Study of the stability of two different fish oils obtained by extraction with supercritical carbon dioxide

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INTRODUCTION

During the last years the evidence of the nutritional benefits of polyunsaturated fatty acids (PUFA), especially omega-3 fatty acids, in the human body has become established. Moreover, the nutrition experts have recently recommended to increase the PUFA consumption in order to maintain an omega-6/omega-3 ratio between 5:1 and 10:1 in the diet [1]. Fish is the major dietary source of ω-3 PUFA; therefore an increase in the consumption of fish is advisable in order to reach the recommended level of the average intake of ω-3 and to improve the ω-6/ω-3 ratio in the diet. However, it is apparently difficult to change the dietary habits towards a higher fish intake, and therefore, many products enriched with ω-3 PUFA have been developed in the last years. For that reason, it is important to find natural sources of ω-3 fatty acids abundant and profitable.

An interesting source of ω-3 rich fish oil can be some by-products of the fish industry such as fish skin. Fat is more concentrated in fish skin than in other parts of their body, as muscle, and its recovery may be economically interesting. Supercritical Fluid Extraction with (SFE) has been shown to be a technique to obtain good quality fish oil from such by-products [2]. The most used SC-solvent is carbon dioxide, which is non-toxic and cheap and has mild critical conditions, which make it suitable for processing thermo-degradable compounds as polyunsaturated fatty acids.

Since fish oil presents a high susceptibility to oxidation, mainly due to its high PUFA content, the aim of this work was to study the stability of oil obtained by SFE from two fish species, under different storage conditions.

MATERIALS AND METHODS

Raw material and pre-treatment

The raw materials used in this work were by-products of the Fish Industry, specifically the offcuts from hake (*Merluccius capensis – Merluccius paradoxus*) and orange roughy (*Hoplostethus atlanticus*), both of them captured in the Namibian coasts and provided by Pescanova, a Spanish food company located in Pontevedra (Spain). The offcuts were obtained by peeling fishes with a TRIO™ peeler in open seas and consisted mainly of skin with some stuck muscle. These by-products were frozen in the fishing boats at -20 ºC and kept frozen until the experiments were performed. In the laboratory, they were cut into small pieces (1 mm-10 mm equivalent diameter, De) with a cutter (CT25, Talleres Cato S.A. Spain), in order to facilitate fish oil extraction, and stored under vacuum at –18 ºC. Before performing the supercritical extraction of oil, fish skin was freeze-dried (FreeZone 12 Liter Console Freeze Dry System with drying chamber, Labconco).

Extraction of fish oil by supercritical fluid

The extraction of oil was carried out in a semi-pilot SFE-plant. Approximately 200 g of freeze-dried fish by-product were placed in the extractor that was later pressurized up to the extraction pressure, 25 MPa, with carbon dioxide (Carburos Metálicos, liquid $CO_2 \geq 99.9$ %). Then, 10 kg CO_2/h were circulated at the extraction temperature, 40ºC, during 3 hours. The solvent was continuously recycled to the extractor after removing the solute in a separator where the solvent power of CO_2 was reduced by reducing pressure down to 5 MPa [2].

Analytical Methods

The effects of time and temperature of storage on fish oil quality were examined. Fish oil was analysed immediately after extraction (day 0) and along 75 days of storage under pro-oxidative conditions (20ºC, sunlight), and conventional conditions (4ºC, without light).

Fatty acids profile was determined by the AOAC method [3] using the equipment and conditions previously reported by Rubio-Rodríguez et al. [2].

Oxidation parameters (Free fatty acids content, peroxide value, thiobarbituric acid reactive substances (TBARS) and anisidine value) were determined by the AOCS official methods Ca 5a-40, Cd 8-53, 19.90 [4] and BS 684-2.24 method [5] respectively.

The odour fingerprint was obtained by means of an electronic nose αFOX 4000 (AlfaMOS, Toulouse, France) with a sensor array of 18 metal oxide sensors. The vials with samples were incubated under agitation (cycles 5 s on and 2 s off and 500 rpm) in an oven at 50ºC for generating the equilibrated headspace. The injection temperature was 60 ºC and the carrier gas was synthetic air that flowed at 150 mL/min.

