Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 96 (2006) 446–451
Food Chemistry
www.elsevier.com/locate/foodchem

Effect of way of cooking on content of essential polyunsaturated fatty acids in muscle tissue of humpback salmon (*Oncorhynchus gorbuscha*)

Michail I. Gladyshev [a,*], Nadezhda N. Sushchik [a], Galina A. Gubanenko [b], Sevilia M. Demirchieva [b], Galina S. Kalachova [a]

[a] *Institute of Biophysics of Siberian Branch of Russian Academy of Sciences, Akademgorodok, Krasnoyarsk 660036, Russia*
[b] *Krasnoyarsk State Trade-Economical Institute, Lidiya Prushinskaya Street, 2, Krasnoyarsk 660075, Russia*

Received 18 October 2004; received in revised form 15 February 2005; accepted 15 February 2005

Abstract

Contents of fatty acids in filets of unfrozen (control), boiled, fried, roasted and boiled in a small amount of water humpback salmon, collected from a wholesale market in Krasnoyarsk city (Siberia, Russia) were analyzed. Special attention was paid to essential polyunsaturated fatty acids of ω3 family: eicosapentaenoic, 20:5ω3 (EPA) and docosahexaenoic, 22:6ω3 (DHA). Heat treatment in general did not decrease content of EPA and DHA in humpback, except a modest reduction during frying. Cooked humpback appeared to be the valuable source of essential ω3 PUFAs, namely EPA and DHA. It was hypothesized that the absence of significant reduction of PUFAs' contents in red flesh of fishes of *Salmonidae* family during heat treatment may be due to a high level of natural antioxidants which formed in the course of evolution as adaptation to their ecological niche.

1. Introduction

In last decades polyunsaturated fatty acids (PUFAs) of ω3 family have been recognized to be essential components of humans' diet. These acids, particularly eicosapentaenoic, 20:5ω3 (EPA) and docosahexaenoic, 22:6ω3 (DHA), appeared to play a key role in ontogenesis, especially neural development, functioning of cardiovascular system and immune systems (e.g., Broadhurst et al., 2002; Lauritzen, Hansen, Jorgensen, & Michaelsen, 2001). Regular consumption of food with appropriate content of EPA and DHA provides prevention and treatment of depressions, cardiovascular and some other diseases (e.g., Arts, Ackman, & Holub, 2001; Okita et al., 2002; Silvers & Scott, 2002).

Aquatic ecosystems are known to be the main source of PUFAs in Biosphere, thereby humans obtain principal part of EPA and DHA consuming fish, aquatic invertebrates and macroalgae (Arts et al., 2001). Meanwhile, unsaturated fatty acids are more susceptible to oxidation than their saturated analogues and PUFAs content in aquatic species was demonstrated to decrease during storage and cooking (Candella, Astiasaran, & Bello, 1998; Ohshima, Shozen, Usio, & Koizumi, 1996; Sant'Ana & Mancini-Filho, 2000; Tarley, Visentainer, Matsushita, & de Souza, 2004). Nevertheless, it was found that PUFAs level remained unchanged in some kinds of products under certain ways of treatment (Candella et al., 1998; Montano, Gavino, & Gavino, 2001). Thereby, kind of fish species and way of cooking may be determinant factors for content of the essential fatty acids in consumed products.

* Corresponding author. Tel.: +7 391 249 4517; fax.: +7 391 243 3400.
E-mail address: glad@ibp.ru (M.I. Gladyshev).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.02.034

The goal of our study was to determine PUFAs' contents in humpback salmon (*Oncorhynchus gorbuscha*) after four ways of cooking.

2. Materials and methods

2.1. Fish samples

Commercially available frozen fish were collected at a local wholesale market at the same time from three trade firms in Krasnoyarsk city (Siberia, Russia). They were caught somewhere in Far East of Russia, and we do not know whether the fish were from the same stocks or locations nor how long and under what conditions they were held before being brought to the market, thereby we collected fish from different firms to randomize the sample. Nevertheless, these free firms are most respectable and large in Krasnoyarsk and they follow all the Federal Standards of storage of fish before the selling. The fish were held at the market prior to being sampled during about three days, under −6 to −8 °C. Fish, collected from the market, were held in refrigerators under −20 °C before the following treatment. Three fishes from each provider were used in each analysis, i.e., nine fishes were sampled under each treatment: control (unfreezing), boiling, frying, roasting and boiling in a small amount of water. Thereby, 45 samples from 45 fishes were analyzed. Muscle tissues (filets) under dorsal fin were taken as the samples. The tissues were thawed under room temperature during about an hour prior to analyses. All skin was removed from the muscle tissue prior to analyses.

