Research Article

Comparison of fatty acid profiles of dried and raw by-products from cultured and wild fishes

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Fish by-products may become alternative sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, due to the high enzymatic activity in these biological tissues, special care must be taken to prevent lipid oxidation and hydrolysis. In this work, several by-products from Chilean fishes (farmed salmon and wild red cusk-eel and yellowtail kingfish) were dried at 105°C for 3 h to remove water and inactivate enzymes. The effect of temperature on EPA and DHA levels was assessed by comparing fatty acid profiles of raw and dried by-products. Drying at 105°C for 3 h was considered an adequate process to obtain dried powders from fish by-products with appreciable amounts of EPA and DHA, even though EPA and DHA values showed a certain decrease after drying. Several methodologies involving food-grade solvents were checked to evaluate their suitability for lipid extraction from dried by-products, being Soxhlet extraction with n-hexane identified as the most suitable process in terms of extraction yield and EPA/DHA values. Cholesterol amount was also studied, being the highest and lowest amounts found in liver and viscera from farmed salmon, respectively.

Practical applications: In fish processing plants, raw by-products are collected after fish evisceration, and they can be transported to oil extraction facilities, although their lipids may be easily degraded unless special precautions are taken to preserve such biomass. Raw fish by-products must be subjected to water removal and enzyme inactivation to prevent lipid degradation and hydrolysis, and it is desirable that such actions are carried out in the processing plants themselves to ensure the maximum oil quality. Drying at 105°C for a short time (3 h) was assayed in this work because of its simplicity, low cost, scalability, and feasibility to be installed in fish processing plants. Soxhlet procedure with n-hexane is effective to extract lipids containing EPA and DHA from dried by-products for nutritional or nutraceutical purposes. Because of water removal, lipid extraction efficiency from dried by-products is enhanced and less solvent is needed, which is economically and environmentally desirable.

Keywords: Docosahexaenoic acid / Eicosapentaenoic acid / Fish by-products / Lipid extraction

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1 Introduction

Polyunsaturated fatty acids from the n-3 family (n-3 PUFAs) are key nutrients whose beneficial effects for the human health are widely known. Eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are the most recognized n-3 PUFA because of their nutritional and physiological relevance [1–3]. EPA and DHA are mainly found in marine oils, especially those coming from fish, microalgae, and some crustaceans [4, 5]. Unfortunately, consumption of EPA and DHA sources such as marine fish is

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Abbreviations: ALA, alpha-linolenic acid; **AA,** arachidonic acid; **DHA,** docosahexaenoic acid; **EPA,** eicosapentaenoic acid; **FA,** fatty acid; **FAME,** fatty acid methyl ester; **GC-FID,** gas chromatography-flame ionization detection; **LA,** linoleic acid; **MA,** myristic acid; **MUFA,** monounsaturated fatty acid; **OA,** oleic acid; **PA,** palmitic acid; **PUFA,** polyunsaturated fatty acid; **SA,** stearic acid; **SFA,** saturated fatty acid

usually much lower in Western populations than what is recommended by dietary guidelines, and daily requirements of EPA and DHA to keep a healthy status are frequently not met. Furthermore, marine fish is foreseen to become a scarce food in a medium term due to overfishing worldwide [4].

Although EPA and DHA can be synthesized by the human metabolism from their precursor alpha-linolenic acid (ALA, 18:3n-3), such conversion is very limited due to the deficient activity of the Δ6-desaturase enzyme, which is involved in the first metabolic step consisting in the desaturation of ALA to yield stearidonic acid (SDA, 18:4n-3) [6, 7]. Thus, the best way to provide EPA and DHA to the organism is directly through the diet. In this way, it is advisable to search for alternative sources of EPA and DHA able to provide such nutrients in a suitable amount for human or animal consumption. Fish by-products from aquaculture or fishery industries are currently used to elaborate fishmeal for animal feeding or they are directly discarded, causing problems regarding environmental protection and sustainability [8]. However, such biomasses have a potential to be used as rich sources of EPA and DHA [8]: several studies have been carried out with by-products from different fish species such as carp (*Cyprinus carpio*) [9], hilsa fish (*Hilsa ilisa*) [10], trout (*Oncorhynchus mykiss*) [11], catfish (*Clarias gariepinus*) [12], gilthead sea bream (*Sparus aurata*), and European sea bass (*Dicentrarchus labrax*) [13], to assess their suitability as EPA and DHA sources.

Chile is an important supplier of both cultured and wild fish, being salmon (*Salmo salar*) one of the most important species, which is mainly exported. Chilean production of salmon was 644 500 Mt in 2014 (Chilean National Service of Fisheries and Aquaculture, SERNAPESCA). To date, however, no studies have been carried out in Chile to assess the values of EPA and DHA contained in fish by-products in order to support a valorization of these residues, allowing the obtaining of products enriched in EPA and DHA with high added value and applications for human and/or animal consumption. In this work, viscera and liver from farmed salmon will be assessed as alternative sources of EPA and DHA together with viscera from wild red cusk-eel (*Genipterus chilensis*) and yellowtail kingfish (*Seriola lalandi*), called "congrio colorado" and "palometa" in Chile, respectively. Although production of red cusk-eel and yellowtail kingfish in Chile is much lower than that of salmon, with 636 and 372 Mt reported in 2014, respectively (SERNAPESCA), both species are commercially interesting: red cusk-eel is a fish with a high demand in the Chilean market due to its exceptional flesh quality [14, 15]. Yellowtail kingfish is popular because of its firm, white, and slightly oily flesh, and it is widely used for sashimi preparation. For this reason, this species has a strong demand in the Asian market [16]. Yellowtail kingfish is a seasonal species which is caught in the summer months in the Chilean coast, whereas red cusk-eel is available throughout the whole year.

Raw viscera is difficult to handle because of its texture and high water content, and such biomass must be carefully stored at low temperatures to prevent lipid degradation (hydrolysis and oxidation processes) due to the high amount of enzymes which are present in the biomass [17]. Extracted oil must be also kept at very controlled conditions of darkness, low temperature, and inert atmosphere to prevent n-3 PUFA oxidation which are very sensitive to oxidative degradation. Furthermore, facilities to deep-freeze large volumes of by-products or to extract their lipid fraction are absent at fish processing plants, especially at small remote ones. This problem often results in the by-products being discarded [17]. However, when by-products are dried and grinded, a fine, inert, and easy-to-handle powder meal containing n-3 PUFA may be obtained. Drying is an easier method to implement directly at the site of recollection/fishing and considerably reduces the cost of producing the powder [18]. The drying process is also advantageous in terms of efficiency when using solvents to extract the lipid fractions from by-products: because of the water removal, lower solvent volumes are needed to produce a given amount of lipids compared to the use of raw biomass. This fact is environmentally desirable.