Volatile compounds were analysed by Solid Phase Dynamic Extraction (SPDE) sampling and GC-MS. SPDE (Chromtech, Idstein, Germany) was performed after equilibration of the samples at 70 ºC for 1 min. The SPDE needle was coated inside by PDMS-AC. Gas chromatographic analyses were carried out with an Agilent 6890N Series GC System coupled to an Agilent Technologies 5973i mass spectrometer (Agilent, Palo Alto. CA. USA). The SPDE syringe was injected and thermally desorbed at 250 ºC. Compounds were separated on a HP5 capillary column (50 m length × 0.32 mm I.D fused silica capillary column coated with 1.05 μm film thickness (Quadrex Corporation. New Haven. USA)). The temperature of the column was increased at a rate of 3 ºC/min from 40 ºC to 240 ºC. The effluent from the capillary column went directly into the mass spectrometer and volatiles compounds were identified comparing its mass spectra with NIST and Wiley spectrum libraries.

RESULTS

Fatty acid profile of fish oils

The fatty acid composition of hake and orange roughy oils were quite different. Hake oil showed the following ranking order for the fatty acids classified according to their unsaturation: monounsaturated (46%) > saturated (28%) ≈ polyunsaturated (26%) fatty acids whereas for the orange roughy oil this order was monounsaturated (92%) >> saturated (3%) ≈ polyunsaturated (5%) fatty acids. Orange roughy had low concentration of polyunsaturated and saturated fatty acids, whereas it had a higher concentration of monounsaturated acids. Table 1 shows that oleic acid (209.2 mg/g oil) was the main monounsaturated fatty acid in orange roughy oil, being the responsible for the high value of this fraction. Hake oil had a higher concentration of monounsaturated fatty acids and a similar concentration of saturated and polyunsaturated fatty acids. Values for C20:5 and C22:6 fatty acids varied widely

between the two species considered in this work. EPA and DHA levels in hake oil were 36.3 and 82.1 mg/g oil, respectively and 2.7 and 4.1 mg/g oil, respectively, in orange roughy oil. The low content of ω-3 fatty acids could make orange roughy fish offcuts unsuitable for their commercial exploitation.

Table 1: Fatty acid composition (mg/g oil) in hake and orange roughy oils

mg/g oil	Hake oil	Orange roughy oil
C14:0	18.79	3.72
C16:0	129.12	6.58
C18:0	20.51	1.99
SFA	168.42	12.29
C16:1	33.85	38.05
C18:1	164.13	209.16
C20:1	36.87	31.45
C22:1	32.22	25.33
C24:1	7.80	9.68
MUFA	274.87	313.67
C18:2	7.05	3.84
C18:3 n-3	4.40	3.35
C18:4	3.18	0.36
C20:3	0.82	0.22
C20:4	5.52	1.77
C20:5 n-3	36.32	2.72
C22:4	4.03	0.20
C22:5	8.03	0.61
C22:6 n-3	82.07	4.06
PUFA	151.42	17.13
Total	594.71	343.09

Oil quality along storage

Oil oxidation was studied taking into account several parameters such as free fatty acids (FFA), peroxide value (PV), anisidine value (AV) and thiobarbituric acid reactive substances (TBARS).

The FFA values in hake oil increased with storage time and temperature (Table 2). The hake oil stored at 20ºC had statistically significant ($p<0.05$) higher FFA values than the samples at 4ºC. Hake oil at initial conditions presented fairly high FFA concentrations, even higher than values found in orange roughy oil after 75 days of storage. In this oil FFA remained almost constant during storage time at the two storage temperatures essayed. In hake oil, the maximum FFA value was 7.56 %. The high values of FFA after 75 days of storage are still within the recommendation of 1-7 % for food grade fish oil [6].

The peroxide values of the two fish oils were shown to significantly increase during storage, but orange roughy oil evidenced a peroxide value much lower than that of hake oil. The limit of acceptability for PV is 8 meq O_2/kg fish oil [7]. Orange roughy oil did not exceed the

acceptability limit along 75 days of storage at 4 ºC or 20 ºC. Hake oil, however, exceeded this limit after 14 days of storage.

