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Quantification of fatty acids in salmon fillets conserved by different methods

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ABSTRACT. Lipid contents and the composition of fatty acids of fillets from Chilean salmon (*Salmo salar*) were determined under different conservation methods: fresh salmon, frozen salmon, water-conserved canned salmon and frozen salmon in long-term storage. Fatty acid contents were determined by gas chromatography. The fillets had high lipid levels, ranging between 9.71 and 12.86%. All samples presented high levels of monounsaturated fatty acids, between 363.69 and 425.30 mg g^{-1} of total lipids, followed by polyunsaturated fatty acids (294.46 - 342.45 mg g^{-1} of total lipids) and saturated fatty acids (203.32 - 223.17 mg g^{-1} of total lipids). Although samples revealed different lipid contents, all proved to be great sources of omega-3 fatty acids, regardless of the manner of conservation.

Keywords: omega-3 fatty acids, *Salmo salar*, total lipids.

Quantificação de ácidos graxos em filés de salmão de diferentes formas de conservação

RESUMO. O conteúdo lipídico e a composição em ácidos graxos foram determinados em filés de salmão (*Salmo salar*), de origem chilena, submetidos a diferentes formas de conservação: salmão fresco, salmão congelado, salmão enlatado conservado em água e salmão congelado armazenado por longo período. A composição em ácidos graxos nos filés foi determinada por cromatografia em fase gasosa. Os filés apresentaram elevado teor lipídico entre 9,71 e 12,86%. Todas as amostras apresentaram níveis mais elevados de ácidos graxos monoinsaturados, de 363,69 a 425,30 mg g^{-1} de lipídios totais, seguido pelos ácidos graxos poli-insaturados (294,46 a 342,45 mg g^{-1} de lipídios totais) e ácidos graxos saturados (203,32 a 223,17 mg g^{-1} de lipídios totais). Apesar de as amostras apresentarem teores lipídicos diferentes, todas se mostraram boas fontes de ácidos graxos ômega-3, independente da forma de conservação.

Palavras-chave: ácidos graxos ômega-3, *Salmo salar*, lipídios totais.

Introduction

World fish consumption has reached a record number of 18.6 kg per capita/year. Since 1980 world aquaculture has grown at an annual average rate of 8.8% and currently provides about 47% of consumed fish worldwide so that global demand for fishing products could be met (Food and Agriculture Organization of the United Nations [FAO], 2012).

The Atlantic salmon (*Salmo salar*) is rich in vitamin A and various B complex vitamins, coupled to several minerals, such as calcium, copper, iron, phosphorus, magnesium, manganese, selenium and zinc (Araujo, 2004). In addition, fish species with high fat levels are rich sources of eicosapentaenoic acid and docosahexaenoic acid since these nutrients are passed on from diatoms and flagellates, at the base of the food chain, to zooplankton and finally to fish (Tocher, 2003). Therefore, oil fish is considered a healthy choice in human diet, since fatty acids have beneficial effects on cardiovascular health; they are a safeguard against chronic inflammatory diseases such as arthritis, psoriasis and asthma; they reduce the occurrence of several types of cancer; and improve the visual function (Calder & Yagoob, 2009, Lecerf, 2009, Milte, Sinn, & Howe, 2009).

Although fish consumption has increased, consumers do not have enough information on whether there are differences among the possible ways of obtaining omega-3 fatty acids, and on the best manner in obtaining them. Current study evaluated fatty acid composition, mainly omega-3, from samples of Chilean salmon fillets stored in different manners (fresh, frozen, canned) obtained on the local market in Maringá, State Paraná, Brazil.

Material and methods

Four samples of Chilean salmon fillets were obtained from different local markets in Maringá PR Brazil, in July 2014. Samples from two distinct batches were fresh salmon (FrS), frozen salmon (FS), water-conserved canned salmon (WS) and frozen salmon in long-term storage (FSS), within the limits of their expiring date, albeit with altered color characteristics.

Preparation of the samples

Skin-less fillets were ground one by one in a food multiprocessor, at room temperature, and stored in vacuum-packed polythene bags at -18°C for further analysis. Analyses were carried out in triplicate. The level of total lipids was gravimetrically measured after extraction by the Bligh and Dyer (1959) method.