In this work, fish by-products from salmon, red cusk-eel, and yellowtail kingfish were dried and their lipid fractions extracted with different systems based on n-hexane, which is a food-grade solvent. As n-hexane is an apolar solvent, isopropanol (also a food-grade solvent) was added to increase the polarity of the extraction system and assess if such modification had an effect on the extraction yield and fatty acid (FA) profiles. Application of high extraction temperature when extracting lipids with n-hexane was carried out in Soxhlet mode, to check if some variation was observed in extraction yield or FA profiles.

The potential influence of drying and the extraction mode on EPA and DHA values was assessed by comparing FA profiles in the dried by-products with those from raw by-products. Additionally, cholesterol amount was measured in the lipids extracted from dried by-products.

2 Materials and methods

2.1 Sample collection

The whole viscera of ten farmed salmons collected from Buill Center and Isla Matilde Center (X and XI Region, respectively, Chile) were provided by an aquaculture company located at Puerto Montt (X Region, Chile). After fish evisceration, by-products were refrigerated and delivered (<18 h) to laboratory facilities. Once in the laboratory, livers were separated from the rest of the viscera to be processed separately. The whole viscera from red cusk-eel (10 fishes) and kingfish yellowtail (8 fishes) were provided by local artisanal fishermen in Coquimbo (IV Region, Chile) and sent

refrigerated to the laboratory. Samples were labelled, placed in food-safe plastic bags and stored at −20°C in the lab until processing.

2.2 Water content

Once each type of biomass was thawed, homogeneous composites of the by-products from each fish were obtained by grinding in a food mixer. A representative aliquot (10 g) of each composite was collected and dried in an oven at 105°C until constant weigh to estimate the water content. Determinations were performed in triplicate for each biomass and results are reported as mean value ± standard deviation (Table 1).

2.3 Drying of raw by-products

Two hundred grams of each composite were placed in an oven at 105°C for 3 h and ground with a blender afterwards until a fine powder was obtained. These powders were stored at −20°C for further processing.

2.4 Lipid extraction

Lipid extraction was carried out according to the following methodologies:

(1) Folch extraction. Raw (5 g) and dried (1 g) by-products were mixed with 100 and 20 mL, respectively of chloroform:methanol (2:1 v/v), magnetically stirred for 20 min and then filtered to remove solids. After that, distilled water was added to the filtrate (20 and 4 mL for filtrates from raw and dried by-products, respectively) and the mix was vortexed for 2 min. The resulting biphasic system was allowed to separate by centrifugation (2750*g*, 5 min). The lower organic phase was collected and filtered through anhydrous sodium sulphate, and the solvent was evaporated in a rotary evaporator at 40°C. The residue was collected, weighed, and stored at −20°C in the darkness and inert atmosphere with nitrogen to prevent lipid degradation.

(2) Hexane extraction. Raw (5 g) and dried (1 g) by-products were mixed with 50 and 10 mL, respectively of n-hexane and magnetically stirred for 30 min. After filtering, the extraction was repeated with other portion of n-hexane for 30 min. Anhydrous sodium sulphate (500 mg) was added to the filtrate and the mix was vortexed for 30 s. After filtration and solvent removal in a rotary evaporator at 40°C, the residue was collected, weighed, and stored at −20°C in the darkness and inert atmosphere.

(3) Hexane:isopropanol extraction. Raw (5 g) and dried (1 g) by-products were mixed with 50 and 10 mL, respectively of n-hexane:isopropanol (3:2 v/v) and magnetically stirred for 30 min. After filtering, the extraction was repeated with other portion of n-hexane: isopropanol for 30 min. Anhydrous sodium sulphate (500 mg) was added to the filtrate and the mix was vortexed for 30 s. After filtration and solvent removal in a rotary evaporator at 50°C, the residue was collected, weighed, and stored at −20°C in the darkness and inert atmosphere.

(4) Soxhlet extraction. This procedure was applied only to dried samples, due to the complexity of handling raw by-products. Dried by-products (10 g) were placed in a Soxhlet apparatus and extracted with 150 mL n-hexane for 4 h. After solvent evaporation in a rotary evaporator at 40°C, the residue was collected, weighed, and stored at −20°C in the darkness and inert atmosphere.

All employed solvents for lipid extraction were from Merck (Darmstadt, Germany).

Table 1. Water and lipid content from fish by-products studied in this work

	Salmon liver	Salmon viscera	Red cusk-eel viscera	Yellowtail kingfish viscera
Water content (g/100 g raw by-product)	68.7 ± 1.7	51.7 ± 1.4	80.4 ± 1.2	70.7 ± 1.9
Dried by-product yield (g/100 g raw by-product)	31.3 ± 1.6	48.3 ±1.4	19.6 ± 1.2	29.3 ± 1.8
Lipid content (g/100 g raw by-product)				
Folch	7.2 ± 0.2	44.3 ± 1.5	5.6 ± 0.2	5.6 ± 0.1
Hexane	3.1 ± 0.1	31.4 ± 0.9	1.6 ± 0.1	1.5 ± 0.3
Hexane:isopropanol	6.6 ± 0.4	27.3 ± 1.2	4.2 ± 0.8	5.7 ± 0.3
Lipid content (g/100 g dried by-product)				
Folch	22.4 ± 0.4	65.0 ± 0.8	27.7 ± 0.2	19.1 ± 0.2
Soxhlet	20.6 ± 0.2	65.0 ± 1.0	27.5 ± 0.4	16.7 ± 0.1
Hexane	12.4 ± 0.2	51.8 ± 1.3	14.6 ± 0.8	11.9 ± 0.4
Hexane:isopropanol	18.8 ± 0.9	59.6 ± 2.0	14.6 ± 0.7	15.5 ± 0.4

Results are expressed as mean value ± standard deviation.

2.5 Fatty acid profiles

Raw and dried by-products (500 and 100 mg, respectively) and extracted lipids by each assayed method (30 mg) from raw and dried by-products were weighed in Pyrex test tubes and derivatized to fatty acid methyl esters (FAME) by adding 2 mL of a mixture of methanol:acetyl chloride (20:1 v/v) and 1 mL n-hexane and then heating at 100°C for 30 min in a hot block [13]. Then, tubes were allowed to reach room temperature and 1 mL distilled water was added to each one. Tubes were then centrifuged (2750*g*, 5 min) and the upper hexane layer was collected for FA analysis by gas chromatography coupled with a flame ionization detector (GC-FID) as described in [13]. Briefly, FAMEs were analyzed using an Agilent 6890N GC and an Omegawax 250 capillary column (30 m × 0.25 mm id × 0.25 μm film thickness; Supelco, Bellefonte, PA, USA). The temperature program was: 1 min at 90°C, heating until 200°C at a rate of 10°C/min, constant temperature at 200°C (3 min), heating until 260°C at a rate of 6°C/min, and constant temperature at 260°C (5 min). The injector temperature was 250°C with a split ratio of 50:1. Injection volume was 1 μL. Detector temperature was 260°C. Nitrogen was used as carrier gas (1 mL/min). Methyl tricosanoate (Sigma–Aldrich, Germany) was used as internal standard. Peaks were identified by retention times obtained from known standards (Supelco 37 component FAME mix and PUFA No. 3 from Sigma–Aldrich, Germany). All solvents were from Merck (Darmstadt, Germany). This process was carried out in duplicate and results are expressed as mean value ± standard deviation (Tables 2–5).

