SFE of Fish Oil from Fish by-Products: A Comparison with other Production Processes

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INTRODUCTION

Fish oil is considered to be an important natural source of polyunsaturated fatty acids (PUFA), especially omega-3, whose benefits on human health have been extensively reported in the literature. In the last years, the demand for omega-3 PUFA has increased noticeably in the pharmaceutical and food industries, and nowadays, the production of good quality fish oil from fish by-products is regarded as a great opportunity to valorise solid wastes and increase the benefits in the fish industry. Wet reduction is the common method to produce fish oil. This method involves three basic steps: cooking at high temperatures (85 ºC – 95 ºC), pressing and centrifuging [1] allowing the production of good quality crude oil from fresh fish by-products of fatty species such as herring, salmon, tuna, etc. However, the use of high temperatures, related to PUFA oxidation, makes this method inappropriate to produce high quality fish oil, specially, when the antioxidant content in the raw material is low. Alternative processes, such as enzymatic extraction with proteases or supercritical fluid extraction, are being developed nowadays to obtain high quality fish oil under mild temperatures and, therefore, minimize PUFA oxidation.

Since it is important to establish the most suitable technology in a specific case, the aim of this study is to compare both yield and quality of the oil extracted from an oily fish (orange roughy) and a lean fish (hake) using different extraction processes: cold extraction, wet reduction, enzymatic treatment and supercritical fluid extraction.

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, 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.

Oil extraction methods

Oil extraction from fish by-products was carried out by four different methods: cold extraction (CE), wet reduction (WR), enzymatic extraction with protease (EE) and SC-CO_2 extraction (SFE). In the cold extraction method, oil was removed from fresh raw material by direct centrifugation (10 min at 1000×g and 20 ºC). Extraction by wet reduction and

enzymatic extraction were also applied over fresh raw material, but using two steps: cooking in a water bath (15 min at 85 ºC– 95 ºC) or enzymatic hydrolysis with Alcalase® (120 min at 56 ºC) respectively, followed by a centrifugation step (10 min at 1000×g and 20 ºC) to remove the oil extracted. SFE was carried out in a semi-pilot SFE plant described elsewhere [2] and following the experimental conditions proposed in a previous study (180 min at 250 bar, 10 kg CO_2/h and 40 ºC) [3]. This method involved water removing as a first step, which was carried out by freeze-drying in order to minimize lipid oxidation and preserve the structure of the raw material allowing an effective contact between oil and SC-CO_2. Finally, the oil extracted by each method was stored in a closed flask in the darkness and at -18 ºC to minimize deterioration before characterization.

Analytical Methods

Water and protein content. Hake and Orange Roughy offcuts were analyzed to determine their water and protein content by the AOAC Official Methods 934.01 and 981.10 respectively. Fat content was also determined by Soxhlet using petroleum ether as solvent in a Büchi extraction system (model B-811).

Physical properties. A comparison of the different extraction methods was made by determining several physical properties of the different oils obtained: density was measured at room temperature in an automated vibrating tube density meter (Anton Paar DMA 5000) with an uncertainty of ± 0.00001 g/cm^3; colour coordinates (CIE L*a*b*) were measured in quadruple by means of reflectance spectra in a spectrophotometer (Konica Minolta); turbidity was determined using a portable turbidimeter (Hach Company, Loveland, CO, USA) and reported in nephelometric turbidity units (NTU) and moisture and total volatile matter content were determined by following the IUPAC Standard Methods [4].

Fatty acids profile was determined by the AOAC method [5]. The fatty acid methyl esters were firstly prepared and then analyzed by gas chromatography (GC) in a Hewlett Packard gas chromatograph (6890 N Network GC System) equipped with an auto-sampler (7683B series) and a flame ionization (FID) detector. The separation was carried out with helium (1.8 mL/min) as carrier gas. A fused silica capillary column (OmegawaxTM-320, 30 m × 0.32 mm i.d.) was used. The column temperature was programmed starting at a constant temperature of 180 ºC during 20 min, heated to 200 ºC at 1 ºC/min, held at 200 ºC during 1 minute, heated again to 220 ºC at 5 ºC/min and finally held at 220 ºC for 20 min. A split injector (50:1) at 250 ºC was used. The FID was also heated at 250 ºC. Most of the fatty acid methyl esters were identified by comparison of their retention times with those of chromatographic standards (Sigma Chemical Co.). Their quantification was made by relating the peaks area to the area of an internal standard (methyl tricosanoate) as indicated by the AOAC method [5]. Calibration curves were made for each pair internal standard + chromatographic standard in order to find the corresponding response factors.

