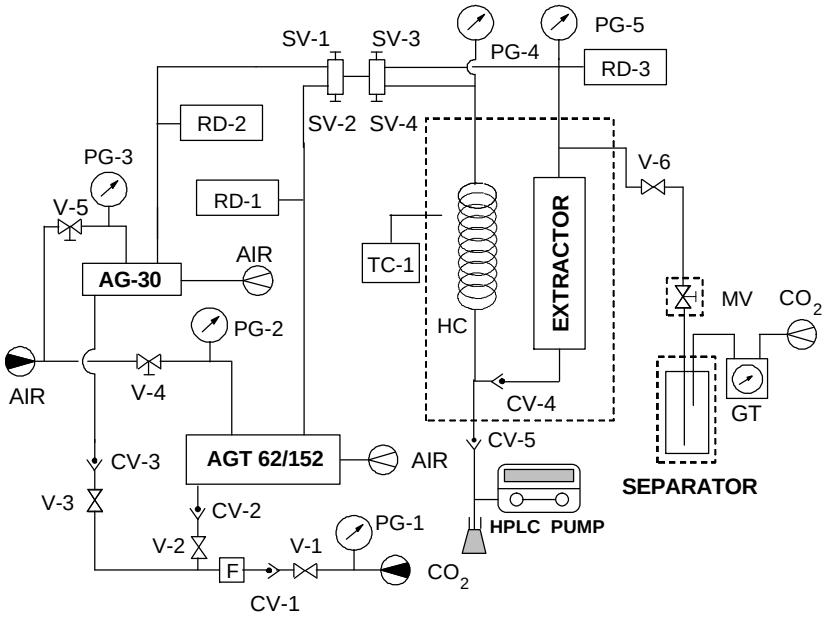

Figure 1. Supercritical fluid extraction system. Dashed lines represents thermostated regions. PG1-5= pressure gauges; V1-6= valves; SV1-5= valves; CV1-5= check valves; F= filter; RD1-3= rupture disks; MV= micrometering valve; AG-30 and AGT62/152= Haskel gas-boosters; TC= thermocouple; HC= heating coil; GT= gas flow meter and gas totalizer.

were prepared using AOAC Method 991.39 [7] and 1 μL of the prepared FAMEs in isooctane was injected onto a DB-23 column (60 m, 0.25 mm diameter, 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA) with He as the carrier gas at the flow rate of 1.4 mL/min. The injector and detector temperatures were set at 250 and 300 °C, respectively, while the makeup gas (He) flow rate was set at 30 mL/min. The column temperature was programmed as follows: 90 °C for 2 min, 10 °C/min to 180 °C, held for 10 min, then 5 °C/min to 240 °C and held for 16 min, for a total analysis time of 49 min. FAMEs were tentatively identified by comparison of their retention times with those of pure standards (Sigma, Milano, Italy) (Matreya, Milano, Italy).

RESULTS AND DISCUSSION

In the literature, SFE of fish oil has been reported, from substrates such as rainbow trout [8], Atlantic mackerel [9] and sardine [10], while fractionation and purification by SC-CO_2 of fish oils extracted with organic solvents has also been studied [11,12]. However, no available data can be found regarding the study of a SFE method applied for the total fat determination in fish products. In order to optimize the SFE process, the influence of several parameters was investigated: percentage of Hydromatrix mixed to the sample, extraction pressure and temperature, addition of ethanol as modifier, fish substrate.

Because the high water content of the different kind of fishes (up to 86% for frozen cod), the use of a suitable water adsorbent was necessary in order to adequately load the extractor

and not to have plugging or channeling problems during the extractions. Initial experiments were conducted on small amounts of matrix (15-20 g) loosely loaded in the extractor, but the recoveries obtained also working at the highest temperatures and compression levels of the supercritical fluid were less than 10% compared to Soxhlet extraction. The addition of sand at different percentages did not result in any improvement of the extraction recoveries; therefore the utilization of Hydromatrix was tested at three different levels, 30, 50 and 60%. While with samples having a relatively low water content (European eel - about 57%; Atlantic salmon - about 63%) already with 30% of Hydromatrix it was possible to obtain a free flowing product, a higher percentage (50%) had to be utilized with those fishes, such as frozen Atlantic cod, much richer in water. The tests showed that while a minimum amount of Hydromatrix was needed, increasing its presence up to 60% did not result in any improvement of fat recovery; therefore the addition of 50% of Hydromatrix was set as a fixed extraction parameter.

