Concentrating n-3 Fatty Acids from Crude and Refined Commercial Salmon Oil

Mª Elsa PANDO[1], Beatriz BRAVO[1], Macarena BERRIOS[1], Andrea GALDAMES[1], Catalina ROJAS[1], Nalda ROMERO[1], Conrado CAMILO[1], Cristian ENCINA[1], Matías RIVERA[1], Alicia RODRÍGUEZ[1] and Santiago P. AUBOURG[2]

[1]Department of Food Science and Chemical Technology. Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile; [2]Department of Food Technology, Instituto de Investigaciones Marinas (CSIC), Vigo, Spain

Abstract

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The urea complexation was used to concentrate n-3 fatty acids (FA) from crude and refined commercial salmon oils. The experimental procedure included salmon oil saponification, free fatty acid (FFA) collection, formation of urea-FFA inclusion complexes, extraction of free n-3 FA and further analysis by gas-liquid chromatography of the corresponding FA methyl esters. Differences between crude and refined salmon oil could be observed. Crude oil provided higher typical odour, viscosity and suspension particle values, whereas crude salmon oil showed higher FFA and impurities content while *p*-anisidine and iodine values, moisture content and formation of conjugated dienes and trienes did not provide any significant differences between both oils; refined oil showed lower *a** and *b** scores when compared to its counterpart crude oil. Related to the n-3 PUFA concentration, a decrease in saturated fatty acids C 14:0, C 16:0, and C 18:0 and monounsaturated fatty acids C 18:1 9c, and C 18:1 11c, as well as a high yield of n-3 PUFA, EPA+DHA and total PUFA recovering could be observed starting from both crude and refined oils, which confirmed salmon oil to be a profitable source of such highly valuable constituents. Factors such as reaction temperature and ureaFFA ratio showed to be markedly significant to achieve higher value concentrations.

Keywords: salmon oil; crude oil; refined oil; n-3 fatty acids; urea complexation; quality

Marine species have attracted considerable attention as a source of high amounts of valuable nutritional components to the human health and nutrition. Among them, essential fatty acids corresponding to the n-3 series have attracted increasing attention, *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA) being the most abundant components (Ackman & Ratnayake 1990). Marine organisms are recognised as the most important natural sources of such polyunsaturated fatty acid (PUFA) series, this arising from the marine phytoplankton as primary producer, and then following the tropic chain up to marine invertebrates and fish (Greene & Selivonchick 1987).

In recent years, evidence that fish-consuming populations have a low prevalence of coronary heart, circulatory and inflammation diseases has generated a great deal of interest in fish oils; in this sense, PUFA presence has been recognised as specially responsible for this positive behaviour (Simopoulos 1991). Thus, Clupeidae, Scombridae and Salmonidae are recognised as the fish families with the highest percentages of EPA and DHA in the foodstuff portion. EPA and DHA presence in humans is recognised as being mostly acquired by external sources (Tvrzicka *et al*. 2011). Fish oils and shellfish are nutritionally considered the major source of n-3 PUFA, especially EPA and DHA; thus, their intake in appropriate

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amounts has been found necessary (Zuta *et al*. 2003; Liu *et al*. 2006; Mbatia *et al*. 2010; Strobel *et al*. 2012). The positive effect of EPA is mainly associated to cardiovascular diseases (Nieto *et al*. 1997; Valenzuela *et al*. 2009), whereas DHA presence in nervous tissue and retina is extremely important for correct development of the corresponding life functions (Bradbury 2011; Kremmyda *et al*. 2011; Tvrzicka *et al*. 2011). Both fatty acids are precursors of a group of eicosanoids having anti-inflammatory, anti-thrombotic, antiarrhythmic and vasodilator properties (Kapoor & Patil 2011).

These beneficial effects for health have raised a great interest in obtaining concentrates of both EPA and DHA (Ganga *et al*. 1998). It has been suggested that the intake of n-3-PUFA concentrates would be better than direct consumption of fish oil due to the low contents of saturated fatty acids (SFA) found in PUFA-concentrated oils (Haagsma *et al*. 1982; Mbatia *et al*. 2010).