2.2. Heat treatments

Common ways of heat treatment were used: boiling under temperature 85–90 °C during 10–15 min, boiling in a small amount of water under 85–90 °C during 10–15 min, frying in sunflower oil under 150–170 °C during 15–20 min and roasting under 250–280 °C during 15–20 min. Before the roasting, fish were fried in sunflower oil during 3 min, no water was added. Sunflower oil was used because it is the most common oil in Russia.

2.3. Analysis

To measure moisture content filets from the same fish individuals of about 10–15 g of wet weight were taken and dried until constant weight under temperature 105 °C.

A fixed volume of an internal standard solution (19:0, nonadecanoic acid) was added to each sample. The samples were kept at −20 °C until further analysis. Lipids were extracted with chloroform:methanol (2:1, v/v) three times simultaneously with mechanical homogenization of the tissues with glass beads. The combined lipid extracts were filtered, dried by passing through anhydrous Na_2SO_4 layer and evaporated at 35 °C. The lipid extract was subjected to acidic methanolysis to prepare fatty acid methyl esters (FAME) as described previously (Gladyshev et al., 2000). FAME were analyzed on a gas chromatograph equipped with a mass spectrometer detector (GCD Plus, Hewlett–Packard, USA) and a 30 m long × 0.25 mm internal diameter capillary column HP-FFAP. The column temperature program was as follows: from 100 to 190 °C at 3 °C/min, 5 min isothermally, to 230 °C at 10 °C/min, and 20 min isothermally. Other instrumental conditions were as described elsewhere (Gladyshev et al., 2000). Peaks of FAME were identified by their mass spectra compare to those in the data base (Hewlett–Packard, USA) and to those of available authentic standards (Sigma, USA). Positions of double bonds in fatty acids were determined by GS-MS of FAME dimehyldisulphide adducts and dimethyloxazoline derivatives of FA prepared as described previously (Sushchik, Gladyshev, Moskvichova, Makhutova, & Kalachova, 2003).

2.4. Statistics

Student's *t*-test and single-factor ANOVA were used for analysis of data. ANOVAs of essential PUFA's contents and ratio were carried out by the conventional way (Campell, 1967) as follows. Control (unfrozen fish) and the four variants of heat treatment were taken as the five levels of factor. Total, residual and between levels sums of squares (s.s.) were calculated. Importance degree of the factor investigated

$$f_x = (\text{between levels s.s.}/\text{total s.s}) \cdot 100\%$$

and the variance ratio, F were calculated. If $f_x > 50\%$ and $F > F_{st}$ (at 0.05 or 0.01 probability levels, according to Fisher's test at the degrees of freedom $v_1 = 4$ and $v_2 = 40$, i.e., 5 levels of factor and 45 samples), it means that importance of the factor investigated (effect of the heat treatment) was higher than that of all the other factors and statistically significant, i.e., variations of FA content within each of the variants were small and negligible, but differences of means between the variants were high and significant at P probability level.

3. Results

Variations in moisture content in all fish samples were less than 10%. Minimum moisture value was characteristic of fried fish (63.9 ± 0.53%) and maximum moisture was found for boiled fish (70.3 ± 0.75%). Moisture contents of unfrozen, boiled in a small amount of water and roasted fish accounted for 68.9 ± 0.35%, 67.1 ± 0.34 and 65.0 ± 0.91%, respectively.

Table 1
Prominent fatty acid content (g/100 g of dry weight) in humpback salmon after different ways of heat treatment: mean values from nine samples ± SE (standard error)