Trace metals (Fe, Cu, Zn, As, Cd, Hg and Pb) were determined by ICP-MS (Agilent 7500i) over samples previously digested with HNO_3 65 % suprapur® (Merck, Germany) in a microwave oven (Ethos Sel, Milestone).

Oxidation parameters (FFA content, peroxide value and anisidine value) were determined by the AOCS official methods Ca 5a-40, Cd 8-53 [6] and BS 684-2.24 method [7] 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.

Sensory analysis was carried out by 10 panellist trained in descriptive analysis of fish off-flavors, using six sensory descriptors (fishy, rancid, cooked, acid, sweet and others) in a intensity range from 0 to 5. Samples (0.5 mg oil) were presented randomised at room temperature in blind bottles numbered with a three digits code.

RESULTS

Influence of the extraction method on oil yield

The oil extraction from a natural matrix, such as fish by-products, can involve the production of several phases: a dense phase formed mainly by solids (proteins) with some water, a lighter phase formed mainly by water, and an oily phase which is the lightest one. Between the aqueous phase and the oil phase, an emulsion is usually formed. Figure 1 presents the weight percentage of each of these phases obtained through the four methods essayed and for the two types of fish used.

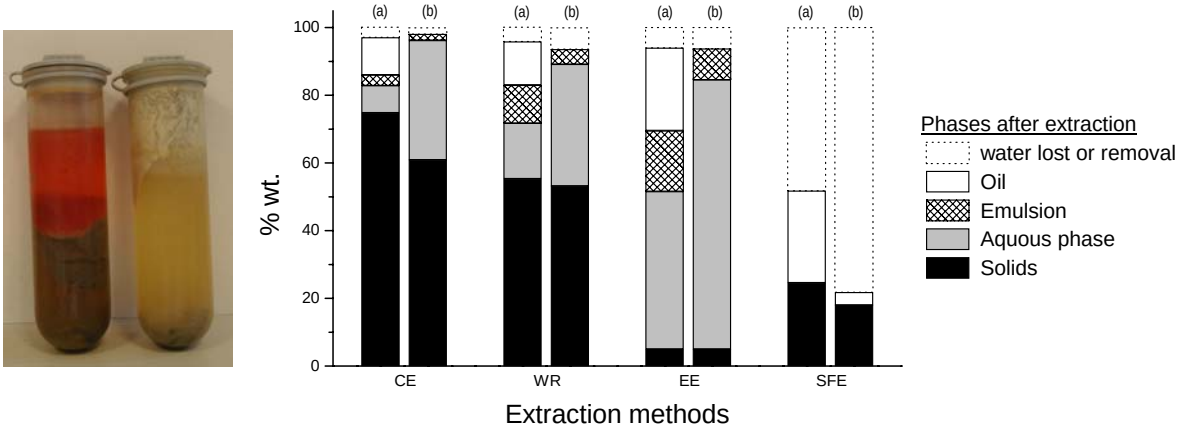

Figure 1. Different phases obtained in oil extraction from orange roughy skin (a) and hake skin (b) by different methods. CE: cold extraction; WR: wet reduction; EE: enzymatic extraction and SFE: supercritical carbon dioxide extraction. The photography corresponds to the results of the EE method

The aqueous phase and the oil-in-water emulsion appear when extraction is performed without previous fish drying (CE, WR and EE) but they do not appear in SFE because water has been previously removed by freeze-drying. The water/oil ratio in hake skin (20.1) is larger than in orange roughy skin (1.7). This ratio difference is also observed for the water to oil phases obtained after extraction, as can be observed in Figure 1.

Whereas oil from orange roughy skin was easily extracted whatever was the method used, SFE was the only method suitable for oil extraction from hake skin. The CE and WR processes include a pressing step at industrial scale that improves yields regarding those obtained in the laboratory.