Table 2. Changes in the quality of hake and orange roughy oils stored up to 75 days at two different temperatures

	FFA (% oleic)	TBARS (mg MA/kg)	PV (meq O_2/kg oil)	AV
Hake oil				
Storage temperature				
4ºC	5.27 a	74.29 a	16.90 a	61.37 a
20ºC	5.96 b	118.33 b	19.76 b	95.85 b
$LSD_{0.05}$	**0.05**	**3.32**	**0.31**	**0.38**
Storage time (days)				
0	4.70 a	7.83 a	4.11 a	7.75 a
6	4.88 ab	36.77 b	10.37 b	22.79 b
14	4.87 ab	34.82 b	12.87 c	34.82 c
32	5.06 b	66.81 c	24.54 e	47.68 d
45	5.59 c	122.68 d	20.68 d	79.73 e
60	6.64 d	142.38 e	26.89 f	105.90 f
75	7.56 e	262.87 f	28.87 g	251.62 g
$LSD_{0.05}$	**0.10**	**6.21**	**0.59**	**0.72**
Orange roughy				
Storage temperature				
4ºC	1.64 a	1.56 a	0.61 a	1.17 a
20ºC	1.65 a	2.77 b	1.54 b	2.06 b
$LSD_{0.05}$	**0.03**	**0.11**	**0.03**	**0.06**
Storage time (days)				
0	1.55 bc	0.12 a	0.20 a	0.00 a
6	1.52 bc	0.41 a	0.38 b	1.44 c
14	1.85 d	2.18 c	0.44 b	1.28 c
32	1.98 d	6.26 d	0.79 c	2.52 d
45	1.89 d	2.05 c	0.86 c	0.57 b
60	1.64 c	1.71 bc	1.58 d	2.42 d
75	1.30 a	1.38 b	1.73 d	1.49 c
$LSD_{0.05}$	**0.07**	**0.27**	**0.06**	**0.12**

Means within the same column and the same main effect with different letters are different ($p<0.05$)

The anisidine and TBARS value allow evaluating secondary lipid oxidation. In general, it was observed that anisidine and TBARS values in hake oil samples gradually increased during storage at both temperatures. The observed increase was considerably lower at 4 ºC than at 20 ºC during storage. These values, however, were very low in orange roughy oil.

The low values found for all the parameters related to lipid oxidation in orange roughy oil, agree with its fatty acid profile, since it is well established that oily fishes are particularly susceptible to lipid oxidation and rancidity development because of their high PUFA content [8]

Volatile compounds

The oxidation of PUFA-containing lipids causes the development of off-flavours and aromas, often referred to as 'rancidity' in fish. The compounds giving rise to rancid flavours and aromas are volatile secondary oxidation products derived from the breakdown of the lipid

hydroperoxides. The main volatile components of fish oil can be grouped into several types of compounds; the most important families are acids, alcohols, aldehydes, alkanes, ketones, furans, amines and amides.