Chromatographic analysis of fatty acids methyl esters

Fatty acids methylation was carried out following method by Santos Júnior et al. (2014). Fatty acids methyl esters were separated in a thermo-gas chromatograph, trace ultra 3300 model, fitted with a flame ionization detector and a fused-silica capillary column CP-7420 (Select Fame, 100 m long, 0.25 mm internal diameter and 0.25 μm of cyanopropyl/polysiloxane). The flow of H_2 (carrier gas) was 1.2 mL min^{-1}, with 30 mL min^{-1} of N_2 (makeup); and 35 and 300 mL min^{-1}, to H_2 and synthetic air, respectively, to the detector flame. Injected volume was 2.0 μL, using 1:80 split, with temperatures at 200°C for the injector and 240°C for detector. Ramp was programmed with initial column temperature at 165°C for 7.0 min and raised to 185°C at a rate of 4°C min^{-1}; it was kept for 4.67 min and raised again to 235°C at a rate of 6°C min^{-1} and kept for 5.0 min, totaling 30.0 min of chromatographic running (Martin, Oliveira, Visentainer, Matsushita, & Souza, 2008). Corresponding retention times of the analytes and peak areas were obtained through integration by Software Chromquest 5.0. Fatty acids were identified by comparing their retention times with Sigma (USA) composition patterns.

Methyl esters absolute quantification of fatty acids was carried out by internal standardization, adopting pattern by Sigma (USA) methyl ester of tricosanoic acid (23:0), as described by Joseph and Ackman (1992). Theoretical correction factors for flame ionization detector (FID) (Visentainer, 2012) calculated fatty acid concentration on samples in mg g^{-1} of total lipids, according to Equation 1:

$$FA = \frac{A_X W_{IS} CF_X}{A_{IS} W_X CF_{AE}} \times 100 \quad (1)$$

where:
FA is the concentration of fatty acids in mg per g of total lipids;
A_X is the peak area (fatty acids);
A_{IS} is the peak area of internal pattern methyl ester of tricosanoic acid (23:0);
W_{IS} is the mass of the internal pattern (in mg) added to the sample;
W_X is the sample mass (in mg);
CF_X is the theoretical correction factor;
CF_{AE} is the conversion factor needed to express the results in mg of fatty acids instead of methyl esters.

Statistical analysis

Results were submitted to analysis of variance (ANOVA) at 5% significance level. The measured rates of lipid levels and fatty acids of samples were compared by Tukey's test.

Results and discussion

Table 1 presents total lipid contents from different salmon samples.

Table 1. Total lipid levels from different samples.

	FrS	WS	FSS	FS
Total Lipids (%)	$12.86^{b} \pm 0.98$	$10.30^{a} \pm 0.79$	$9.71^{a} \pm 1.16$	$12.36^{b} \pm 0.48$

Results are given as average ± standard deviation of triplicate. Rates with different letters in the same row indicate significant differences ($p < 0.05$) according to Tukey's test. FrS: fresh salmon; WS: water-conserved canned salmon; FSS: frozen salmon in long-term storage; FS: frozen salmon.

A significant difference ($p < 0.05$) was reported in total lipid content, or rather, FrS and FS had higher rates than WS and FSS, with FrS and FS as the samples with the highest level (12.86 and 12.36%, respectively). Consequently, based on the above results, salmon may be classified as a high fatty fish according to the classification by Ackman (1989), with lipid level above 8%.

Measuring total lipid level presents a difficult comparison in studies since differences of this level may be caused by diet, reproductive cycle, capturing site and season (Moreira, Visentainer, Souza, & Matsushita, 2001, Erkan & Ozden, 2007). Further, the method employed for lipid extraction may affect the quantity of total extracted lipids. Lipid percentage in current assay was close to rates by Salán, Galvão, and Oetterer (2006) (11.48%) and those by Tonial et al. (2010) (10.82%), both using *in*

natura salmon samples. Moreover, results corresponded to lipid rates at the Brazilian Food Database, with 9.7 g of lipids/100 g of raw fillets (*Tabela Brasileira de Composicao de Alimentos* [Taco], 2011).

Table 2 shows the composition of fatty acids analyzed in samples of Chilean salmon where 22 fatty acids were found in total lipids. Some differences may be observed among the samples.

Results are given as average ± standard deviation of triplicate. Rates with different letters in the same row indicate significant differences ($p < 0.05$) according to Tukey's test. FrS: fresh salmon; WS: water-conserved canned salmon; FSS: frozen salmon in long-term storage; FS: frozen salmon; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; ∑SFA: total saturated fatty acid; ∑MUFA: total monounsaturated fatty acid; ∑PUFA: total polyunsaturated fatty acid; n-6: total omega-6 fatty acid; n-3: total omega-3 fatty acid; n-6/ n-3: omega-6/ omega-3 ratio.