Influence of the extraction method on oil quality

The influence of the extraction method on oil quality was evaluated by means of some physical parameters, fatty acids profile, trace metals content, oxidation parameters and sensory properties. Only the orange roughy oil obtained by the four methods is compared since hake oil could only be obtained by SFE.

Physical properties: Some physical properties such as density, turbidity, colour and moisture and total volatile compounds have been determined for the oils obtained by the different

methods. All of them depend on the extraction method. The oil extracted by SFE presented the lowest density and the higher turbidity value (see Table 1) that may be related to the CO_2 dissolved in the oil, since after keeping oils under vacuum conditions, the turbidity of the four oils reached similar values. Colour coordinates: luminosity, redness and yellowness were similar in the oils extracted by the different methods, except for the oil obtained by SFE, which presented higher luminosity and redness than the other three oils.

Table 1. Physical properties found in orange roughy oil extracted by different methods

	Fish oil extraction method			
	CE	**WR**	**EE**	**SFE**
Density (kg/m^3)	871.42 ± 0.01	866.03 ± 0.03	866.58 ± 0.01	865.82 ± 0.05
Turbidity (NTU)	95 ± 1	81 ± 1	1.9 ± 0.3	633 ± 1
Moisture and total volatile compounds (% wt.)	0.7 ± 0.1	0.8 ± 0.1	0.4 ± 0.1	0.6 ± 0.1

Fatty acids (FA) profile: A total of 20 fatty acids (C14:0, C16:0, C16:1, C18:0, C18:1n-9, C18:1n-7, C18:2n-6, C18:3n-6, C18:3n-3, C18:4n-3, C20:1n-9, C20:3n-6, C20:4n-6, C20:5n-3, C22:1n-11, C22:1n-9, C22:4n-6, C22:5n-3, C22:6n-3, C24:1) were detected in GC analysis for orange roughy. It was observed that the oil extracted by the four different methods presented a similar fatty acid profile, composed by a 92 % of monounsaturated FA, a 5 % of PUFA and a 3 % of saturated FA. The most abundant fatty acid was oleic acid (54 %), followed by gadoleic (13 %) and palmitoleic acid (11 %). Among SFA, palmitic acid presented the higher concentration (1.6 %), whereas myristic and stearic acids were in a low proportion (1 % and 0.6 % respectively). The amount of omega-3 and omega-6 fatty acids was also similar (around 3 %), and the EPA and DHA content was around 0.8 % and 2 % respectively.

Trace metals content: Trace metals such Fe, Cu, Zn, As, Cd, Hg and Pb were investigated in fish by-products and in the oil extracted by different methods (Table 2). Zn was the most abundant metal in the raw material, followed by Fe, As and Cu, whereas As was the only element detected in the oil obtained by CE, WR and EE methods. This can be because Fe, Cu and Zn are included in several metalloproteins and enzymes involved in cellular biochemistry [8], which are stored inside the cells and are not soluble in the lipid phase; therefore, their extraction together with the extracellular oil by physical methods such as cold extraction, wet reduction or enzymatic extraction is quite difficult. However, arsenic is present in seawater as inorganic compounds such arsenate or arsenite, or in marine organisms [9] and fish oil [10] as lipid soluble arsenic compounds, which can be easily extracted together with the oil by physical methods.

Table 2. Trace metals found in orange roughy skin and skin oil extracted by different methods

Metal	**Raw material (ppm)**	**Fish oil extraction method**			
		CE	**WR**	**EE**	**SFE**
Fe	1.73 ± 0.09	n.d.	n.d.	n.d.	n.d.
Cu	0.27 ± 0.08	n.d.	n.d.	n.d.	n.d.
Zn	10 ± 3	n.d.	n.d.	n.d.	1.5 ± 0.6
As	0.85 ± 0.08	0.82 ± 0.03	0.80 ± 0.01	0.94 ± 0.06	0.26 ± 0.03
Cd	n.d.	n.d.	n.d.	n.d.	n.d.
Hg	n.d.	n.d.	n.d.	n.d.	n.d.
Pb	n.d.	n.d.	n.d.	n.d.	n.d.

