Concentrating *n*-3 fatty acids from crude and refined commercial salmon oil

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ABSTRACT

In the present research, 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. As a result, differences between crude and refined salmon oil could be observed. Thus, crude oil provided higher typical odour, viscosity and suspension particle values. Concerning chemical analyses, crude salmon oil showed a higher FFA content and iodine value. Related to physical colour assessment, refined salmon oil showed lower a* and b* scores when compared to its counterpart crude oil. A high yield of *n*-3 FA recovering could be observed in both cases, which confirmed salmon oil to be 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.

Keywords: Salmon oil, crude and refined, *n*-3 fatty acids, urea complexation, quality.

INTRODUCTION

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 an increasing attention, being cis-5,8,11,14,17-eicosapentaenoic acid (EPA) and cis-4,7,10,13,16,19-docosahexaenoic acid (DHA) 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 on 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. Among them, Coho salmon (*Oncorhynchus kisutch*) has received a great attention because of its increasing farming production (FAO 2007a) in parallel to important capture production (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 handling of large quantities of materials in simple equipment and being a relatively inexpensive method (RUBIO-RODRÍGUEZ *et al.* 2010). In the present research, urea complexation was used to concentrate *n*-3 PUFA from crude (raw) and refined commercial Coho salmon oils; comparison of results obtained from both kinds of oils was achieved.

MATERIAL AND METHODS

Raw material and chemicals employed. Crude (total *n-3* fatty acids > 12 %) and refined salmon oil were obtained from Salmonoil S.A. (Puerto Montt, Chile). Fatty acid methyl esters (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-Tritricosanoin (CAS: 86850-72-8) was employed as internal standard. Urea, ethanol, n-hexane, methanol, and α-tocopherol employed were analytical grade and obtained from Merck (Santiago, Chile).

Characterization of crude and refined salmon oil. Characterization of starting crude and refined salmon oils was carried out by assessment of free fatty acids (FFA; AOCS 1993, official method Ca 5a-40), conjugated dienes (CD) and trienes (CT) (KIM & LABELLA 1987), peroxide value (PV; AOCS 1993, official method Cd 8b-90), 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 Hunterlab method; VINAGRE *et al*. 2011).

Preparation of FAME. For analysis of FA composition on triglycerides (TG) by GLC, sodium methylate was added into the salmon oil or n-3 concentrates. Then, the 1,2,3-tritricosanoin (23:0-23:0-23:0 TG) solved in n-hexane was added as internal standard in the sample. In order to convert the TG fatty acids into FAME, 10mL of sodium methylate and 50μL of internal standard (100mg/mL) were added. A fused silica capillary column 100m×0.25mm i.d., coated with SP™-2560 was employed. GLC setting conditions were as following: injection temperature at 250 ºC, flame ionisation detector (FID) temperature at 250 ºC, flow rate of carrier gas (N_2) of 1.2 mL/min, and oven temperature from 160 ºC to 220 ºC with an increasing rate of 2 °C/min. DataApex Clarity™ software for chromatogram analysis was used. The concentration of FAME was determined from the relation to the internal standard added (1, 2, 3-Tritricosanoin: Internal standard) by assessment of the peak/area ratio. Quantification of all kinds of FA was achieved according to the AOCS Official Method (AOCS 2009, Ce 1j-7).

Free n-3 PUFA concentration from salmon oil. The procedure included salmon oil saponification, FFA collection, formation of urea-FFA inclusion complexes, and extraction of free *n*-3 PUFA. The final concentrated *n-3* PUFA was flushed with nitrogen and stored at -70ºC with 100 ppm of α-tocopherol (HAAGSMA *et al*. 1982; ZUTA *et al*. 2003). EPA and DHA, ratio on variations of total *n*-3 PUFA, and *trans* FA were determined.

Statistical analysis. Physical and chemical analyses were performed in triplicate (n=3). The 95% confidence intervals of each quality parameter was 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 case of significant differences, a multiple range comparison was carried out by means of the Tukey test. Statgraphics Plus® 5.1 software (Manugistics Inc., Rockville, USA) was used.

RESULTS AND DISCUSSION

Table 1 shows the characterization of the starting crude (raw) and refined oil from salmon. Quality differences as a result of the refining process can be observed. Concerning chemical analyses, crude salmon oil showed a higher FFA content and IV. 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.

With the aim of being fit for human nutrition, 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 fin the case of 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.