Among fish species, Coho salmon (*Oncorhynchus kisutch*) has received great attention because of its increasing farming production in countries such as Chile, Japan and Canada (FAO 2007a) in parallel to important capture production in countries such as USA, Russian Federation, Canada, and Japan (FAO 2007b); additionally, high and profitable n-3 PUFA contents have been reported for this species (Aubourg *et al*. 2005; Vinagre *et al*. 2011).

Several methods have been reported for concentrating PUFA in marine oils, with varied yields (Haagsma *et al*. 1982; Zuta *et al*. 2003; Rubio-Rodríguez *et al*. 2010).

Among them, urea complexation has been applied extensively, as allowing the handling of large quantities of materials in simple equipment and being a relatively inexpensive method (Rubio-Rodríguez *et a*l. 2010). Present research concerns the preparation of n-3 PUFA concentrates from available marine sources. In it, urea complexation was used to concentrate n-3 PUFA from crude (raw) and refined commercial salmon oils; comparison of results obtained from both kinds of oils was made.

MATERIAL AND METHODS

Raw material and chemicals employed. Crude (total n-3 fatty acids > 12%) and refined salmon oil was obtained from Salmonoil S.A. (Puerto Montt, Chile). Fatty acid methyl ester (FAME) standards and fatty acid (FA) standards were purchased from NU-CHEK PREP, Inc. (Elysian, USA), these including methyl esters of 52 different FA ranging between C4:0 and C24:1 (GLC Reference standard 463; Lot 021-U). 1,2,3-Tritricosanoylglycerol (CAS: 86850-72-8) was employed as internal standard. Urea, ethanol, *n*-hexane, methanol, and α-tocopherol employed were of analytical grade and obtained from Merck (Santiago, Chile).

Characterisation of crude and refined salmon oil. Characterisation of starting crude and refined salmon oils was carried out by assessment of free fatty acids (FFA; AOCS 1993, official method Ca 5a-40), peroxide value (PV; AOCS 1993, official method Cd 8b-90), *p*-anisidine value (AV; AOCS 1993, official method Cd 18-19), insoluble impurities (AOCS 1993, official method Ca 3a-46), unsaponifiable matter (UM; AOCS 1993, official method Ca 6b-53), iodine value (IV; AOCS 1993, official method Cd 1-25), moisture and volatile matter (AOCS 1993, official method Ca 2d-25) and colour parameters (L^*, a^*, b^* values by CIE $L^*a^*b^*$ method; Vinagre *et al*. 2011).

Additionally, conjugated diene (CD) and triene (CT) formation was measured at 233 nm and 268 nm, respectively (Kim & Labella 1987). Results are expressed according to the following formula: CD (or CT) = B × V/w, where: B – absorbance reading at 233 nm (or 268 nm), V – volume (ml), w – mass (mg) of oil measured.

Preparation of FAME. In order to convert the FA included in triacylglycerols into FAME, 10 ml of sodium methylate and 50 µl of internal standard (1,2,3-tritricosanoylglycerol, 23/0-23/0-23/0; 100 mg/ml) were added to 100 mg of oil. A fused silica capillary column 100 m × 0.25 mm *i.d*., coated with SP^{TM}-2560 (Supelco, Bellefonte, PA, USA) was employed. GLC setting conditions were as follows: injection temperature at 250°C, flame ionisation detector (FID) temperature at 250°C, flow rate of carrier gas (H_2) of 1.2 ml/min, and oven temperature from 160°C to 220°C with an increasing rate of 2°C/minutes. DataApex Clarity^{TM} software (DataApex Ltd., Prague, Czech Republic) was used for chromatogram analysis. The concentration of FAME was determined from the calibration curves by assessment of the peak/area ratio. Quantification of all kinds of FA was performed according to the AOCS Official Method (AOCS 2009, Ce 1j-7; Fahy *et al*. 2005).