The effect of pressure and temperature was tested for CO_2 densities ranging from 0.869 g/cm^3 (35 MPa/60°C) to 0.992 g/cm^3 (70 MPa/60°C) [13, 14]. Initial tests were conducted on samples of Sea bass (150 $\pm$ 25 g) having a fat content of 8.4%. The extractions were run in triplicate, and the calculated recoveries at 70 MPa/60°C, 50 MPa/80°C, 50 MPa/60°C and 35 MPa/60°C were 85.7%, 81.5%, 80.8 and 83.7, respectively. Statistical analysis of the data showed that the extractions were very reproducible, with calculated RSD values less than 1%.

Because the recoveries were lower than those obtained by Soxhlet extraction with petroleum ether, the addition of ethanol as modifier was tested. The positive effect of the use of ethanol on the total fat recovery has been reported for the SFE of vegetable or animal matrices, and different percentages of EtOH addition have been suggested for maximization of fat recovery [3, 8, 15, 16]. In this work the use of ethanol was studied using the solvent at the following levels: 2.5, 5.0 and 10.0 mole %. In order to optimize the extraction process several tests were conducted on samples of Sea bass of different sizes and of European eel, having a fat content ranging from 8.4 to 25.0 %; however, due to the pressure limits of the HPLC pump utilized for EtOH addition (maximum working pressure 40 MPa) the use of the modifier was studied only at the extraction pressure of 35 MPa. When EtOH was added at 2.5 mole %, the percentages of lipid recovery did not show any appreciable increase; conversely, at 5 and 10 mole % the fat recovery showed different increments, that were higher for the samples with higher fat content. Because the percentages of recovery were not substantially different using EtOH at 5 or 10 mole %, and because the use of the higher modifier levels required a much longer treatment of the sample for complete removal of the solvent residues, the addition of EtOH at 5 mole % was set as an extraction parameter.

Fish can contain different levels of fat according to the species, but also to the stage of growth; therefore, in this study several species of fish were studied, and for one fish of special interest for fish farmers (European Sea bass) the study was also conducted at four different levels of growth, differentiating the samples according to the size. In Figure 2 the extraction efficiencies of four extraction methods, namely Soxhlet (with petroleum ether), pure SC-CO_2 (35MPa/60°), SC-CO_2+ EtOH (5 mol %) and Bligh and Dyer (B&D), are compared. The data are calculated assuming as 100% recovery the amount of fat extracted using the Soxhlet system. The utilization of pure SC-CO_2 resulted always in lower recoveries, while the data obtained using the Soxhlet or the B&D extraction methods were always very similar, with the exception of the samples of cod and albacore tuna, characterized by a low fat content ($\leq$ 0.3%). However, with the B&D method the calculated RSD values were always much higher than those obtained with the Soxhlet extraction, and it has been reported in the literature that the B&D method can lead to high variability in the results [17].

The use of EtOH caused an increase in fat recovery, but only for the eel samples the amounts extracted were similar to those obtained with the B&D method. In the case of the commercial frozen cod and for the tuna, characterized by a very low fat content, SFE with the use of EtOH resulted in recoveries up to three fold higher than those obtained with the Soxhlet method. However, the fat percentages assessed by SFE were 0.47 and 0.52%, similar