FAME chromatograms belonging to commercial crude and refined salmon oil and their *n*-3 FA concentrates are given in Figure 1. The effect of urea on percentage of *n*-3 FA in the crude (B) and refined (D) concentrates can be observed. Thus, the *n*-3 FA

concentrate content increased due low-temperature crystallization and the urea inclusion compound formation. The urea fractionation of the fatty acids is mainly based on the degree of unsaturation: the more 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 (g/100g FAME) are shown in Table 2.

The most abundant fatty acids found in crude salmon oil (g/100g oil): C 14:0 (1.97), C 16:0 (8.07), C 16:1 9C (2.69), C 18:0 (2.26), C 18:1 9C (17.27), C 18:2 9C12C (9.49), C 18:3 n3; α-linolenic (2.02), C 20:5 (2.89), C 22:5 (1.40) and C 22:6 (3.06). Whereas FA showing a higher presence in refined salmon oil were (g/100g oil): C 14:0 (1.56), C 16:0 (6.48), C 16:1 9C (2.22), C 18:0 (1.78), C 18:1 9C (13.81), C 18:2 9C12C (7.64), C 18:3 **C** 18:3 n3; α-linolenic (16.69), C 20:5 (2.37), C 22:5 (1.11) and C 22:6 (2.49). Crude and refined salmon oil *trans* FA contents (g/100g oil) were C 16:1 9T (0.13 and 0.11), C 18:2 9C12T (0.08 and 0.07), C 18:2 9T12C (0.02 and 0.03) showing contents of 0.40 and 0.44 when expressed as g/100g FAME, respectively (Table 3).

AUBOURG *et al.* (2005) found that the most abundant FA in coho salmon farmed in the South of Chile were C 18:1 n9 and C 16:0 fatty acids (19.3 and 20.7 g/100 g total FAME, respectively), followed by C 22:6 n3, C 16:1 n7 and C 20:5 n3 (14.8, 7.7 and 7.1 g/100 g total FAME, respectively).

In the present research, composition of crude salmon oil FA concentrate was (g/100g oil): C 14:0 (0.14), C 16:0 (0.06), C 16:1 9C (1.66), C 18:0 (0.48), C 18:1 9C (1.73), C 18:2 9C12C (11.2), C 18:3 C 18:3 n3; α-linolenic (2.81), C 20:5 (5.59), C 22:5 (2.26) and C 22:6 (6.23); whereas composition of refined salmon oil fatty acid concentrate was (g/100g oil): C 14:0 (0.45), C 16:0 (0.34), C 16:1 9C (3.18), C 18:0 (0.04), C 18:1 9C (7.42), C 18:2 9C12C (20.24), C 18:3 C 18:3 n3; α-linolenic (4.47), C 18:4 (2.03), C 20:2 (1.84), C 20:5 (10.66), C 22:5 (3.95) and C 22:6 (13.42). The crude and refined salmon oil *trans* fatty acid concentrates were C 16:1 9T (0.008 and 0), C 18:2 9C12T (0.08 and 0.16), C 18:2 9T12C (0 and 0.11) showing contents of 0.23 and 0.36 when expressed as g/100g FAME, respectively (Table 3).

Saturated fatty acid content decreased after urea inclusion whereas unsaturated fatty acid content increased specially in the case of the *n*-3 fatty acids. Saturated fatty acids of refined and crude oil such as C 12:0, C 14:0 and C 18:0, as well as monounsaturated fatty acids such as C 18:1 9C formed adducts due to urea inclusion.

The total PUFA content of crude and refined salmon oil concentrate was 31.10 and 55.56 g/100g oil, respectively, being the EPA+ DHA content of 11.82 and 24.08 g/100g oil, respectively.

The crude and refined oil concentrate presented 0.09 and 0.28 g of *trans* FA/100g oil, respectively. The *trans* FA content from industrial sources in food must be lower than 2% of the total fat content of the product.

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). The best result obtained for the total *n*-3 PUFA of refined salmon oil concentrate was 51.3 g/100g FAME, this containing 36.31 g/100g oil (Table 3).

Related to the *n*-3 PUFA concentration, a high yield of *n*-3 PUFA recovering could be observed in all cases, which confirmed salmon oil to be 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 analyses, crude salmon oil showed a higher FFA content and IV. 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 *n*-3 PUFA concentration, a high yield of n-3 PUFA recovering could be observed in all cases, which confirmed salmon oil to be a profitable source of such highly valuable constituents.