Preparation of crude and refined salmon oils. Figure 1 shows the flowchart of the industrial process for obtaining the crude and refined salmon oils. Thus, for obtaining the crude salmon oil, the oil fraction obtained from the press liquor in the tricanter step, this including the polished oil fraction of tail water,

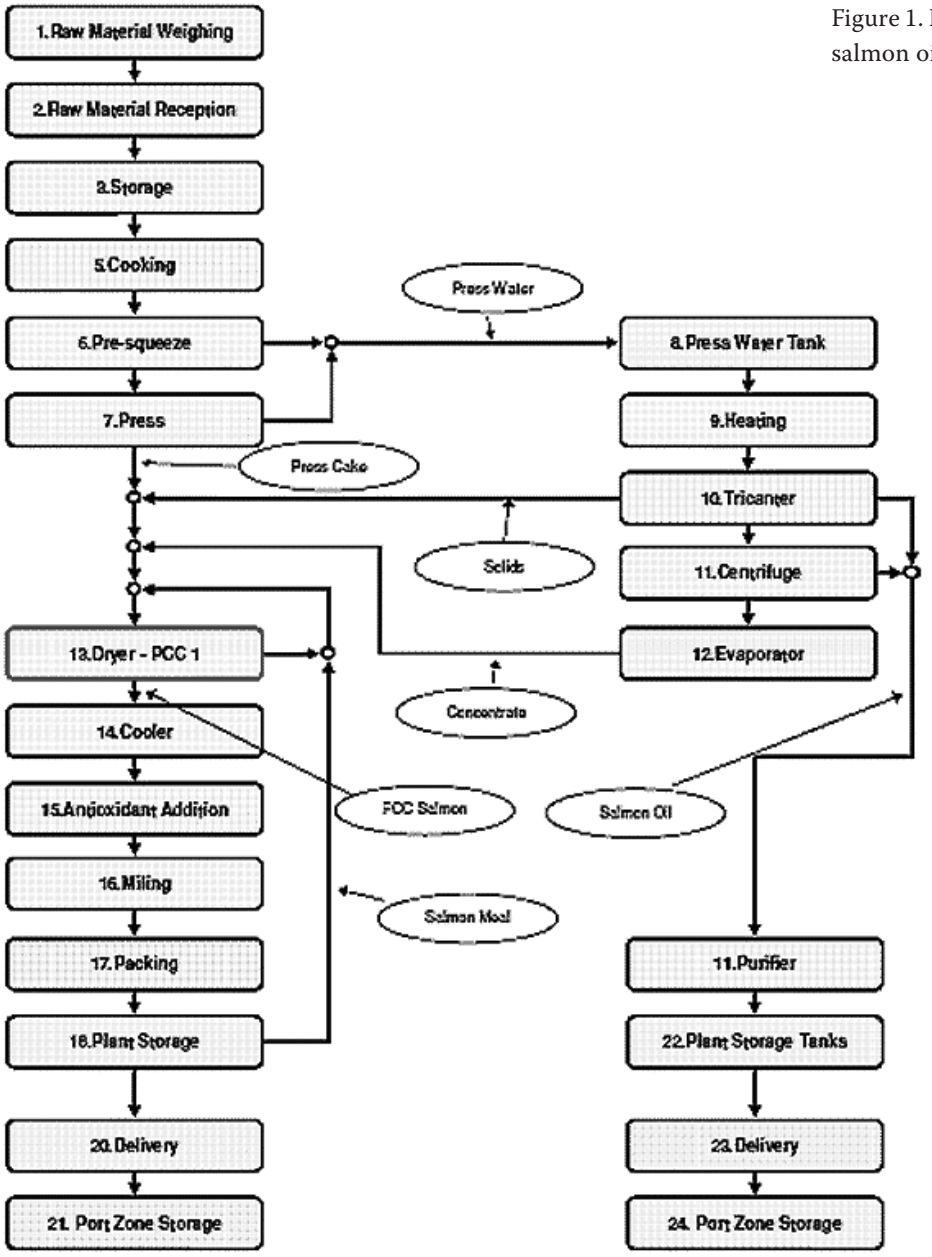

Figure 1. Flowchart of crude and refined salmon oil processing

is finally recovered from the centrifuge step. In the case of significant deviations of acidity, partial neutralisation is carried out by NaOH addition followed by removal of soaps by centrifugation. For obtaining the refined oil, neutralisation followed by a second polishing (water washing and centrifugation) and drying in a vacuum tank are carried out. Finally, the oil is filtered by means of an earth filter. The analysis of both oils by GLC was performed after previous FAME preparation.

Preparation of n-3-PUFA concentrates from salmon oils. The procedure included salmon oil saponification, FFA collection, formation of urea-FFA inclusion complexes, and extraction of free n-3 PUFA. Urea complexation was carried out by adduct formation according to the following procedure: 35 g of FFA were mixed with urea at a ratio of 1:3.5 (w/w), respectively, and ethanol was added at a ratio of 1:3.7 (w/v) based on the weight of urea. The mixture was heated at 60°C with stirring to dissolve all the urea, producing a clear homogeneous solution. Initially, the urea-fatty acid adduct was allowed to crystallise at room temperature and then the sample was held at 4°C for 18 h without agitation for further crystallisation. Urea crystals were separated by filtration and the non-urea-complexing fraction was diluted with 300 ml of distilled water, acidified to pH 4.5 with 6N HCl, and extracted twice with 150 ml of hexane. Hexane extracts were combined and then dried over anhydrous sodium sulphate. The solvent

was removed using a rotary evaporator. The final concentrated n-3 PUFA were flushed with nitrogen and stored at –70°C with 100 ppm of α-tocopherol (Haagsma *et al*. 1982; Zuta *et al.* 2003). The analysis of oil concentrates by GLC was performed after previous FAME preparation.