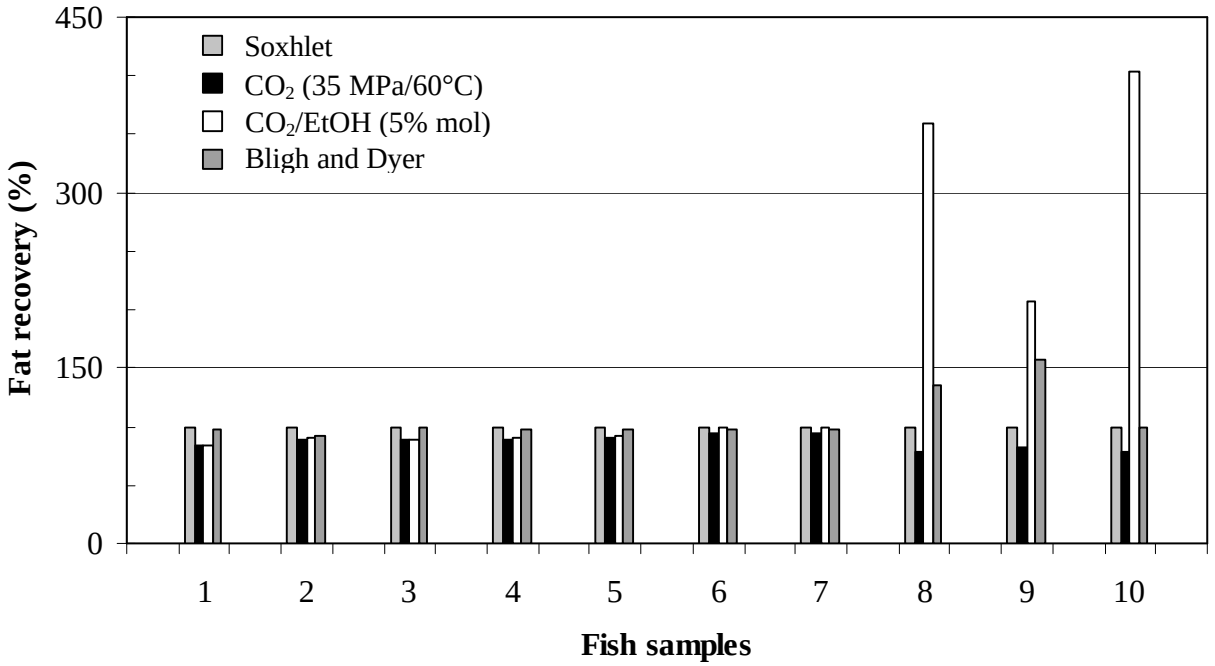

Figure 2. Extraction efficiencies of the different tested methods calculated on a 100% recovery for Soxhlet extraction. Numbers refer to: 1-European sea bass (150 ± 25 g); 2-European sea bass (210 ± 25 g); 3-European sea bass (340 ± 25 g); 4-European sea bass (475 ± 25 g); 5-Atlantic salmon; 6-European eel partially skinned; 7-European eel completely skinned; 8-Atlantic cod brand "A"; 9-Atlantic cod bran "B"; 10-albacore tuna.

to the value of 0.5% declared by the producers on the labels. Also the B&D data were higher than those obtained with Soxhlet extraction; however, the resulting fat percentages were about 2/3 of those declared on the labels, and while for the SFE experiments the calculated RSD reached a maximum value of 1.5%, with the B&D the RSD values were well above 13%.

The determination of the fat content in fish products is frequently associated with the determination of the fatty acid composition, because of the importance of the n-3 and n-6 fatty acids for the human diet. In order to point out any possible influence of the extraction methods on the fatty acid composition of the recovered fatty fraction, GC analysis of the extracts was also performed. The fatty acid profile of each sample extracted with the four tested methods is reported in Table 2 and 3, and from the analytical data no major differences could be pointed out for the recovered extract.