The oil extracted by SFE contained both Zn and As, although their concentration was substantially lower than in raw material. It is reported that SFE extraction of metals is possible when metals are as organometallic compounds, and their solubility in SC-CO_2 depends of the organic ligand type [10]. Fortunately, the metals present in the raw material show a poor solubility in SC-CO_2 and, therefore, the metal content in the oil extracted was reduced noticeably.

Oxidation parameters: Oil oxidation was studied taking into account several parameters such as free fatty acids (FFA) content, peroxide value (PV) or anisidine value (AV), besides CIE L*a*b* colour coordinates and volatile compound profile. The bi-plot of PC1 and PC2, obtained from Principal Components Analysis (PCA), shows a clear difference between the oil extracted by SFE, which presented negative values for PC1, and the oil extracted by the other methods, which presented positive values for PC1 (Fig. 2). In addition, it may be observed that the oil extracted by SFE is characterised by the variables relative to hydrolysis (FFA), colour parameters and volatile alkanes, acids and amines, whereas the oil extracted by the other methods is characterised by the variables relative to primary and secondary oxidation such PV and volatile aldehydes. Therefore, it can be concluded that the oil extracted by SFE is the least oxidized. This can be explained by considering that the SFE method is carried out at mild temperatures and under a non-oxidant atmosphere.

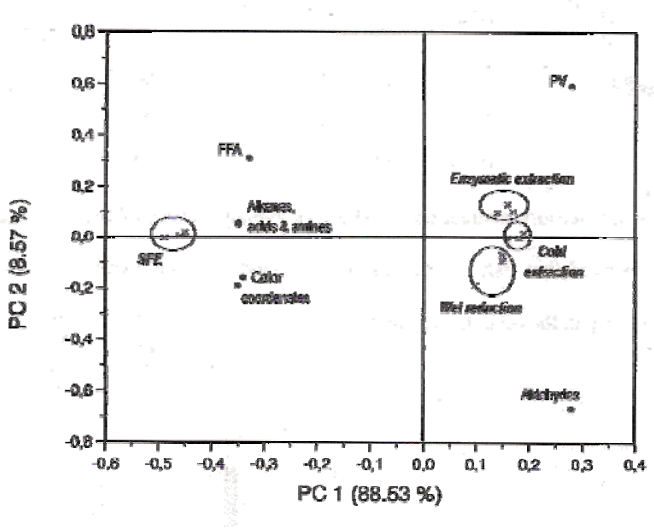

Figure 2.- Bi-plot of PC1 and PC2, obtained from Principal Components Analysis (PCA) of oxidation data found in orange roughy oil extracted by different methods.

Sensory analysis. Analysis of the data obtained both by the electronic nose system and by the trained panel revealed a clear difference between the oil extracted by the SFE method and the oil extracted by the other methods. It was found that the oil extracted by SFE presents the most intense fishy odour, which is related with the presence of volatile amines detected in the volatile compounds profile. These amines, originated in the raw material, can be removed when extraction procedures are carried out in open system, but remain in the oil when the extraction occurs in a closed system as in SFE method.

CONCLUSIONS

Fish oil production has become an interesting way for valorising fish by-products, since it is a natural source of omega-3 fatty acids, beneficial for human health.

The common method to produce fish oil is wet reduction, although it involves the use of high temperatures responsible for lipid oxidation. Enzymatic extraction and SFE have been

proposed as alternative methods to extract fish oil under soft temperatures and therefore to produce a high quality oil.

In this study, it was observed that oil from orange roughy skin, with a low water/oil ratio, was easily extracted whatever was the method used, whereas SFE was the only method suitable for oil extraction from hake skin, with a high water/oil ratio.

Comparing the oil quality, it was found that SFE, not only permitted to reduce oxidation, but also heavy metals content, although volatile compounds such amines, responsible for the fishy odour, were not removed by SFE as much as by the other methods.

In any case, although SFE could be a useful tool for fish oil extraction, especially to produce a non-oxidised oil with low heavy metal content, the requirement of a previous drying step or the intense fishy odour found in the final products makes necessary a deeper study before scaling up of the process.

Finally, economical considerations such as investment and operation costs are necessary to decide the profitability of the process on large scale.

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