Statistical analysis. Physical and chemical analyses were performed in triplicate. The 95% confidence intervals of each quality parameter were calculated, taking into account the number of replications and considering the standard deviation (SD) of each sample. Results obtained were analysed by a multifactorial analysis of variance (MANOVA). In the case of significant differences, a multiple range comparison was carried out by means of Tukey's test. Statgraphics Plus® 5.1 software (Manugistics Inc., Rockville, USA) was used.

RESULTS AND DISCUSSION

Table 1 shows the characterisation of the starting crude (raw) and refined oil from salmon. Quality differences as a result of the refining process can be observed. Concerning chemical and physical analyses, crude salmon oil provided higher typical odour, viscosity and suspension particle values, showed a higher FFA content and IV while *p*-anisidine and iodine values, moisture content and formation of conjugated dienes and trienes did not provide any significant differences between both oils ($P > 0.05$). With the aim of being fit for human nutrition, the fat composition should not exceed relevant limitations expressed in legislation. According to Minsal (2012), accepted values of FFA in oil for human consumption should be lower than 0.25% (expressed as oleic acid) and the PV scores should be lower than 10 meq active oxygen/kg oil. Meantime, values lower than score 20 are required for the AV (Goed 2012). Accordingly, both kinds of present oils agree with such nutritional requirements. Related to physical colour assessment, refined salmon oil showed lower *a** (redness loss) and *b** (yellowness loss) values when compared to its counterpart crude oil.

FAME chromatograms corresponding to commercial crude and refined salmon oil and their n-3 FA concentrates are given in Figure 2. The effect of urea on the percentage of n-3 FA in crude (B) and refined (D) concentrates can be observed. Thus, the n-3 FA concentrate content increased due to the low-temperature crystallisation and the urea inclusion compound formation. The urea fractionation of the FA is mainly based on the degree of unsaturation; thus, the more the unsaturated, the less they will be included in the urea crystals (Haagsma *et al*. 1982).