Fatty acid	European sea bass															
	(150 ± 25 g)				(210 ± 25 g)				(340 ± 25 g)				(475 ± 25 g)			
	Extraction method				Extraction method				Extraction method				Extraction method			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
C 14:0	7.6	7.5	7.0	7.0	6.6	7.0	7.0	6.7	7.0	6.5	6.9	6.6	6.7	6.6	6.6	6.5
C 15:0	0.4	0.5	0.7	0.4	0.6	0.7	0.7	0.3	0.6	0.6	0.6	0.0	0.6	0.6	0.6	0.6
C 16:0	23.2	24.2	23.3	23.0	21.8	22.5	22.4	22.6	23.0	22.6	23.0	23.3	23.6	23.1	23.2	22.5
C 17:0	1.2	1.2	1.2	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.0	1.1	1.0	1.1
C 18:0	3.1	3.3	3.2	3.4	3.1	3.1	3.1	3.4	3.1	3.2	3.1	3.5	3.3	3.3	3.4	3.2
Total Satured	35.5	36.7	35.4	34.9	33.2	34.4	34.3	34.1	34.8	34.0	34.7	34.5	35.2	34.7	34.8	33.9
C 16:1	7.8	8.0	7.7	7.5	7.3	7.5	7.6	7.5	7.6	7.3	7.5	7.4	7.4	7.3	7.3	7.5
C 17:1	0.8	0.9	0.8	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.7	0.8	0.8	0.8	0.8
C 18:1n-9	18.4	19.1	18.5	18.4	18.3	18.5	18.6	18.6	18.7	18.9	19.0	19.0	20.0	20.2	20.5	17.8
C 18:1n-7	3.0	3.2	3.0	2.9	2.9	2.9	3.1	3.0	2.9	3.0	3.0	3.0	2.9	2.9	2.9	3.0
C 20:1n-9	4.0	4.0	4.0	4.0	4.0	3.8	4.0	3.9	3.6	3.6	3.6	4.0	3.6	3.6	3.7	3.9
C 20:1n-7	0.7	0.4	0.7	0.6	0.7	0.7	0.7	0.7	0.7	0.6	0.6	0.7	0.6	0.6	0.6	0.7
C 22:1n-11	3.5	3.7	3.4	3.4	3.6	3.5	3.3	3.3	3.3	3.1	3.0	3.2	3.0	3.1	3.1	3.4
Total Monouns	38.2	39.3	38.1	37.5	37.6	37.7	38.1	37.8	37.6	37.3	37.5	38.0	38.3	38.5	38.9	37.1
C 18:2	3.9	3.8	3.9	3.9	3.7	3.7	3.7	3.9	3.6	3.7	3.7	4.0	3.4	3.5	3.5	3.8
C 20:2	tr	tr	tr	0.0	tr	tr	tr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C 20:4	0.6	0.4	0.5	0.4	0.6	0.5	0.6	0.5	0.5	0.6	0.6	0.0	0.5	0.5	0.5	0.6
Total n-6	4.5	4.2	4.4	4.3	4.3	4.2	4.3	4.4	4.1	4.3	4.3	4.0	3.9	4.0	4.0	4.4
C 18:3	1.2	1.7	1.2	1.2	1.2	1.1	1.2	1.2	1.2	1.2	1.2	1.2	1.1	1.1	1.1	1.2
C 18:4	1.8	1.7	1.8	1.7	1.9	1.8	1.8	1.8	1.9	1.9	1.9	1.7	1.8	1.8	1.8	1.8
C 20:3	0.6	0.5	0.6	0.8	0.7	0.6	0.6	0.8	0.6	0.6	0.7	0.8	0.6	0.6	0.6	0.8
C 20:5	7.8	6.9	7.6	7.6	8.1	7.6	7.3	7.8	8.0	8.0	8.1	7.7	7.3	7.5	7.4	8.3
C 21:5	0.4	tr	tr	0.0	tr	tr	tr	0.0	tr	0.3	tr	0.0	0.0	0.2	0.0	0.0
C 22:4	0.0	0.0	0.0	0.0	tr	tr	tr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C 22:5	1.4	1.3	1.4	1.4	1.5	1.4	1.3	1.4	1.3	1.3	1.4	1.4	1.3	1.3	1.3	1.4
C 22:6	8.2	7.1	8.1	8.4	8.8	8.4	8.3	8.8	8.0	8.0	8.0	8.8	7.5	7.5	7.4	9.2
Total n-3	21.4	19.2	20.7	21.1	22.2	20.9	20.5	21.8	21.0	21.3	22.3	21.6	19.6	20.0	19.6	22.7
n-3/n-6	4.8	4.6	4.7	4.9	5.2	5.0	4.8	4.9	5.1	5.0	4.9	5.4	5.0	5.0	4.9	5.2
C 16:4 n1	0.4	0.4	0.3	0.0	0.5	0.5	0.5	*0.2*	0.6	0.5	0.5	0.0	0.5	0.6	0.5	0.0
Unidentified peaks	2.1	2.2	2.1	1.8	2.4	2.6	2.3	2.2	2.5	2.5	2.4	2.1	2.7	2.5	2.5	1.9