Fatty acids	Control (unfrozen)	Boiled	Fried	Boiled in a small amount of water	Roasted
14:0	0.207 ± 0.054	0.235 ± 0.184	0.145 ± 0.079	0.249 ± 0.148	0.266 ± 0.105
15:0	0.028 ± 0.009	0.033 ± 0.023	0.020 ± 0.009	0.033 ± 0.015	0.037 ± 0.013
16:0	0.832 ± 0.218	0.964 ± 0.391	0.726 ± 0.272	1.031 ± 0.850	1.167 ± 0.319
16:1ω7	0.237 ± 0.091	0.267 ± 0.150	0.152 ± 0.077	0.310 ± 0.278	0.215 ± 0.082
16:2ω4	0.013 ± 0.007	0.012 ± 0.009	0.008 ± 0.005	0.015 ± 0.014	0.010 ± 0.004
16:3ω4	0.003 ± 0.003	0.004 ± 0.003	0.001 ± 0.001	0.002 ± 0.002	0.000
17:0	0.023 ± 0.008	0.029 ± 0.016	0.018 ± 0.007	0.032 ± 0.018	0.031 ± 0.007
18:0	0.181 ± 0.085	0.197 ± 0.052	0.324 ± 0.183	0.303 ± 0.471	0.528 ± 0.296
18:1ω9	0.475 ± 0.159	0.509 ± 0.240	0.473 ± 0.149	0.556 ± 0.411	0.702 ± 0.155
18:1ω7	0.118 ± 0.061	0.131 ± 0.052	0.074 ± 0.034	0.143 ± 0.132	0.114 ± 0.028
18:2ω6	0.069 ± 0.022	0.092 ± 0.055	0.831 ± 0.482	0.096 ± 0.053	0.624 ± 0.336
18:3ω6	0.004 ± 0.003	0.004 ± 0.003	0.001 ± 0.001	0.007 ± 0.005	0.004 ± 0.002
18:3ω3	0.058 ± 0.016	0.066 ± 0.042	0.139 ± 0.066	0.065 ± 0.034	0.124 ± 0.044
18:4ω3	0.173 ± 0.068	0.198 ± 0.101	0.123 ± 0.047	0.181 ± 0.071	0.151 ± 0.065
20:1ω11	0.557 ± 0.208	0.608 ± 0.318	0.366 ± 0.145	0.603 ± 0.294	0.445 ± 0.155
20:1ω9	0.040 ± 0.045	0.057 ± 0.049	0.063 ± 0.018	0.082 ± 0.034	0.080 ± 0.017
20:2ω6	0.019 ± 0.006	0.026 ± 0.015	0.014 ± 0.005	0.021 ± 0.009	0.019 ± 0.007
20:3ω3	0.009 ± 0.002	0.010 ± 0.004	0.006 ± 0.002	0.010 ± 0.005	0.007 ± 0.002
20:4ω6	0.025 ± 0.003	0.032 ± 0.005	0.019 ± 0.004	0.031 ± 0.017	0.025 ± 0.005
20:4ω3	0.077 ± 0.025	0.096 ± 0.037	0.059 ± 0.022	0.091 ± 0.045	0.070 ± 0.025
20:5ω3	0.536 ± 0.121	0.621 ± 0.195	0.383 ± 0.148	0.590 ± 0.377	0.463 ± 0.161
22:1ω11	0.796 ± 0.380	0.840 ± 0.566	0.586 ± 0.227	0.878 ± 0.471	0.650 ± 0.247
22:1ω9	0.017 ± 0.019	0.093 ± 0.145	0.029 ± 0.014	0.046 ± 0.025	0.034 ± 0.010
21:5ω3	0.026 ± 0.005	0.032 ± 0.012	0.021 ± 0.007	0.030 ± 0.013	0.023 ± 0.009
22:5ω6	0.009 ± 0.004	0.014 ± 0.003	0.008 ± 0.002	0.011 ± 0.005	0.009 ± 0.004
22:5ω3	0.110 ± 0.041	0.160 ± 0.040	0.092 ± 0.031	0.149 ± 0.089	0.113 ± 0.036
22:6ω3	1.056 ± 0.152	1.411 ± 0.398	0.810 ± 0.162	1.021 ± 0.271	0.961 ± 0.240
24:1	0.115 ± 0.036	0.141 ± 0.049	0.085 ± 0.036	0.125 ± 0.062	0.089 ± 0.025
Total FA	6.018 ± 1.140	7.156 ± 2.659	5.796 ± 1.835	7.020 ± 3.846	7.252 ± 1.884
ω3/ω6	16.2 ± 0.9	15.9 ± 1.2	2.2 ± 0.2	10.4 ± 0.5	3.4 ± 0.5

In all samples 58 fatty acids (FA) were identified. Quantitatively prominent FAs are given in Table 1. The two essential PUFAs, EPA and DHA, comprised 6.4–8.9% and 13.1–19.4% of the total, respectively.