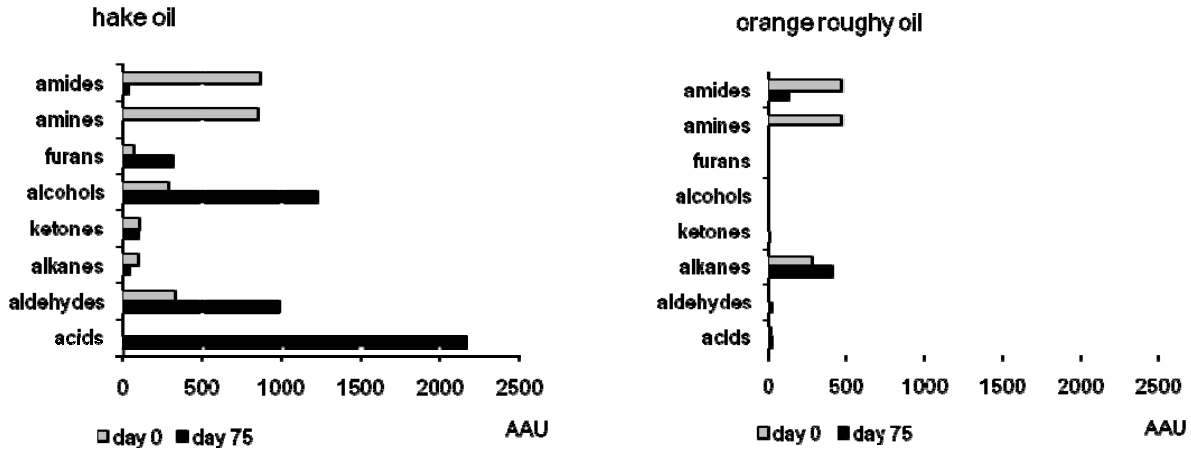

Figure 1. Volatile compounds in hake and orange roughy oils by families of compounds at initial conditions and at the end of storage at 20 ºC

Hake oil presented a larger number and a much higher concentration of volatile compounds than orange roughy oil (Figure 1). At initial conditions, orange rough oil contained acid compounds unlike hake oil, on the contrary furans, alcohols, ketones and aldehydes are only present in hake oil. Amides, amines and alkanes were identified in both oils; the two first families were more abundant in hake oil and alkanes in orange roughy oil. In general, in hake oil, volatile compounds suffered an increment along storage, except for amides and amines. At the end of storage the predominant groups were acids, aldehydes, alcohols and furans. Orange rough oil hardly showed modifications, only alkanes and aldehydes increased, but its concentration was low.

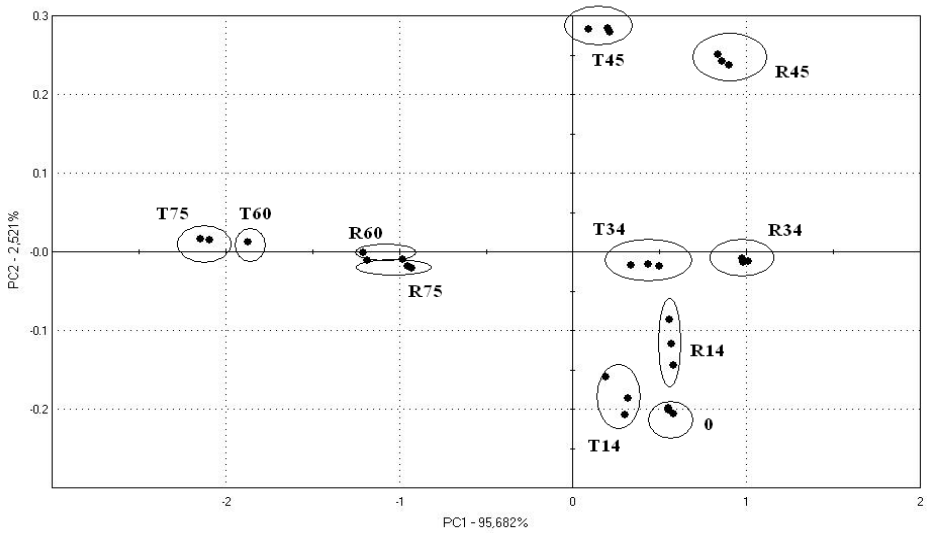

Figure 2. PCA of hake oil during storage time and temperatures 4 ºC (R) and 20 ºC (T)

Odour fingerprint

Since, the major diversity and quantity of volatile compounds correspond to hake oil, the results of odour fingerprint of this oil obtained by electronic nose are shown (Figure 2).

The PCA score plot for the electronic nose sensors responses, clearly separated samples at days 60 and 75 from earlier storage times along PC1, which explained 95 % of the variance. From initial time untill day 45, samples mainly differed in PC2 that only explains 2.5 % of the variance and they are similar in PC1 coordinates.

CONCLUSIONS

The fatty acid compositions of the two fish by-products studied in this work differ greatly, being hake the most appropriate raw material for SFE of rich omega-3 fatty acids oil. However, orange roughy oil showed a much better stability against oxidation than hake oil that was observed to have a high oxidative deterioration. This behaviour is probably due to the higher content of omega-3 PUFA in hake. PUFA are fairly unstable and, as a consequence, the oil that contains a high proportion of them is unstable as well. Therefore, some techniques to stabilize fish oils with a high PUFA content are necessary in order to avoid their oxidation along time.

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