Table 2. Fatty acid percentage composition of extracts obtained with different extraction methods from European sea bass (samples of four different sizes). (A-Soxhlet; B-CO_2 at 35 MPa/60 °C; C-CO_2 at 35 MPa/60 °C + EtOH 5% mol; D-Bligh and Dyer).

Fatty acid	Atlantic salmon				European eel (1)				European eel (2)				Atlantic cod "A"				Atlantic cod "B"				Albacore tuna			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
C 14:0	7.6	7.7	7.8	7.2	4.6	4.9	4.9	4.8	5.4	5.8	5.4	4.6	3.9	4.4	3.0	4.1	3.4	2.4	2.3	3.4	7.2	6.8	4.8	7.3
C 15:0	0.5	0.5	0.3	0.0	0.6	0.6	0.6	0.6	0.6	0.7	0.6	0.5	0.9	0.8	0.7	0.9	0.0	0.0	0.2	0.0	1.9	1.7	1.6	1.8
C 16:0	17.7	18.0	18.0	18.3	22.2	22.7	23.0	23.7	22.4	22.4	22.2	22.0	31.7	30.7	31.5	30.9	22.8	23.4	24.8	22.9	35.2	34.9	35.3	35.5
C 17:0	1.1	1.2	1.2	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.0	1.0	1.0	1.1	1.0	0.8	0.7	0.8	0.8	2.6	2.1	2.5	2.2
C 18:0	3.0	2.9	2.9	3.1	4.0	3.9	4.0	4.3	3.8	3.6	3.8	4.3	5.0	4.9	5.8	5.0	3.1	3.2	3.4	3.0	10.2	10.9	15.3	10.8
C 24:0	0.0	0.0	0.0	0.0	0.9	0.9	0.9	0.9	0.7	0.7	0.8	1.0	0.0	0.7	0.4	0.0	0.0	0.3	0.2	0.0	0.0	0.0	0.0	0.0
Total Satured	29.9	30.3	30.2	29.7	33.4	34.1	34.5	35.4	34.0	34.3	33.9	33.4	42.5	42.5	42.5	41.9	30.1	30.0	31.7	30.1	57.1	56.4	59.5	57.6
C 14:1	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C 15:1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.3	0.4	0.0	0.0	0.0	1.0	0.0
C 16:1	8.0	8.3	8.2	7.9	10.0	10.3	10.1	10.0	9.7	9.9	9.6	9.5	5.1	5.2	4.0	5.2	6.9	4.9	4.5	6.7	6.5	6.8	4.4	7.1
C 17:1	0.7	0.7	0.7	0.3	0.7	0.7	0.7	0.7	0.8	0.8	0.8	0.6	0.3	0.3	0.0	0.0	0.0	0.6	0.5	0.0	1.1	0.4	1.0	0.0
C 18:1n-9	14.8	14.9	15.0	15.0	33.9	33.6	33.2	34.5	30.1	29.7	30.0	33.2	15.1	14.6	15.8	15.3	19.9	19.0	18.9	19.6	15.6	17.6	17.4	20.2
C 18:1n-7	3.5	3.6	3.6	3.6	4.5	4.4	4.4	4.5	4.2	4.1	4.2	4.5	3.1	3.0	3.1	3.2	3.2	3.3	3.1	3.3	2.7	3.3	3.0	3.3
C 20:1n-9	6.1	6.0	6.2	6.0	1.1	1.0	1.1	1.1	2.1	2.0	2.2	1.2	2.2	2.3	2.0	2.3	5.9	5.1	4.9	6.0	0.4	1.1	0.5	0.6