Results of ANOVAs for effect of heat treatment on contents of PUFAs and their ratio are given in Table 2. Respective means of the values for the five levels of the factor investigated, i.e., for unfrozen, boiled, fried, boiled in a small amount of water and roasted fish are given in Table 1. In general, there was no significant effect of the heat treatment on contents of EPA, DHA and their sum: values of importance of the factor investigated, f_x, were less than 50% (Table 2). Meanwhile the way of treatment significantly affected the ratio ω3/ω6, which is of a high dietetic importance (Table 2). The ratio decreased in result of all the treatments compare to that of unfrozen fish, especially during frying and roasting (Table 1).

Table 2
Single-factor ANOVAs for content of PUFAs (g/100 g of dry weight) and the ratio in humpback salmon after different ways of heat treatment

PUFA	Total s.s.	Between levels s.s.	f_x	F
20:5ω3	227.7	33.8	14.8	3.48
22:6ω3	448.3	177.1	39.5	13.06
Σ20:5ω3 + 22:6ω3	1096.8	341.70	31.1	9.05
Σω3/ω6	1908.8	1701.2	89.1	163.90*

* $f_x > 50\%$, $F > F_{st}$ and $P < 0.05$ (see text for details).

To reveal a probable particular difference between a pair of variants, which may be damped in overall ANOVA, pair analysis of PUFAs' contents in control (unfrozen fish) and those in cooked fish using Student's t -test was carried out. In general, the pair analysis confirmed the results of ANOVAs: there were no significant differences between the control and boiled, boiled in a small amount of water and roasted fish. Nevertheless, contents of EPA and DHA in fried fish were significantly lower, than those in unfrozen fish (Table 1): t values were 2.41 and 3.34, respectively, $P < 0.01$ under degree of freedom 43 for both values.

4. Discussion

Thereby, among the ways of heat treatment, only frying resulted in statistically significant decrease in the two essential PUFAs' contents compare to unfrozen fish.

Meanwhile, the decrease in the PUFA's contents was not quantitatively substantial (Table 1). To obtain officially recommended appropriate intake of EPA + DHA for humans about 1 g per day (e.g., Ahlgren, Blomqvist, Boberg, & Gustafsson, 1994; Arts et al., 2001), it is necessary to consume 233 g of fried humpback, or 200 g of roasted, or 187 g of boiled in a small amount of water, or 167 g of boiled humpback. Hence, cooked humpback is beneficial to human health.

We did not consider a possible decrease of PUFAs' content in humpback due to freezing and storage in the study. Nevertheless, it does not seem to be significant. We can compare our data on unfrozen humpback with those for fresh fish species from the same family *Salmonidae*, available from the literature. For instance, in fresh filets of Atlantic salmon (*Salmo salar*), maximum content of EPA was about 2.2 mg/g of wet weight, DHA – 7.7 mg/g of wet weight and ratio of ω3/ω6 was 3.3 (Torstensen, Froyland, Ornsrud, & Lie, 2004). The average values for unfrozen humpback are 1.7 mg/g of wet weight of EPA and 3.3 mg/g of wet weight of DHA (see the moisture content and Table 1) in this study. In dorsal muscles of rainbow trout (*Oncorhynchus mykiss*) from several Canadian lakes and reservoirs average concentrations of EPA ranged in 2.0–4.1 mg/g of dry weight, DHA – 6.5–14.7 mg/g of dry weight (Kainz, Arts, & Mazumder, 2004). For the unfrozen humpback average contents of EPA and DHA in the same units were 5.36 mg/g of dry weight and 10.56mg/g of dry weight, respectively (transformed from Table 1). Hence, contents of PUFAs in the unfrozen humpback studied were practically equal to those in closely related fish species from the same genius *Oncorhynchus*.

It is interesting to compare PUFAs' content in humpback with those in other products. In fried salmon (*Salmo salar*) total content of EPA and DHA was 1.7 g/100 g of food, in fried sardine (*Sardine pilchardus*) – 0.88 g/100 g of food, in fried Spanish mackerel (*Scomberomorus commersoni*) – 0.39 g/100 g of food (Candella et al., 1998). According to our data in fried humpback EPA and DHA content was 0.43 g/100 g of food, and in boiled humpback – 0.60 g/100 g of food.