Crude and refined salmon oil compositions, as well as their concentrate counterparts are shown in Table 2. The most abundant FA found in crude salmon oil were (g/100 g total FA): C 14:0 (3.42), C 16:0 (14.03), C 16:1 9c (4.68), C 18:0 (3.93), C 18:1 9c (30.04), C 18:2 9c,12c (16.50), C 18:3 n-3; α-linolenic (3.51), C 20:5 n-3 (5.03), C 22:5 n-3 (2.44), and C 22:6 n-3 (5.32). Whereas FA showing the higher presence in refined salmon oil were (g/100 g total FA): C 14:0 (3.47), C 16:0 (14.40), C 16:1 9c (4.94), C 18:0 (3.96), C 18:1 9c (30.70), C 18:2 9c,12c (16.99), C 18:3 n-3; α-linolenic (0.38), C 20:5 n-3 (5.27), C 22:5 n-3 (2.47), and C 22:6 n-3 (5.54).

Table 1. Characterisation[1] of the starting crude and refined salmon oil[2]

Quality parameter	Crude salmon oil	Refined salmon oil
Free fatty acids (FFA; g oleic acid/100 g oil)	1.78 ± 0.04[a]	0.23 ± 0.00[b]
Conjugated dienes (CD)	0.006 ± 0.007[a]	0.019 ± 0.010[a]
Moisture (g/100 g oil)	0.27 ± 0.19[a]	0.26 ± 0.15[a]
Impurities (g/100 g oil)	0.43 ± 0.28[b]	0.12 ± 0.02[a]
Iodine value (IV; g iodine/100 g oil)	146.35 ± 0.35[a]	146.55 ± 0.21[a]
Conjugated trienes (CT)	0.007 ± 0.006[a]	0.039 ± 0.040[a]
Peroxide value (PV; meq active oxygen/kg oil)	2.73 ± 0.36[a]	3.54 ± 0.16[b]
p-Anisidine value (AV)	5.33 ± 0.50[a]	5.14 ± 1.02[a]
Unsaponifiable matter (g/100 g oil)	0.86 ± 0.39[a]	1.51 ± 0.38[b]
*a** colour value	3.10 ± 0.50[a]	1.13 ± 0.33[b]
*b** colour value	7.95 ± 0.49[a]	6.87 ± 1.12[b]
*L** colour value	10.91 ± 1.20[a]	11.33 ± 0.73[a]

[1]mean values ($n = 9$) ± standard deviations; [2]for each quality parameter; [a,b]mean values preceded by different letters denote significant differences