2.6 Cholesterol quantification

Lipids (700 mg) extracted from dried by-products by the Soxhlet method were saponified under reflux for 1 h after addition of 14 mL 1 M potassium hydroxyde in ethanol 96%. After that, the mixture was cooled at room temperature and 7 mL distilled water were added. Unsaponifiable matter was isolated through three consecutive extractions with n-hexane (7 mL each). The hexane extracts were put together and the solvent was removed using a rotary evaporator under vacuum. The residue was reconstituted in acetonitrile: isopropanol (65:35 v/v). Quantitative analysis were carried out by HPLC coupled with a diode-array detector (DAD) according to [13]. A Finnigan Surveyor (Thermo Electron, Cambridge, UK) equipped with a Hypersil Gold C18 column (250 × 4.6 mm, 5 μm i.d.) was employed, and a mixture of acetonitrile:isopropanol (65:35 v/v) was used as mobile phase in isocratic regime (1 mL/min). Injection volume was 20 μL and column temperature was set at 30°C. Detection was carried out at 210 nm. A cholesterol standard (≥99% purity) (Sigma–Aldrich, Germany) was used to build a calibration curve for quantification purposes. Determinations were performed by duplicate and results are reported as mean value ± standard deviation (Fig. 1).

2.7 Statistical analysis

The Shapiro–Wilk test was carried out to assess normality within data. Two-way ANOVA and Tukey's post hoc test were used to evaluate statistical significance ($p < 0.05$). The IBM SPSS Statistics for Windows software package version 21.0 was used to perform statistical analysis.

3 Results and discussion

In this work, fish visceral by-products were processed as a whole, in the way they are obtained after fish evisceration. Tissue separation is time consuming and not economically feasible in industrial terms, and it was not intended in this work. Only with salmon by-products, livers were separated from the rest of the visceral mass because they were easily identified and separable from the viscera.

FA profiles in fish tissues can vary depending of several issues such as the harvest season, water temperature, etc. However, the focus of this work was to compare FA profiles of raw and dried by-products to check the effect of high temperature during drying, and therefore variability due to sampling season and other factors was not considered.

After drying and grinding of by-products, a fine powder was obtained, except in the case of salmon viscera, where an oily paste was produced due to the high amount of lipids in this by-product. Drying is a simple way to increase the efficiency of the lipid extraction because when water is removed, interaction of solvents with by-products is enhanced and lower solvent amounts are needed to extract lipids. It has been recently pointed out the advantages of obtaining powders from dried fish by-products with nutritional purposes because they can be handled, transported, and stored in an easier way than raw by-products or oils [19]. In such study, authors carried out a characterization of several dried by-products from tuna using 55°C and 8 h as drying conditions, although FA profiles were not assessed and therefore the potential influence of drying conditions on n-3 PUFA values were not reported. In our study, drying conditions were 105°C for 3 h. It has been reported the use of freeze-drying to remove water from by-products prior to processing. Freeze-drying keeps lipids preserved as this technique works at low temperatures and high vacuum. However, when intended to be scalable to large amount of biomass, this technique is complex to adapt and quite expensive, whereas application of high temperature for a short time is an easier and cheaper way to remove water from by-products.

3.1 Water and lipid content in fish by-products

The highest water amount among the studied fish by-products was contained in viscera from red cusk-eel (80.4 g H_2O/100 g raw biomass) followed by yellowtail kingfish

Table 2. Fatty acid profiles of salmon liver and its lipid fractions, both in raw and dried state (in percentage of total FAs)

	Salmon liver									
	Raw					Dried				
Fatty acid	Direct	Folch	Hexane	Hexane:iProp	Direct	Folch	Hexane	Hexane:iProp	Soxhlet
14:0	1.16 ±0.03^{ab}	0.98 ±0.04^{a}	1.28 ± 0.01^{ab}	0.98 ±0.01^{a}	1.07 ±0.23^{ab}	1.15 ±0.01^{ab}	1.22 ±0.01^{ab}	1.30 ±0.02^{b}	1.22 ±0.02^{ab}
16:0	12.49 ±0.10^{a}	12.49 ±0.23^{a}	11.54 ±0.08^{bc}	12.16 ±0.07^{ab}	13.30 ±0.35^{d}	12.56 ±0.04^{ae}	11.39 ±0.10^{c}	13.19 ±0.09^{de}	11.72 ±0.06^{bc}
18:0	4.87 ±0.04^{a}	5.76 ±0.10^{b}	4.55 ±0.06^{c}	5.58 ±0.01^{bde}	6.51 ±0.06^{f}	5.65 ±0.04^{be}	5.55 ±0.05^{de}	5.42 ±0.01^{d}	5.70 ±0.01^{be}
16:1n7	1.79 ±0.03^{ab}	1.51 ±0.12^{a}	2.06 ±0.00^{b}	1.55 ±0.02^{a}	1.68 ±0.33^{ab}	1.79 ±0.01^{ab}	1.93 ±0.01^{ab}	1.87 ±0.03^{ab}	1.95 ±0.04^{ab}
18:1n9	28.68 ±0.31^{a}	29.79 ±0.25^{ab}	30.90 ±0.49^{bcd}	28.92 ±0.07^{a}	29.89 ±0.78^{ab}	30.15 ±0.21^{abc}	32.22 ±0.08^{de}	31.40 ±0.11^{cde}	32.43 ±0.46^{e}
18:1n7	2.75 ±0.02^{a}	2.81 ±0.03^{ab}	2.79 ± 0.04^{ab}	2.73 ±0.03^{a}	2.98 ±0.34^{ab}	3.01 ±0.01^{ab}	3.17 ±0.02^{ab}	3.23 ±0.06^{b}	3.14 ±0.03^{ab}
20:1n9	1.19 ±0.04^{a}	1.43 ±0.03^{abc}	1.35 ±0.06^{ab}	1.40 ±0.04^{ab}	1.64 ±0.66^{abc}	1.80 ±0.01^{abc}	2.30 ±0.00^{c}	1.96 ±0.09^{abc}	2.20 ±0.02^{bc}
18:2n6	12.06 ±0.08^{a}	11.36 ±0.07^{b}	12.53 ±0.07^{c}	11.17 ±0.17^{b}	10.35 ±0.19^{d}	10.48 ±0.07^{d}	11.47 ±0.03^{b}	11.17 ±0.18^{b}	11.01 ±0.07^{b}
20:4n6	2.79 ±0.07^{a}	2.80 ±0.03^{a}	2.86 ±0.05^{a}	2.99 ±0.03^{a}	2.80 ±0.62^{a}	2.31 ±0.02^{ab}	2.18 ±0.02^{ab}	1.73 ±0.03^{b}	2.25 ±0.04^{ab}
18:3n3	4.77 ±0.14^{a}	4.09 ± 0.04^{bc}	4.40 ± 0.04^{ab}	4.02 ± 0.01^{bcd}	3.55 ±0.28^{e}	3.70 ±0.01^{cde}	3.79 ±0.01^{cde}	3.62 ±0.09^{de}	3.69 ±0.01^{cde}
20:4n3	0.93 ±0.04^{a}	0.82 ±0.02^{abc}	0.90 ± 0.00^{ab}	0.88 ± 0.03^{abc}	0.77 ±0.01^{c}	0.86 ±0.01^{abc}	0.80 ±0.00^{abc}	0.77 ±0.08^{bc}	0.80 ±0.02^{abc}
20:5n3	7.70 ±0.28^{a}	6.96 ±0.06^{abc}	7.29 ± 0.21^{ab}	7.06 ± 0.04^{abc}	6.23 ±0.95^{bcd}	5.72 ±0.05^{cd}	5.25 ±0.06^{d}	5.84 ±0.05^{cd}	5.53 ±0.10^{d}
22:5n3	2.39 ±0.01^{a}	2.43 ±0.01^{a}	2.03 ± 0.03^{b}	2.47 ± 0.04^{a}	2.48 ±0.06^{a}	2.74 ±0.01^{c}	2.38 ±0.04^{a}	2.23 ±0.02^{d}	2.41 ±0.06^{a}
22:6n3	14.91 ±0.10^{a}	14.79 ±0.46^{a}	13.40 ± 0.30^{b}	14.83 ± 0.13^{a}	13.98 ±0.69^{ab}	14.18 ±0.18^{ab}	12.87 ±0.12^{b}	13.34 ±0.29^{b}	12.96 ±0.31^{b}
Others	1.54 ±0.04^{a}	2.01 ±0.11^{a}	2.14 ± 0.01^{a}	3.28 ± 0.08^{bc}	2.80 ±0.05^{b}	3.95 ±0.04^{d}	3.53 ±0.33^{cd}	2.97 ±0.30^{bc}	3.03 ±0.18^{bc}