All samples presented higher levels of monounsaturated fatty acids (MUFA), between 363.69 and 425.30 mg g^{-1} of total lipids, followed by polyunsaturated fatty acids (PUFA), between 294.46 and 342.45 mg g^{-1} of total lipids and saturated fatty acids (SFA), between 203.32 and 223.17 mg g^{-1} of total lipids. Larsen, Quek, and Eyres (2010) also observed the same trend in *in natura* salmon from New Zealand.

Among the Chilean salmon samples analyzed, MUFA had the highest quantities in oleic acid (18:1n-9). Its concentration varied from 298.58 to 333.13 mg g^{-1} of total lipids, with FSS with the lowest level ($p > 0.05$) when compared to WS. Results were similar to those by Larsen et al. (2010) and Tonial et al. (2010).

Among the PUFA, linoleic acid (18:2n-6) was the most abundant omega-6, with concentrations between 155.99 and 169.48 mg g^{-1} of total lipids, with no significant difference ($p < 0.05$) between samples. The alpha-linolenic acid (18:3n-3) (from 43.99 to 99.87 mg g^{-1} of total lipids), followed by EPA (from 18.70 to 29.41 mg g^{-1} of total lipids) and DHA (from 29.07 to 38.22 mg g^{-1} of total lipids) were the most abundant omega-3 fatty acids in Chilean salmon samples regardless of the conservation method. In fact, all analyzed samples are excellent sources of EPA and DHA. Moreover, there were no significant differences among the samples. The *Agência Nacional de Vigilância Sanitária* [Anvisa] (2012) characterizes food with contents greater than 80 mg in 100 g of serving as food with high contents of omega-3. Consequently, all samples are sources due to their high contents of fatty acids. In fact, EPA and DHA contents totalized 787.16 mg 100 g^{-1}, 492.03 mg 100 g^{-1}, 529.39 mg 100 g^{-1} and 827.63 mg 100 g^{-1} of sample respectively for FrS, WS, FSS and FS. When compared to the European Food Safety Authority (EFSA, 2010), the recommendation of 250 mg of EPA+DHA per day in 100 g of fish corresponds to daily intake of these fatty acids.

Table 2. Fatty acids composition of different Chilean salmon samples analyzed.