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Table 1. Characterization* of the starting crude and refined salmon oil**

Quality parameter	*Crude salmon oil*	*Refined salmon oil*
Free fatty acids (FFA)	[a]1.78±0.03	[b]0.24±0.02
Conjugated dienes (CD)	[a]0.006±0.007	[a]0.019±0.002
Moisture (%)	[a]0.27±0.21	[a]0.26 ± 0.10
Impurities	[a]0.43±0.28	[a]0.12±0.02
Iodine value (IV)	[b]196.9±13.3	[a]154.0±1.1
Conjugated trienes (CT)	[a]0.007±0.006	[a]0.04 ± 0.04
Peroxide value (PV; meq active oxygen/Kg oil)	[a]2.73 ± 0.36	[b]3.54 ± 0.16
p-anisidine value (AV)	[a]5.33 ± 0.50	[a]5.14 ± 1.02
Unsaponifiable matter	[a]0.86 ± 0.38	[b]1.51 ± 0.38
a* colour value	[a]3.1 ± 0.5	[b]1.13 ± 0.33
b* colour value	[a]7.95 ± 0.49	[b]6.87 ± 1.12
L* colour value	[a]10.91± 1.20	[a]11.33 ± 0.73

* Mean values (n=3) ± standard deviations.

** For each quality parameter, mean values preceded by different letters (a, b) denote significant differences.

Table 2. Fatty acid methyl ester (FAME) composition (g/100g FAME) of commercial crude and refined salmon oil and their corresponding concentrates

FA or FA group	Crude salmon oil	Concentrated crude salmon oil	Refined salmon oil	Concentrated refined salmon oil
C 12:0	0.10	0.10	0.0	0.07
C 14:0	3.26	0.36	3.20	0.56
C 16:0	13.59	0.15	13.53	0.44
C 16:1 9T	0.23	0.02	0.24	0
C 16:1 9C	4.56	4.29	4.68	4.09
C 16:1 11C	0.07	0.38	0.08	0.08
C 17:0	0.22	0.08	0.25	0
C 16:1 13C	0.13	0.25	0.08	0.17
C 17:1	0.45	0	0.47	1.06
C 18:0	3.87	1.26	3.78	0.06
C 18:1 9C	29.71	4.54	29.43	9.69
C 18:1 11C	2.97	0.58	3.20	0.92
C 18:2 9C12T	0.14	0.21	0.14	0.22
C 18:2 9T12C	0.03	0.08	0.06	0.15
C 18:2 9C12C	16.44	29.64	16.4	26.62
C 18:2 9C15C	0.30	0.50	0.27	0.42
C 20:0	0.27	2.29	0.26	0
C 18:3 n6; γ-linolenic	0.20	0.55	0.17	0.56
C 20:1 8C	0	0	0.06	0
C 20:1 11C	1.98	1.02	2.02	1.13
C 18:3 n3; α-linolenic	3.53	7.49	3.45	5.92
C 18:4 n3	0.73	2.52	0.81	2.55
C20:2	1.43	0	1.42	2.44
C22:0	0.14	0	0.15	0
C 20:3 n6	0.33	0.94	0.33	0.85

C 22:1	0.23	0	0.24	0.01
C 20:3 n3	0.20	0.94	0.19	0.23
C 20:4 n3	0.44	1.23	0.43	1.12
[(1)] C 23:0	7.50	0	9.20	0
C 22:2	0.16	0.30	0.16	0.21
C 20:5 n3	5.63	16.60	5.73	15.75
C 24:1	0.23	0.08	0.26	0
C22:4	0.10	0.20	0.09	0.17
C 22:5 n3	2.64	6.50	2.59	5.60
C 22:6 n3	5.66	17.60	5.73	18.90

[(1)]1, 2, 3-Tritricosanoin: Internal standard.

Table 3. **Fatty acid groups composition of commercial crude and refined salmon oil and their corresponding concentrates**

Fatty acid group	Crude salmon oil		Concentrated crude oil		Refined salmon oil		Concentrated refined oil	
	(g/100g FAME)	(g/100g oil)	(g/100g FAME)	(g/100g oil)	(g/100g FAME)	(g/100g oil)	(g/100g FAME)	(g/100g oil)
EPA + DHA	11.30	5.95	34.21	11.82	11.45	4.87	34.61	24.08
Σ *n*-3 PUFA	18.97	10.22	52.89	18.63	19.06	23.39	50.71	35.89
Σ PUFA	38.10	21.24	84.97	31.10	37.97	32.18	76.73	55.56
***trans* FA**	0.40	0.23	0.23	0.09	0.44	0.21	0.36	0.28