Results are expressed as mean value ± standard deviation. Within each FA, values sharing the same letter indicate no significant differences ($p > 0.05$) after applying a two-way ANOVA and Tukey's post hoc test.

viscera and salmon liver (70.7 g and 68.7 g H_2O/100 g raw biomass, respectively) (Table 1). The lowest moisture amount was found in salmon viscera (51.7 g H_2O/100 g raw biomass). Concerning lipid content, the highest amount (according to Folch extraction) was found by far in salmon viscera, followed by salmon liver, red cusk-eel, and yellowtail kingfish viscera (Table 1).

Lower water and higher lipid contents were found in this work in viscera from farmed specimens when being compared to wild fishes, in agreement with previous studies. For instance, high moisture percentages in viscera from some wild-caught species such as Alaskan Pollock (63.5%), Pacific cod (76.5%), pink salmon (81.2%), and liver of jumbo squid (70.0%) have been reported [20, 21], whereas for viscera from farmed gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*), only 49.0% moisture was found [13]. However, lipid content in the same species were higher in farmed fishes: 34% for farmed European sea bass and gilthead sea bream against 2% for pink salmon viscera, 8% for Pacific cod viscera and Jumbo squid liver and 19% for Alaskan Pollock viscera [13, 20, 21]. This fact is probably related to the different lipid content of the diets of wild and farmed fishes. Fish oil is traditionally an ingredient for fish feeding in aquaculture, but reducing the amount of fish oil is critical for sustainability and economic reasons [22]. This way, fish oils are often replaced by vegetable oils [23]. Furthermore, fish farmers tend to increase the lipid content of feed in order to promote growth and save on proteins, which result in a higher lipid concentration in fish tissues [23], particularly in viscera [24]. This could explain the high lipid content observed in viscera of farmed salmon in relation to the other by-products assayed in this work, as it has been previously reported that farmed specimens contain much more lipids than their wild counterparts because of the strong influence of feed composition on lipid occurrence in fish tissues [23, 25, 26].

The use of food-grade solvents is still one of the most employed extraction methods for lipids at industrial scale, and n-hexane is a popular choice due to its relatively low cost and high extraction efficiency [27]. In addition, n-hexane fulfils other desirable conditions for a solvent extraction such as water insolubility, low boiling point to facilitate its removal after extraction, and considerable different density than water [27]. Although there are some concerns about the use of n-hexane as extraction solvent because it is a petroleum-based substance which can be explosive if not operated correctly, its low boiling point allows an effective evaporation after extraction, minimizing the occurrence of solvent traces in the extracted lipids and avoiding the exposure of lipids to higher temperatures which may lead to their thermal degradation. Using other extractants classified as "green solvents" such as ethanol and d-limonene [28, 29] is a recent trend which is being explored for lipid extraction with promising preliminary

Table 3. Fatty acid profiles of salmon viscera and its lipid fractions, both in raw and dried state (in percentage of total FAs)