Fatty acids	Fatty acids level (mg g^{-1} of total lipids)			
	FrS	WS	FSS	FS
14:0	21.65^{a} ± 2.71	20.09^{a} ± 1.23	18.17^{a} ± 2.48	21.59^{a} ± 1.77
15:0	35.15^{a} ± 3.90	27.07^{b,c} ± 1.08	21.60^{c} ± 3.67^{c}	31.14^{a,b} ± 2.14
16:0	101.43^{a} ± 10.75	114.89^{a} ± 5.76	110.12^{a} ± 6.80	114.71^{a} ± 7.30
18:0	28.57^{a} ± 3.25	30.41^{a} ± 1.99	33.25^{a} ± 1.85	33.76^{a} ± 2.86
22:0	13.11^{a} ± 1.46	9.67^{a} ± 0.81	13.56^{a} ± 0.49	14.63^{a} ± 1.07
24:0	5.59^{a,c} ± 0.66	4.56^{c} ± 0.19	6.62^{a,b} ± 0.28	7.33^{b} ± 0.37
∑SFA	205.50^{a} ± 21.70	206.69^{a} ± 10.29	203.32^{a} ± 12.41	223.17^{a} ± 13.88
14:1n-9	0.30^{a} ± 0.06	0.17^{b} ± 0.03	0.21^{b,c} ± 0.01	0.27^{a,c} ± 0.04
16:1n-9	2.21^{a} ± 0.58	3.81^{b} ± 0.17	2.07^{a} ± 0.24	1.97^{a} ± 0.13
16:1n-7	24.98^{a} ± 2.93	33.70^{b} ± 1.52	26.25^{a} ± 2.25	24.59^{a} ± 1.62
18:1n-9	319.74^{a,b} ± 25.03	333.13^{b} ± 19.79	298.58^{a} ± 4.98	320.31^{a,b} ± 17.43
18:1n-7	68.19^{a} ± 1.21	25.09^{b} ± 1.78	25.20^{b} ± 1.33	28.00^{b} ± 1.31
22:1n-9	9.88^{a} ± 1.18	5.19^{b} ± 0.34	11.39^{c} ± 0.96	10.47^{a,c} ± 0.76
∑MUFA	425.30^{a} ± 29.53	401.09^{a} ± 23.14	363.69^{a} ± 8.10	385.62^{a} ± 20.67
18:2n-6	163.41^{a} ± 17.51	169.48^{a} ± 7.67	155.99^{a} ± 25.48	156.18^{a} ± 8.34
18:3n-3	62.39^{a} ± 6.85	99.87^{c} ± 4.10	67.64^{a} ± 4.26	43.99^{b} ± 2.44
20:2n-6	6.23^{a} ± 0.87	6.23^{a} ± 0.27	5.77^{a} ± 0.16	5.40^{a} ± 0.30
20:3n-3	2.77^{a} ± 0.32	2.89^{a} ± 0.15	3.78^{b} ± 0.11	3.67^{b} ± 0.22
20:3n-6	3.03^{a} ± 0.46	2.94^{a} ± 0.12	3.08^{a} ± 0.13	3.12^{a} ± 0.16
20:4n-6	1.80^{a} ± 0.57	0.54^{a} ± 0.07	0.53^{a} ± 0.07	1.47^{a} ± 0.20
20:5n-3 (EPA)	29.41^{a} ± 3.67	18.70^{a} ± 0.48	23.88^{a} ± 1.22	28.74^{a} ± 1.43
22:2n-6	3.63^{a} ± 0.56	2.85^{b} ± 0.21	4.76^{c} ± 0.11	2.79^{b} ± 0.18
22:5n-3	10.46^{a} ± 1.13	9.88^{a} ± 0.51	11.93^{a} ± 0.48	10.89^{a} ± 0.63
22:6n-3 (DHA)	31.80^{a} ± 4.73	29.07^{a} ± 1.21	30.64^{a} ± 4.66	38.22^{a} ± 1.42
∑PUFA	314.92^{a} ± 35.44	342.45^{a} ± 12.56	308.00^{a} ± 28.38	294.46^{a} ± 14.82
n-6	178.10^{a} ± 19.23	182.04^{a} ± 7.68	170.13^{a} ± 25.51	168.96^{a} ± 9.36
n-3	136.83^{a} ± 16.15	160.41^{a} ± 4.89	137.87^{a} ± 9.80	125.51^{a} ± 6.01
n-6/n-3	1.30^{a} ± 0.03	1.14^{a} ± 0.02	1.23^{a} ± 0.21	1.35^{a} ± 0.02

There was no significant difference among the samples with regard to n-6/n-3 ratio (between 1.14 and 1.35). Results were similar to those related by Friesen, Higgs, and Devlin (2015) for farm-raised salmon, whereas these values varied between 0.05 and 0.12 for wild salmon. This is due to the fact that rations for fish diets increased their dependence on alternatives, such as plants and animals as replacements of fish meal and sea fish oil (Tacon, 2005, Friesen, Ikonomou, Higgs, Ang, & Dubetz, 2008). Although vegetable oils are high in polyunsaturated fatty acids, they do not contain high quantities of EPA and DHA. These oils often contain higher levels of omega-6 fatty acids. Animal fats may often be high in saturated fatty acids, although they are good sources of monounsaturated fatty acids, albeit very poor sources of EPA and DHA (Ikonomou et al., 2007, Friesen et al., 2008, Strobel, Jahreis, & Kuhnt, 2012).

Moreover, the n-6/n-3 ratio is used to analyze the nutritional rate of oils and fats, and is recommended by the Department of Health and Social Security (1994) at rates below 4.0. According to Simopoulos (2008), a lower ratio of n-6/n-3 fatty acids is desirable to reduce the risk of many chronic diseases. A ratio of 4/1 was associated with secondary prevention of cardiovascular diseases, with a 70% decrease in total mortality. A ratio of 2.5/1 reduced rectal cell proliferation in patients with colorectal cancer. A ratio of 2-3/1 suppressed inflammation in patients with rheumatoid arthritis, whilst a 5/1 ratio had beneficial effects on patients with asthma. Therefore, results in current study are within the recommended patterns.

Conclusion

Current analysis showed no significant difference in the composition of fatty acids, particularly omega-3, among the different conservation manners available on the local market of raw salmon (fresh/chilled, frozen or canned). All samples were great sources of EPA and DHA, omega-3 family fatty acids. Results suggest that the analyzed samples would be farm-raised due to a reduction in omega-3 fatty acids and an increase in omega-6 fatty acids.

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