Per cent levels of EPA and DHA in humpback were comparable with those in condiments, prepared from salt-fermented Philippines shrimp alamang (*Acetes* spp.) (Montano et al., 2001), in some processed edible seaweeds (Sanchez-Machado, Lopez-Cervantes, Lopez-Hernandez, & Paseiro-Losada, 2004), in claw and breast meat of boiled blue crab (*Callinectes sapidus*) (Celik et al., 2004), in common sole (*Solea solea*) (Gokce, Tasbozan, Celik, & Tabakoglu, 2004), in liver oil of two rayfish species (*Dasyatis brevis* and *Gymnura marmorata*) commercially captured in the Gulf of California (Navarro-Garcia, Pacheco-Aguilar, Bringas-Alvarado, & Ortega-Garcia, 2004) and in canned sardines (*Sardinella brasiliensis*) (Tarley et al., 2004), but significantly higher than in a small Brazil fish "pacu"(*Piaractus mesopotamicus*) (Sant'Ana & Mancini-Filho, 2000). All the marine products, mentioned above, were regarded to be suitable items in the human diet concerning EPA and DHA content. Hence, cooked humpback is also a valuable source of essential ω3 PUFAs, namely EPA and DHA.

It is interesting to remark, that in a product of terrestrial origin, muscles of Iberian pigs, per cent levels of EPA and DHA were 10–20 times lower, than that in humpback, although these pigs were specially reared in pasture, characterized by high natural levels of linolenic acid and these meat products were included in the diet intended to improve plasmatic indicators of coronary and vascular disease (Muriel, Ruiz, Ventanas, & Antequera, 2002).

Besides content of PUFAs, ratio of sum ω3/ω6 acids is known to be of dietetic importance because it is the key factor for balanced synthesis of eicosanoids in organism (e.g., Steffens, 1997). According to current WHO recommendations, daily ratio of ω3/ω6 in total human diet should be no higher than 1:5, i.e., 0.2 (e.g., Vujkovic, Karlovic, Vujkovic, Vorosbaranyi, & Jovanovic, 1999). In tissues of marine fish the ratio on the average varied from 5 to 10, and in freshwater fish – from 1 to 4 (Ahlgren et al., 1994; Steffens, 1997). These values are evidently higher, than recommended, but it should be taken into account that in most part of other products ω3/ω6 ratio is substantially lower than necessary.

In our study the ratio was about 16 in unfrozen fish but was reduced during all heat treatments, especially during frying (probably, due to sunflower oil). Other authors also reported a decrease of ω3/ω6 ratio in salmon during frying from about 8.3 to 1.4, in mackerel – from 8.3 to 0.2 and in sardines – from 14.4 to 0.2 (Candella et al., 1998). In boiled blue crab ω3/ω6 ratio varied from about 3.2 to 2.3 (Celik et al., 2004) and in canned sardines – from 2.2 to 0.2 (Tarley et al., 2004). These values are close to those obtained for fried and roasted humpback in our study.

One of the most prominent results of our study is believed to be the absence of significant decrease of EPA and DHA contents in humpback during heat treatment, except the modest reducing during frying. Some other authors reported a decrease of the PUFAs' levels in fish during grilling (Ohshima et al., 1996) and canning (Tarley et al., 2004). This discrepancy may be due to differences in ways of cooking (chopping, temperature, oil, etc.), but also it may be species specific. For instance, Candella et al. (1998) found that threefold decrease of content of EPA and DHA in sardines and mackerel occurred, while in salmon no significant changes in EPA and DHA contents took place under the same way of frying.

We hypothesize that in muscles of the salmon (*Salmo salar*) and humpback, and probably in all species of

Salmonidae family, there are high levels of natural antioxidants in their red-colored flesh, which prevent PUFAs' degradation during heat treatments. This speculation has a reasonable biological and ecological background. *Salmonidae* fishes come for reproduction from seas into oligotrophic streams, where they die just after the spawning and their carcasses provide the principal food source (directly or via a food chain) for their juveniles. Thus, for a month or two essential diet components, including PUFAs, must be preserved in the carcasses. Indeed, carcasses of salmonids (namely humpback *Oncorhynchus gorbuscha*) were found to be valuable source of ω3 PUFAs for stream inhabitants (Heintz et al., 2004). Thereby, in the course of evolution salmonids had to form specific biochemical adaptations to their ecological niche. A high level of antioxidants may be one of such adaptations.

5. Conclusion

Heat treatment in general did not decrease content of EPA and DHA in humpback, except a modest reduction during frying. Cooked humpback appeared to be the valuable source of essential ω3 PUFAs, namely EPA and DHA. It was hypothesized, that the absence of significant reduction of PUFAs' contents in red flesh of fish of *Salmonidae* family during heat treatment may be due to a high level of natural antioxidants which formed in the course of evolution as adaptation to their ecological niche.