	Salmon viscera								
	Raw				Dried				
Fatty acid	Direct	Folch	Hexane	Hexane:iProp	Direct	Folch	Hexane	Hexane:iProp	Soxhlet
14:0	1.87 ± 0.02^{a}	1.95 ± 0.00^{b}	2.00 ± 0.02^{b}	2.01 ± 0.00^{b}	2.01 ± 0.01^{b}	2.00 ± 0.02^{b}	1.99 ± 0.01^{b}	2.02 ± 0.04^{b}	2.01 ± 0.01^{b}
16:0	10.52 ± 0.44^{ab}	10.27 ± 0.05^{a}	10.89 ± 0.03^{abc}	11.05 ± 0.08^{bc}	11.21 ± 0.00^{c}	11.12 ± 0.03^{bc}	11.06 ± 0.01^{bc}	11.22 ± 0.23^{c}	11.14 ± 0.07^{bc}
18:0	3.01 ± 0.21^{a}	3.51 ± 0.02^{b}	3.86 ± 0.01^{c}	3.92 ± 0.05^{c}	4.04 ± 0.03^{c}	3.97 ± 0.01^{c}	3.96 ± 0.01^{c}	3.96 ± 0.04^{c}	3.97 ± 0.03^{c}
16:1n7	2.59 ± 0.05^{a}	2.58 ± 0.01^{a}	2.56 ± 0.01^{a}	2.56 ± 0.02^{a}	2.56 ± 0.00^{a}	2.54 ± 0.01^{a}	2.54 ± 0.01^{a}	2.54 ± 0.01^{a}	2.55 ± 0.02^{a}
18:1n9	39.46 ± 0.38^{a}	41.06 ± 0.06^{b}	40.55 ± 0.14^{ab}	40.83 ± 0.04^{b}	41.09 ± 0.28^{b}	40.47 ± 0.06^{ab}	40.54 ± 0.07^{ab}	40.83 ± 0.76^{b}	40.74 ± 0.06^{b}
18:1n7	2.71 ± 0.06^{a}	3.16 ± 0.00^{b}	3.13 ± 0.02^{b}	3.17 ± 0.01^{b}	3.23 ± 0.10^{b}	3.13 ± 0.01^{b}	3.13 ± 0.01^{b}	3.16 ± 0.06^{b}	3.15 ± 0.02^{b}
20:1n9	2.00 ± 0.18^{a}	2.44 ± 0.18^{b}	2.32 ± 0.01^{ab}	2.17 ± 0.01^{ab}	2.37 ± 0.04^{ab}	2.23 ± 0.12^{ab}	2.33 ± 0.01^{ab}	2.24 ± 0.10^{ab}	2.25 ± 0.10^{ab}
18:2n6	19.11 ± 0.11^{a}	17.10 ± 0.06^{b}	17.04 ± 0.04^{b}	17.12 ± 0.04^{bc}	17.47 ± 0.04^{c}	16.89 ± 0.01^{b}	16.90 ± 0.06^{b}	16.97 ± 0.21^{b}	16.93 ± 0.06^{b}
20:4n6	0.54 ± 0.30^{a}	0.35 ± 0.02^{a}	0.37 ± 0.01^{a}	0.41 ± 0.05^{a}	0.37 ± 0.00^{a}	0.43 ± 0.01^{a}	0.42 ± 0.00^{a}	0.41 ± 0.02^{a}	0.38 ± 0.03^{a}
18:3n3	6.03 ± 0.07^{a}	5.10 ± 0.03^{b}	4.97 ± 0.01^{bc}	5.03 ± 0.01^{bc}	4.91 ± 0.02^{c}	4.93 ± 0.03^{c}	4.93 ± 0.02^{c}	4.94 ± 0.04^{c}	4.92 ± 0.03^{c}
20:4n3	0.99 ± 0.06^{a}	0.72 ± 0.01^{b}	0.63 ± 0.01^{bc}	0.60 ± 0.01^{c}	0.63 ± 0.01^{bc}	0.62 ± 0.01^{c}	0.63 ± 0.01^{bc}	0.62 ± 0.02^{c}	0.60 ± 0.04^{c}
20:5n3	3.49 ± 0.07^{a}	3.06 ± 0.04^{b}	2.82 ± 0.01^{c}	2.87 ± 0.03^{c}	2.81 ± 0.02^{c}	2.83 ± 0.02^{c}	2.83 ± 0.01^{c}	2.82 ± 0.04^{c}	2.78 ± 0.04^{c}
22:5n3	1.60 ± 0.04^{a}	1.22 ± 0.00^{b}	1.24 ± 0.01^{b}	1.23 ± 0.02^{b}	1.23 ± 0.00^{b}	1.22 ± 0.01^{b}	1.23 ± 0.00^{b}	1.20 ± 0.06^{b}	1.21 ± 0.00^{b}
22:6n3	4.41 ± 0.05^{a}	4.00 ± 0.05^{b}	3.87 ± 0.04^{b}	3.96 ± 0.02^{b}	3.91 ± 0.04^{b}	3.95 ± 0.03^{b}	3.95 ± 0.15^{b}	3.90 ± 0.06^{b}	3.82 ± 0.06^{b}
Others	1.71 ± 0.21^{a}	3.51 ± 0.02^{bc}	3.79 ± 0.11^{c}	3.12 ± 0.04^{abc}	2.17 ± 0.19^{ab}	3.71 ± 0.05^{c}	3.61 ± 0.08^{bc}	3.20 ± 1.09^{abc}	3.58 ± 0.18^{bc}

Results are expressed as mean value ± standard deviation. Within each FA, values sharing the same letter indicate no significant differences ($p > 0.05$) after applying a two-way ANOVA and Tukey's post hoc test.

results, but their use is not yet as extended as n-hexane in industrial applications [30]. Furthermore, such solvents have usually higher boiling points than n-hexane, thus requiring more energy and higher temperatures for solvent removal after extraction [30]. Although other extraction methodologies are available, for instance those employing supercritical fluids or enzymes [31], they are rather expensive and not fully adapted to large scale yet. In this work, three methodologies based on food-grade solvents were employed and compared in terms of extraction efficiency and fatty acid composition of extracted lipids from raw and dried by-products: extraction with n-hexane, extraction with n-hexane:isopropanol (3:2 v/v) and extraction with n-hexane in Soxhlet mode. Adding isopropanol to n-hexane was carried out to increase the polarity of the extraction system compared to extracting only with n-hexane, whereas Soxhlet extraction was performed to check the potential effect of an increased temperature compared to the use of n-hexane at room temperature. Folch extraction [32] was carried out as reference method.

Folch extraction offered the highest yield in all cases both in raw and dried by-products (Table 1). Soxhlet extraction was only carried out for dried by-products due to the difficulty to perform the process with the raw materials, and it was shown to be as effective as Folch extraction for salmon and red cusk-eel viscera, offering close figures to Folch extraction for salmon liver and yellowtail kingfish viscera (Table 1). Hexane and hexane:isopropanol extractions at room temperature showed lower yields than Soxhlet, although adding isopropanol improved generally the efficiency compared to the use of only n-hexane. This way, in terms of lipid extraction efficiency, increasing the operating temperature (Soxhlet) seems to play a more relevant role than increasing polarity of the solvent system (isopropanol addition) in n-hexane-based solvent extraction from dried fish by-products.

3.2 Fatty acid profiles

3.2.1 Salmon liver

Fatty acid profiles of raw and dried salmon liver and lipids extracted from this by-product by different solvent systems are reported in Table 2. The main FAs found in all samples were palmitic acid (PA, 16:0), oleic acid (OA, 18:1n9), linoleic acid (LA, 18:2n-6), EPA, and DHA. Both EPA and DHA levels were lower after drying, but the difference was only significant ($p < 0.05$) for EPA (7.70% vs 6.23% of total FAs in raw and dried liver, respectively). EPA and DHA levels found in extracted lipids from dried liver were lower than in dried liver, but differences were not significant in any case ($p > 0.05$). It means that extraction method had no influence on EPA and DHA profiles when extracting lipids from dried salmon liver. In contrast, DHA level in lipids

Table 4. Fatty acid profiles of red cusk-eel viscera and its lipid fractions, both in raw and dried state (in percentage of total FAs)