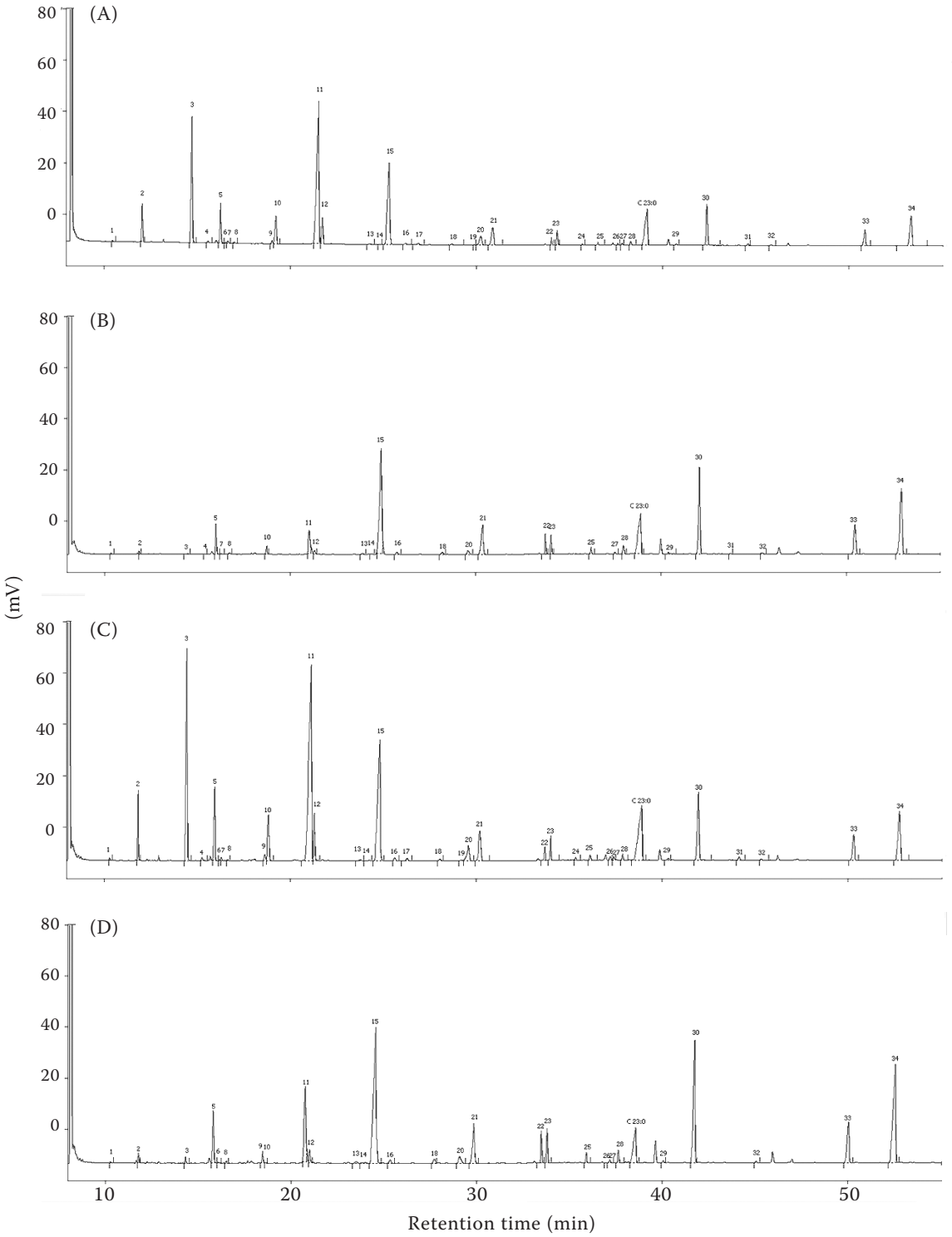

Figure 2. Fatty acid methyl ester chromatograms corresponding to crude and refined salmon oil and their n-3 fatty acid concentrates: (A) crude salmon oil, (B) n-3 concentrates of crude salmon oil, (C) refined salmon oil, and (D) n-3 concentrates of refined salmon oil*

*FAME number or chromatographic peak. **1**: C 12:0; **2**: C 14:0; **3**: C 16:0; **4**: C 16:1 9t; **5**: C 16:1 9c; **6**: C 16:1 11c; **7**: C 17:0; **8**: C 16:1 13c; **9**: C 17:1c; **10**: C 18:0; **11**: C 18:1 9c; **12**: C 18:1 11c; **13**: C 18:2 9c,12t; **14**: C 18:2 9t,12c; **15**: C 18:2 9c,12c; **16**: C 18:2 9c,15c; **17**: C 20:0; **18**: C 18:3 n-6 (γ-linolenic); **19**: C **20**:1 8c; **20**: C 20:1 11c; **21**: C 18:3 n-3 (α-linolenic); **22**: C 18:4 n-3; **23**: C 20:2 n-6; **24**: C 22:0; **25**: C 20:3 n-6; **26**: C 22:1; **27**: C 20:3 n-3; **28**: C 20:4 n-3; **29**: C 22:2 n-6; **30**: C **20**:5 n-3; **31**: C 24:1; **32**: C22:4 n-6; **33**: C 22:5 n-3; **34**: C 22:6 n-3

Table 2. Fatty acid (FA) composition and different FA groups in crude and refined salmon oils and their corresponding concentrates (expressed as g/100 g total FA)