C 20:1n-7	0.6	0.6	0.6	0.0	0.4	0.4	0.2	0.0	0.5	0.5	0.6	0.5	0.0	0.0	0.0	0.0	1.0	1.0	0.9	1.1	0.0	0.0	0.0	0.0
C 22:1n-11	5.6	5.4	5.5	5.3	0.0	0.0	0.0	0.0	0.8	0.7	0.8	0.2	1.0	1.1	0.9	1.1	4.2	3.5	3.1	3.7	0.0	0.0	0.0	0.0
Total Monouns.	39.4	39.4	39.8	38.2	50.5	50.7	49.6	50.8	48.1	47.8	48.0	49.7	26.9	26.6	26.6	27.1	41.1	37.5	36.3	40.5	26.3	29.3	27.2	31.1
C 18:2	4.8	4.8	4.8	4.9	2.5	2.4	2.4	2.5	2.5	2.5	2.5	2.4	1.6	1.6	1.4	1.7	1.0	1.0	0.9	1.2	2.2	2.1	1.9	1.9
C 20:2	0.0	0.0	0.0	0.0	0.6	0.6	0.7	0.6	0.5	0.5	0.6	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C 20:4	1.4	1.4	1.5	1.5	0.7	0.7	0.8	0.7	0.9	0.9	1.0	0.8	0.0	0.0	0.0	0.0	0.4	0.6	0.6	0.4	0.0	0.0	0.0	0.0
Total n-6	6.2	6.2	6.2	6.4	3.8	3.7	3.9	3.7	3.9	3.9	4.1	4.1	1.6	1.6	1.4	1.7	1.4	1.5	1.4	1.6	2.2	2.1	1.9	1.9
C 18:3	1.4	1.4	1.4	1.4	0.8	0.8	0.9	0.8	0.8	0.8	0.9	0.9	0.8	0.9	0.0	0.9	0.0	0.2	0.4	0.0	0.4	1.0	0.0	0.0
C 18:4	2.2	2.2	2.2	2.2	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.2	0.0	0.0	0.0	0.0	0.0	0.5	0.2	0.3	0.4	0.0	0.0	0.0
C 20:3	0.2	0.2	0.0	0.0	2.3	2.1	2.3	2.1	1.7	1.7	1.8	2.4	2.4	2.0	2.6	2.2	1.3	1.5	1.3	1.3	1.8	1.5	1.2	1.4
C 20:5	7.5	7.3	7.4	7.8	3.2	3.0	3.4	2.8	3.2	3.2	3.4	3.5	7.8	7.3	6.1	7.5	7.9	6.5	6.2	7.7	3.3	2.3	1.8	2.3
C 22:5	2.8	2.7	2.8	2.8	2.1	2.0	2.0	1.9	2.1	2.1	2.2	2.1	1.3	1.4	1.4	1.3	2.1	1.6	1.5	2.0	0.0	0.0	0.0	0.0
C 22:6	8.2	8.0	8.1	9.1	1.7	1.5	1.6	1.5	3.0	3.0	3.1	1.8	16.8	15.4	17.0	15.1	14.6	18.4	18.3	14.9	8.5	7.5	5.2	5.8
Total n-3	22.3	21.9	21.8	23.2	10.0	9.4	10.2	9.1	11.3	11.3	11.8	10.9	29.1	26.9	27.0	27.0	26.0	28.7	28.0	26.2	14.5	12.2	8.2	9.5
n-3/n-6	3.6	3.6	3.5	3.6	2.6	2.5	2.6	2.5	2.9	2.9	2.9	2.7	18.2	16.8	19.3	15.9	18.6	19.1	20.0	16.4	6.6	5.8	4.3	5.0
C16:4n-1	0.5	0.6	0.3	0.0	0.4	0.4	0.2	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C16:3n-4	0.0	0.0	0.0	0.0	0.4	0.4	0.2	0.0	0.6	0.6	0.6	0.4	0.0	0.0	0.0	0.0	1.5	2.0	2.3	1.6	0.0	0.0	0.0	0.0
C16:4n-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unidentified peaks	1.8	1.8	1.8	2.5	1.5	1.4	1.6	1.2	2.0	2.0	1.7	1.5	0.0	2.2	2.5	2.3	1.5	2.3	2.8	1.6	0.0	0.0	3.2	0.0