	Red cusk-eel viscera							
	Raw				Dried			
Fatty acid	Direct	Folch	Hexane	Hexane:iProp	Direct	Folch	Hexane	Hexane:iProp	Soxhlet
14:0	3.19 ± 0.13^{a}	4.38 ± 0.02^{b}	4.38 ± 0.01^{b}	4.37 ± 0.01^{b}	3.44 ± 0.03^{c}	4.43 ± 0.02^{b}	4.49 ± 0.02^{b}	4.45 ± 0.09^{b}	4.46 ± 0.02^{b}
16:0	17.05 ± 0.04^{a}	19.36 ± 0.15^{b}	17.67 ± 0.12^{a}	19.19 ± 0.02^{b}	19.23 ± 0.13^{b}	19.56 ± 0.23^{b}	19.42 ± 0.25^{b}	19.44 ± 0.42^{b}	18.94 ± 0.11^{b}
18:0	5.61 ± 0.65^{abc}	5.78 ± 0.05^{abc}	4.80 ± 0.03^{a}	5.61 ± 0.11^{abc}	6.22 ± 0.03^{c}	5.68 ± 0.07^{abc}	5.54 ± 0.12^{abc}	5.56 ± 0.11^{abc}	5.27 ± 0.05^{ab}
16:1n7	9.86 ± 0.37^{ab}	9.60 ± 0.16^{a}	10.26 ± 0.11^{b}	9.67 ± 0.02^{a}	9.95 ± 0.04^{ab}	9.90 ± 0.06^{ab}	9.99 ± 0.05^{ab}	9.78 ± 0.06^{ab}	9.85 ± 0.04^{ab}
18:1n9	16.47 ± 0.16^{a}	16.84 ± 0.39^{ab}	17.11 ± 0.16^{abc}	17.08 ± 0.06^{abc}	17.46 ± 0.10^{bc}	17.46 ± 0.32^{bc}	17.82 ± 0.17^{c}	17.43 ± 0.28^{bc}	16.97 ± 0.04^{abc}
18:1n7	5.24 ± 0.16^{a}	6.81 ± 0.03^{b}	6.71 ± 0.11^{b}	6.82 ± 0.03^{b}	7.02 ± 0.05^{b}	6.97 ± 0.06^{b}	7.03 ± 0.11^{b}	6.92 ± 0.06^{b}	6.95 ± 0.02^{b}
20:1n9	0.66 ± 0.11^{a}	1.14 ± 0.08^{ab}	1.07 ± 0.01^{ab}	1.19 ± 0.01^{ab}	1.59 ± 0.04^{b}	1.17 ± 0.02^{ab}	1.22 ± 0.01^{ab}	1.18 ± 0.00^{ab}	1.56 ± 0.52^{b}
18:2n6	1.20 ± 0.04^{a}	1.05 ± 0.03^{a}	1.07 ± 0.01^{a}	1.09 ± 0.01^{a}	1.05 ± 0.02^{a}	1.15 ± 0.11^{a}	1.22 ± 0.05^{a}	1.15 ± 0.05^{a}	1.04 ± 0.01^{a}
20:4n6	4.28 ± 0.03^{a}	2.85 ± 0.04^{bc}	2.93 ± 0.02^{bc}	2.86 ± 0.05^{bc}	2.96 ± 0.04^{b}	2.66 ± 0.07^{d}	2.64 ± 0.05^{d}	2.78 ± 0.03^{cd}	2.66 ± 0.04^{d}
18:3n3	0.27 ± 0.01^{a}	0.35 ± 0.01^{a}	0.38 ± 0.01^{a}	0.32 ± 0.03^{a}	0.34 ± 0.01^{a}	0.40 ± 0.05^{a}	0.36 ± 0.08^{a}	0.36 ± 0.01^{a}	0.37 ± 0.01^{a}
20:4n3	0.40 ± 0.06^{ab}	0.43 ± 0.03^{ab}	0.49 ± 0.01^{b}	0.50 ± 0.01^{b}	0.37 ± 0.01^{a}	0.47 ± 0.00^{ab}	0.48 ± 0.02^{ab}	0.49 ± 0.02^{b}	0.47 ± 0.04^{ab}
20:5n3	13.31 ± 0.70^{a}	11.30 ± 0.06^{b}	12.05 ± 0.44^{ab}	11.48 ± 0.16^{b}	11.09 ± 0.06^{b}	11.20 ± 0.34^{b}	11.06 ± 0.38^{b}	11.25 ± 0.45^{b}	11.35 ± 0.06^{b}
22:5n3	4.11 ± 0.01^{ab}	4.43 ± 0.02^{bc}	4.52 ± 0.16^{c}	4.50 ± 0.03^{bc}	4.00 ± 0.01^{a}	4.34 ± 0.16^{abc}	4.38 ± 0.12^{abc}	4.41 ± 0.15^{bc}	4.46 ± 0.01^{bc}
22:6n3	15.32 ± 0.28^{a}	13.00 ± 0.05^{bc}	13.71 ± 0.10^{b}	13.30 ± 0.09^{bc}	12.97 ± 0.11^{bc}	12.56 ± 0.38^{c}	12.40 ± 0.35^{c}	12.82 ± 0.51^{bc}	12.91 ± 0.11^{bc}
Others	3.07 ± 0.52^{a}	2.71 ± 0.83^{a}	2.90 ± 0.42^{a}	2.05 ± 0.28^{a}	2.34 ± 0.47^{a}	2.09 ± 0.05^{a}	2.00 ± 0.06^{a}	2.02 ± 0.06^{a}	2.78 ± 0.64^{a}

Results are expressed as mean value ± standard deviation. Within each FA, values sharing the same letter indicate no significant differences ($p > 0.05$) after applying a two-way ANOVA and Tukey's post hoc test.

extracted with n-hexane from raw liver is significantly lower than for raw liver and lipids extracted with the Folch or the hexane:isopropanol methods. Other PUFAs such as LA and ALA, showed significant lower values ($p < 0.05$) in dried than in raw liver, whereas monounsaturated FA (MUFA) levels were not significantly modified and saturated FAs (SFAs) such as PA and stearic acid (SA, 18:0) were significantly increased in dried liver ($p < 0.05$).

3.2.2 Salmon viscera

Fatty acid profiles of raw and dried salmon viscera and lipids extracted from this by-product by different solvent systems are reported in Table 3. The main FAs found in all samples were PA, OA, LA, and ALA, whereas EPA and DHA accounted for less than 5% of total FA each. This by-product contains the lowest levels of EPA and DHA among by-products analyzed in this work, which may be due to low amounts of EPA and DHA in the meals used to feed farmed fishes because of the replacement of fish oils for vegetable oils (which do not content EPA nor DHA) to elaborate the meals [33]. Contrarily to what was observed for salmon liver, where only significant decrease of EPA but not DHA was found after drying, significant lower values for both n-3 PUFA were found in dried viscera when compared to raw one ($p < 0.05$). This fact can be explained when considering FA loss percentages in relative terms; relative loss of a given FA is calculated as (absolute FA loss percentage × 100)/initial FA percentage in the raw form.