No.[1]	FA or FA group	Crude salmon oil	Concentrated crude salmon oil	Refined salmon oil	Concentrated refined salmon oil
1	C 12:0	0.10	0.13	0.11	0.08
2	C 14:0	3.42	0.35	3.47	0.61
3	C 16:0	14.03	0.16	14.40	0.47
4	C 16:1 9t	0.23	0.02	0.24	0.00
5	C 16:1 9c	4.68	4.56	4.94	4.32
6	C 16:1 11c	0.07	0.00	0.09	0.08
7	C 17:0	0.23	0.08	0.27	0.00
8	C 16:1 13c	0.14	0.26	0.09	0.19
9	C 17:1c	0.45	0.00	0.49	1.12
10	C 18:0	3.93	1.34	3.96	0.06
11	C 18:1 9c	30.04	4.75	30.70	10.09
12	C 18:1 11c	2.99	0.55	3.33	0.96
13	C 18:2 9c,12t	0.14	0.22	0.15	0.22
14	C 18:2 9t,12c	0.03	0.09	0.07	0.15
15	C 18:2 9c,12c	16.50	30.49	16.99	27.53
16	C 18:2 9c,15c	0.30	0.53	0.29	0.44
17	C 20:0	0.28	0.00	0.27	0.00
18	C 18:3 n-6 (γ-linolenic)	0.21	0.58	0.18	0.57
19	C 20:1 8c	0.03	0.00	0.07	0.00
20	C 20:1 11c	1.98	1.07	2.09	1.17
21	C 18:3 n-3 (α-linolenic)	3.51	7.79	0.38	6.08
22	C 18:4 n-3	0.77	2.76	0.87	2.76
23	C 20:2 n-6	1.43	2.38	1.47	2.50
24	C 22:0	0.14	0.00	0.16	0.00
25	C 20:3 n-6	0.30	0.89	0.31	0.79
26	C 22:1	0.23	0.00	0.24	0.01
27	C 20:3 n-3	0.17	0.21	0.18	0.22
28	C 20:4 n-3	0.40	1.17	0.40	1.05
29	C 22:2 n-6	0.16	0.30	0.15	0.21
30	C 20:5 n-3	5.03	15.49	5.27	14.50
31	C 24:1	0.23	0.08	0.27	0.00
32	C22:4 n-6	0.09	0.20	0.09	0.18
33	C 22:5 n-3	2.44	6.28	2.47	5.38
34	C 22:6 n-3	5.32	17.27	5.54	18.26
	EPA+DHA	10.35	32.76	10.83	32.76
	∑ total n-3	17.64	50.97	15.13	48.25
	∑ PUFA	36.33	85.81	34.31	80.03
	∑ trans-isomers	0.40	0.33	0.46	0.37

[1]FA number or chromatographic peak

Crude and refined salmon oil *trans* FA were detected (g/100 g total FA, respectively): C 16:1 9t (0.23 and 0.24), C 18:2 9c, 12t (0.14 and 0.15), C 18:2 9t, 12c (0.03 and 0.07); total trans isomer FA content was 0.40 and 0.46, respectively (Table 2). It is worth pointing out that the trans isomer FA content of oils from industrial sources in food must be lower than 2% of the total fat content of the product.

Aubourg *et al.* (2005) found that the most abundant FA in Coho salmon farmed in the south of Chile were C 18:1 n-9 and C 16:0 fatty acids (19.3 and 20.7 g/100 g FAME, respectively), followed by

C 22:6 n-3, C 16:1 n-7 and C 20:5 n-3 (14.8, 7.7 and 7.1 g/100 g FAME, respectively).

In the present research, FA composition in the crude salmon oil concentrate was (g/100 g total FA): C 14:0 (0.35), C 16:0 (0.16), C 16:1 9c (4.56), C 18:0 (1.34), C 18:1 9 c (4.75), C 18:2 9c,12c (30.49), C 18:3 n-3; α-linolenic (7.79), C 20:5 n-3 (15.49), C 22:5 n-3 (6.28) and C 22:6 n-3 (17.27); whereas FA composition in the refined salmon oil concentrate was (g/100 g total FA): C 14:0 (0.61), C 16:0 (0.47), C 16:1 9c (4.32), C 18:0 (0.06), C 18:1 9c (10.09), C 18:2 9c,12c (27.53), C 18:3 n-3 (6.08), C 18:4 n-3 (2.76), C 20:2 n-6 (2.50), C 20:5 n-3 (14.50), C 22:5 n-3 (5.38) and C 22:6 n-3 (18.26).