Table 3. Fatty acid percentage composition of extracts obtained with different extraction methods from Atlantic salmon, European eel partially skinned (1); European eel completely skinned (2); Atlantic cod brand "A"; Atlantic cod brand "B"; albacore tuna. Letters A-D see Table 1.

CONCLUSIONS

In this work the utilization of SC-CO_2 for analytical purposes has been studied for the determination of the total fat content of fish products, fresh or commercially frozen. The efficiency of the extraction method has been found to be function of the water content, of the fat content, as well as of the matrix. With the available equipment, optimal conditions were found to be: addition of Hydromatrix to the sample prior to the extraction step (50% by weight); extraction pressure 35 MPa; extraction temperature 60°C; use of EtOH as modifier at 5% mol/mol. The analytical results were similar to those obtained with the Bligh and Dyer method, but it was possible to obtain much lower values of RDS within the three determinations carried out on each sample. However, with some matrices the extraction recoveries did not reach the same levels obtained with the other methods, and further work is needed to clarify this problem.

REFERENCES

[1] BLIGH, E.G., DYER, W.S., Can. J. Biochem. Physiol., Vol. 37, **1959**, 911.
[2] FOLCH, J., LEES, M., SLOANE-STANLEY, G.H., J. Biol. Chem., Vol. 226, **1957**, p. 497.
[3] BERG, H., MÅGÅRD, M., JOHANSSON, G., MATHIASSON, L., J. Chromatogr. A, Vol. 785, **1997**, 345.
[4] TAYLOR, S.L., ELLER, F., KING, J.W., Food Res. Int., Vol. 30, **1997**, 365.
[5] ANKLAM, E., BERG, H., MATHIASSON, L., SHARMAN, M., ULBERTH, F., Food Addit. Contam., Vol. 15, **1998**, 729.
[6] DIONISI, F., HUG, B., AESCHLIMANN, J.M., HOULLEMAR, A., J. Food. Sci., Vol. 64, **1999**, 612.
[7] AOAC "Official Methods of Analysis" 15th Ed., Association of Official Analytical Chemists, Arlington, Virginia, USA, **1990**.
[8] HARDARDOTTIR, I., KINSELLA J.H., J. Food Sci., Vol. 53, **1988**, 1656.
[9] TEMELLI, F., LEBALNC, E., FU, L., J. Food Sci., Vol. 60, **1995**, 703.
[10] ESQUIVEL, M.M., BANDARRA, N.M., FONTAN, I., BERNARDO-GIL, M.G., BATISTA, I., NUNES, M.L., EMPIS, J.A., Lebesm. Wiss. Technol., Vol. 30, **1997**, 715.
[11] CATCHPOLE, O.J., GREY J.B., NOERMARK K.A., J. Supercrit. Fluids, Vol. 19, **2000**, 25.
[12] ALKIO, M., GONZALEZ, C., JANTTI, M., AALTONEN, O., J. Am. Oil Chem. Soc., Vol. 77, **2000**, 315.
[13] ISCO Software SFSOLVER, **1991**, ISCO Inc., Lincoln, Nebraska, USA.
[14] KENNEDY, G.C., Am. J. Sci., Vol. 252, **1954**, p. 225.
[15] TEMELLI, F., J. Food Sci., **1992**, Vol. 57, 440.
[16] JOHNSON, R.B., BARNETT, H.J., Aquaculture, Vol. 216, **2003**, 263.
[17] MANIRAKIZA, P., COVACI, A., SCHEPENS, P., J. Food Comp. Anal., Vol. 14, **2001**, 93.