For instance, absolute DHA loss percentage in salmon viscera after drying is 0.50% (from 4.41 to 3.91%) whereas in salmon liver is 0.93% (from 14.91 to 13.98% of total FAs). However, DHA decrease is statistically significant ($p < 0.05$) in salmon viscera but not in liver, in spite of the absolute DHA loss percentage is lower in viscera than in liver. Considering the DHA loss percentage in relative terms, DHA decrease in dried salmon liver is 6.2% of that existing in raw liver (not significant according to the statistical analysis) and 11.3% in the case of salmon viscera (significant according to the statistical analysis).

No differences on EPA and DHA values were found between dried viscera and lipids extracted from dried viscera. Other PUFAs such as LA and ALA showed significant lower values in dried than in raw viscera, whereas OA level was significantly higher in dried viscera ($p < 0.05$). SFAs such as myristic acid (MA, 14:0), PA, and SA were significantly increased in dried viscera ($p < 0.05$).

3.2.3 Red cusk-eel viscera

Fatty acid profiles of raw and dried red cusk-eel viscera and lipids extracted from this by-product by different solvent systems are reported in Table 4. The main FAs found in all samples were PA, OA, EPA, and DHA. EPA and DHA values were significantly lower in dried than in raw viscera,

Table 5. Fatty acid profiles of yellowtail kingfish viscera and its lipid fractions, both in raw and dried state (in percentage of total FAs)

	Yellowtail kingfish viscera								
	Raw				Dried				
Fatty acid	Direct	Folch	Hexane	Hexane:iProp	Direct	Folch	Hexane	Hexane:iProp	Soxhlet
14:0	2.39 ± 0.02^{a}	2.40 ± 0.06^{a}	3.06 ± 0.16^{b}	2.76 ± 0.02^{ab}	2.67 ± 0.01^{ab}	3.21 ± 0.04^{b}	3.12 ± 0.03^{b}	3.25 ± 0.45^{b}	2.87 ± 0.09^{ab}
16:0	18.31 ± 0.06^{a}	18.88 ± 0.35^{ab}	20.11 ± 0.91^{bcd}	20.07 ± 0.05^{abcd}	19.81 ± 0.25^{abc}	21.76 ± 0.13^{de}	22.42 ± 0.08^{e}	22.58 ± 0.74^{e}	20.98 ± 0.52^{cde}
18:0	8.56 ± 0.22^{a}	8.54 ± 0.08^{a}	8.28 ± 0.34^{a}	8.96 ± 0.07^{ab}	9.08 ± 0.21^{ab}	10.79 ± 0.04^{c}	10.39 ± 0.03^{cd}	9.59 ± 0.50^{bd}	9.14 ± 0.11^{ab}
16:1n7	3.58 ± 0.12^{a}	3.38 ± 0.09^{a}	4.32 ± 0.30^{bc}	3.97 ± 0.06^{ab}	3.85 ± 0.07^{ab}	4.42 ± 0.01^{bc}	4.69 ± 0.01^{c}	4.43 ± 0.36^{bc}	3.99 ± 0.15^{ab}
18:1n9	18.35 ± 0.11^{ab}	19.94 ± 0.67^{bcd}	17.75 ± 0.85^{a}	18.74 ± 0.23^{ab}	18.21 ± 0.10^{a}	19.29 ± 0.23^{abc}	21.57 ± 0.06^{d}	20.44 ± 0.26^{cd}	19.37 ± 0.46^{abc}
18:1n7	3.23 ± 0.01^{abc}	3.24 ± 0.07^{abc}	3.03 ± 0.23^{a}	3.04 ± 0.01^{ab}	2.98 ± 0.11^{a}	3.46 ± 0.01^{bc}	3.57 ± 0.01^{c}	3.33 ± 0.15^{abc}	3.40 ± 0.12^{abc}
20:1n9	0.88 ± 0.06^{a}	1.12 ± 0.03^{b}	0.92 ± 0.08^{ac}	0.97 ± 0.02^{abc}	0.94 ± 0.02^{abc}	1.04 ± 0.01^{abc}	1.10 ± 0.01^{bc}	1.04 ± 0.08^{abc}	1.01 ± 0.04^{abc}
18:2n6	1.24 ± 0.03^{a}	1.85 ± 0.16^{b}	1.30 ± 0.02^{a}	1.21 ± 0.04^{a}	1.17 ± 0.02^{a}	1.21 ± 0.08^{a}	1.24 ± 0.00^{a}	1.37 ± 0.21^{a}	1.20 ± 0.02^{a}
20:4n6	2.67 ± 0.13^{a}	2.40 ± 0.08^{ab}	2.36 ± 0.15^{abc}	2.37 ± 0.04^{abc}	2.36 ± 0.08^{abc}	1.95 ± 0.03^{cd}	1.91 ± 0.01^{d}	2.01 ± 0.23^{bcd}	2.32 ± 0.07^{abcd}
18:3n3	0.61 ± 0.04^{a}	0.76 ± 0.02^{a}	0.68 ± 0.05^{a}	0.58 ± 0.01^{a}	0.57 ± 0.02^{a}	0.53 ± 0.09^{a}	0.52 ± 0.01^{a}	0.82 ± 0.35^{a}	0.58 ± 0.01^{a}
20:4n3	0.52 ± 0.15^{a}	0.61 ± 0.01^{a}	0.57 ± 0.07^{a}	0.53 ± 0.01^{a}	0.53 ± 0.01^{a}	0.41 ± 0.01^{a}	0.47 ± 0.01^{a}	0.52 ± 0.01^{a}	0.52 ± 0.02^{a}
20:5n3	6.93 ± 0.18^{a}	5.90 ± 0.08^{ab}	6.03 ± 0.69^{ab}	5.89 ± 0.01^{ab}	5.77 ± 0.20^{ab}	5.26 ± 0.06^{b}	5.45 ± 0.08^{b}	5.30 ± 0.66^{b}	6.01 ± 0.15^{ab}
22:5n3	3.05 ± 0.15^{a}	3.02 ± 0.05^{a}	2.68 ± 0.01^{ab}	2.72 ± 0.04^{ab}	2.71 ± 0.08^{ab}	2.01 ± 0.02^{c}	2.11 ± 0.01^{c}	2.35 ± 0.30^{bc}	2.82 ± 0.01^{a}
22:6n3	24.93 ± 0.19^{a}	21.90 ± 0.96^{bcd}	22.50 ± 0.95^{abc}	23.03 ± 0.60^{ab}	22.96 ± 0.25^{ab}	19.52 ± 0.31^{de}	18.88 ± 0.13^{e}	19.96 ± 1.03^{cde}	21.02 ± 0.73^{bcde}
Others	4.81 ± 0.01^{ab}	6.11 ± 0.37^{b}	6.44 ± 0.05^{b}	5.21 ± 1.05^{b}	6.44 ± 0.53^{b}	5.18 ± 0.19^{b}	2.60 ± 0.01^{c}	3.05 ± 0.87^{ac}	4.83 ± 0.46^{ab}

Results are expressed as mean value ± standard deviation. Within each FA, values sharing the same letter indicate no significant differences ($p > 0.05$) after applying a two-way ANOVA and Tukey's post hoc test.

although no different EPA and DHA values were found between dried viscera and its extracted lipids, no matter which method was used for extraction. In contrast, significant different values for these PUFAs were observed between raw viscera and its extracted lipids.