The crude and refined salmon oil trans fatty acid concentrates were (g/100 g total FA): C 16:1 9t (0.02 and 0), C 18:2 9c, 12t (0.22 and 0.22), C 18:2 9t, 12c (0.09 and 0.15); total trans FA content was 0.33 and 0.37, respectively (Table 2).

SFA content decreased after urea inclusion, whereas unsaturated fatty acid content increased especially in the case of the n-3 FA. SFA of refined and crude oil such as C 14:0, C 16:0 and C 18:0, as well as monounsaturated fatty acids such as C 18:1 9c and C 18:1 11c, formed adducts due to urea inclusion.

The total n-3 PUFA content (g/100 g total FA) of crude and refined salmon oil concentrate was 50.97 and 48.25, respectively; being the EPA+DHA content of 32.76 for both concentrates (Table 2).

According to Haagsma *et al.* (1982), a maximal efficiency of 82% was found when the urea/FA (w/w) ratio was near to score 3. Similar results were observed in the present research (Table 2); thus, the best result obtained for the total PUFA content (g/100 g total FA) of crude salmon oil concentrate was 85.81 and 80.03, respectively (Table 2).

Related to the n-3 PUFA concentration, a high yield of n-3 PUFA recovering could be observed when starting from both crude and refined oils. It could be concluded that salmon oil can provide a profitable source of such highly valuable constituents. Factors such as temperature of reaction and urea/FFA ratio showed to be markedly significant in order to achieve a higher value concentration.

CONCLUSIONS

The composition and properties of crude and refined fish oil and their corresponding n-3 concentrates showed to depend on the composition of the raw material employed. As a result, differences between crude and refined salmon oil could be observed. Thus, crude oil provided higher a^* and b^* colour values. Concerning chemical and physical analyses, crude salmon oil showed a higher FFA content and IV, provided a higher typical odour, viscosity and suspension particle values, while *p*-anisidine and iodine values, moisture content and formation of conjugated dienes and trienes did not provide any significant differences between both oils. Related to physical colour assessment, refined salmon oil showed lower a^* (redness loss) and b^* (yellowness loss) values when compared to its counterpart crude oil. Related to the n-3 PUFA concentration, SFA content decreased after urea inclusion, whereas unsaturated fatty acid content increased especially in the case of the n-3 FA. SFA of refined and crude oil such as C 14:0, C 16:0 and C 18:0, as well as monounsaturated fatty acids such as C 18:1 9c and C 18:1 11c, formed adducts due to urea inclusion. Meantime, the total n-3 FA content of 50.97 and 48.25 g/100 g total FA in concentrate crude and refined oil respectively could be observed when starting from both crude and refined oils. Both in concentrate crude and refined oil, the EPA + DHA content was of 32.76 g/100 g total FA. Thus, urea complexation of both crude and refined oils resulted in an increase in the total PUFA content up to 85.81 and 80.03 g/100 g total FA, respectively, in the non-urea complexed fractions, which confirmed salmon oil to be a profitable source of such highly valuable constituents. Therefore, a high yield of n-3 PUFA recovering could be observed in all cases, when starting from both crude and refined oils. It could be concluded that salmon oil can provide a profitable source of n-3 long-chain polyunsaturated fatty acids.

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Corresponding author:

Prof Alicia Verónica Rodríguez, University of Chile, Faculty of Chemical and Pharmaceutical Sciences, Department of Food Science and Chemical Technology, Santos Dumont Nº 964. Independencia, Santiago, Chile; E-mail: arodrigm@uchile.cl