No differences were found between raw and dried viscera for LA and ALA, whereas significant differences ($p < 0.05$) were found for arachidonic acid (AA, 20:4n-6) in raw (4.28% of total FAs), and dried viscera (2.96% of total FAs). OA, MA, and PA proportions were significantly higher in dried than in raw viscera ($p < 0.05$).

3.2.4 Yellowtail kingfish viscera

Fatty acid profiles of raw and dried yellowtail kingfish viscera and lipids extracted from this by-product by different solvent systems are reported in Table 5. The main FAs found in all samples were PA, OA, and DHA, whereas EPA accounted for 5–7% of total FAs. DHA proportion was the highest among all assayed by-products in this work. EPA and DHA proportions did not decrease significantly between raw and dried viscera. Whereas EPA level was similar between dried viscera and its lipids extracted by the four different methods, DHA proportion was significantly lower for lipids extracted with chloroform:methanol (Folch) (19.52%), n-hexane (18.88%), and n-hexane:isopropanol (19.96%) at room temperature, but when Soxhlet extraction was carried out, DHA level (21.02%) was closer to that found in dried viscera (22.96%). LA and ALA proportions were similar that these found in red cusk-eel viscera, showing no differences between raw and dried viscera. OA and PA levels were not significantly higher in dried than in raw viscera.

Among all assayed dried by-products in this work, yellowtail kingfish viscera showed the highest DHA proportion (22.96%), followed by salmon liver (13.98%), red cusk-eel viscera (12.97%), and salmon viscera (3.91%). Only for red cusk-eel and salmon viscera, DHA levels were significantly lower than for raw viscera, and even in these cases, decreases were not very pronounced, indicating that drying had a limited effect on DHA levels. Concerning EPA, the highest proportion among all assayed dried by-products was found in red cusk-eel viscera (11.09%), followed by salmon liver (6.23%), kingfish yellowtail viscera (5.77%), and salmon viscera (2.81%). EPA values were significantly decreased for dried red cusk-eel viscera and salmon liver and viscera compared to raw by-products, but as in the case of DHA, such decreases were not sharp.

LA and ALA proportions in dried by-products were much higher in liver and viscera from farmed salmon than in wild red cusk-eel and yellowtail kingfish viscera. This fact can be explained considering that farmed fishes are often fed with formulations including vegetable oils rich in LA and/or ALA such as sunflower, corn, canola, linseed, camelina, or soybean oils, which replace the traditional fish oils used for fishmeal elaboration [33] because of the current lower

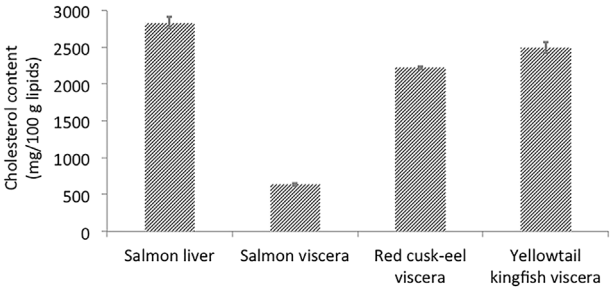

Figure 1. Cholesterol content in the lipid fractions extracted from dried fish by-products (mg cholesterol/100 g lipids).

availability and higher prices of fish oil. This way, LA and ALA values in tissue lipids from farmed fishes are usually higher than those found in wild specimens, as it was found in this study.

3.3 Cholesterol content

Cholesterol is naturally occurring in fish oils, being cholesterol amounts in most commercial fish oils (extracted from fish flesh) between 500 and 800 mg/100 g oil (USDA Food Composition Database). In our work, the highest concentration was found in salmon liver (2835 mg cholesterol/100 g oil), followed by yellowtail kingfish viscera (2490 mg/100 g oil), red cusk-eel viscera (2220 mg/100 g oil), and salmon viscera (640 mg/100 g oil) (Fig. 1). This way, cholesterol amounts were higher than 2000 mg/100 g oil in all cases except for salmon viscera. Such results are in agreement with previous evidence reporting that fish viscera may contain even three times more cholesterol than fish muscle [34].

Due to the high cholesterol content, it is advisable that cholesterol is removed from the lipid fraction whether it is intended to be used for nutritional purposes. Several methods have been described to remove cholesterol, for instance vacuum stripping is able to remove free cholesterol from oils, but it may be necessary a prior treatment with cholesterol esterase to convert the esterified cholesterol intro free cholesterol [35]. Other option would be the use of beta-cyclodextrins, which have been used to effectively remove cholesterol from animal products [31, 32]. Through complex formation, cholesterol separation process can be achieved by simultaneously recovering cholesterol and beta-cyclodextrins. Cyclodextrins are inexpensive enzyme-modified starch derivatives, industrially produced. They are non-toxic, not absorbed in the upper gastrointestinal tract, and completely metabolized by the colon microflora [36, 37].

4 Conclusions

Drying of fish by-products to obtain fine and inert powders may suppose a suitable action to facilitate handling, transport, and storage of products containing EPA and DHA. It increases extraction efficiency when using solvents to extract lipids and allows the use of lower solvent amounts, which is economically and environmentally desirable. However, application of high temperatures (>100°C) may affect FA profiles of such material, especially EPA and DHA because of their high susceptibility to thermal degradation. In this work, salmon liver, red cusk-eel viscera, and kingfish yellowtail viscera were successfully dried to obtain powders. However, salmon viscera, due to its high lipid content, resulted in an oily paste which sets it apart from the rest of the by-products studied in this work in term of ease of handling and storage.

Although drying decreased EPA and DHA proportions in all assayed by-products, such variations were not considerable and we consider that the advantages provided by drying overcome the slightly lower values of EPA and DHA found in dried by-products.

When comparing the different extraction processes to yield the lipid fraction from dried by-products, extraction with n-hexane using Soxhlet seems to be the best option of all food-grade assayed methods: no significant differences concerning EPA and DHA values were detected between lipids extracted with Soxhlet and their corresponding dried by-products in any case. Also in terms of lipid yield, Soxhlet was shown to be the most effective option among food-grade methods.

To the best of our knowledge, this is the first study reporting FA composition and cholesterol content in fish by-products from Chile. Further research is needed to assess other parameters in the lipid fractions such as contaminants (heavy metals, pesticides, dioxins, PCBs) as well as FA distribution in the lipid fraction (polar or neutral lipids) and oxidative stability. The use of "green solvents" such as isopropyl alcohol, ethyl acetate, or terpenes such as limonene is worth to be explored to assess their efficiency in terms of lipid extraction yields and fatty acid profiles when processing fish by-products.

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