Acknowledgments

We used GS-MS of Joint Equipment Unit of Krasnoyarsk Scientific Centre of Siberian Branch of Russian Academy of Sciences. The work was supported by a personal grant for young scientists of the Siberian Branch of the Russian Academy of Science, by personal Grant MK-1846.2003.04 for young candidates of science (Ph.D.) of the President of the Russian Federation, by award No. REC-002 of the US Civilian Research & Development Foundation for the Independent States of the Former Soviet Union (CRDF) and the Ministry of Education and Science of the Russian Federation, and by personal award from the Russian Science Support Foundation.

References

Ahlgren, G., Blomqvist, P., Boberg, M., & Gustafsson, I. B. (1994). Fatty acid content of the dorsal muscle – an indicator of fat quality in freshwater fish. *Journal of Fish Biology, 45*, 131–157.

Arts, M. T., Ackman, R. G., & Holub, B. J. (2001). "Essential fatty acids" in aquatic ecosystems: a crucial link between diet and human health and evolution. *Canadian Journal of Fisheries and Aquatic Sciences, 58*, 122–137.

Broadhurst, C. L., Wang, Y., Crawford, M. A., Cunnane, S. C., Parkington, J. E., & Schmidt, W. F. (2002). Brain-specific lipids from marine, lacustrine, or terrestrial food resources: potential impact on early African Homo sapiens. *Comparative Biochemistry and Physiology, 131*, 653–673.

Campell, R. C. (1967). *Statistics for biologists*. Cambridge: University Press.

Candella, M., Astiasaran, I., & Bello, J. (1998). Deep-fat frying modifies high-fat fish lipid fraction. *Journal of Agricultural and Food Chemistry, 46*, 2793–2796.

Celik, M., Tureli, C., Celik, M., Yanar, Y., Erdem, U., & Kucukgulmez, A. (2004). Fatty acid composition of the blue crab (*Callinectes sapidus* Rathbun, 1896) in the north eastern Mediterranean. *Food Chemistry, 88*, 271–273.

Gladyshev, M. I., Emelianova, A. Y., Kalachova, G. S., Zotina, T. A., Gaevsky, N. A., & Zhilenkov, M. D. (2000). Gut content analysis of *Gammarus lacustris* from Siberian lake using biochemical and biophysical methods. *Hydrobiologia, 431*, 155–163.

Gokce, M. A., Tasbozan, O., Celik, M., & Tabakoglu, S. S. (2004). Seasonal variations in proximate and fatty acid compositions of female common sole (*Solea solea*). *Food Chemistry, 88*, 419–423.

Heintz, R. A., Nelson, B. D., Hudson, J., Larsen, M., Holland, L., & Wipfli, M. (2004). Marine subsidies in freshwater: effects of salmon carcasses on lipid class and fatty acid composition of juvenile coho salmon. *Transactions of the American Fisheries Society, 133*, 559–567.

Kainz, M., Arts, M. T., & Mazumder, A. (2004). Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. *Limnology and Oceanography, 49*, 1784–1793.

Lauritzen, L., Hansen, H. S., Jorgensen, M. H., & Michaelsen, K. F. (2001). The essentiality of long chain $n-3$ fatty acids in relation to development and function of the brain and retina. *Progress in Lipid Research, 40*, 1–94.

Montano, N., Gavino, G., & Gavino, V. C. (2001). Polyunsaturated fatty acid contents of some traditional fish and shrimp paste condiments of the Philippines. *Food Chemistry, 75*, 155–158.

Muriel, E., Ruiz, J., Ventanas, J., & Antequera, T. (2002). Free-range rearing increases ($n-3$) polyunsaturated fatty acids of neutral and polar lipids in swine muscles. *Food Chemistry, 78*, 219–225.

Navarro-Garcia, G., Pacheco-Aguilar, R., Bringas-Alvarado, L., & Ortega-Garcia, J. (2004). Characterization of the lipid composition and natural antioxidants in the liver oil of *Dasyatis brevis* and *Gymnura marmorata* rays. *Food Chemistry, 87*, 89–96.

Ohshima, T., Shozen, K., Usio, H., & Koizumi, C. (1996). Effects of grilling on formation of cholesterol oxides in seafood products rich in polyunsaturated fatty acids. *Lebensmittel-Wissenschaft und-Technologie – Food Science and Technology, 29*, 94–99.

Okita, M., Sasagawa, T., Tomioka, K., Hasuda, K., Ota, Y., Suzuki, K., et al. (2002). Habital food intake and polyunsaturated fatty acid deficiency in liver cirrhosis. *Applied Nutritional Investigation, 18*, 304–308.

Sanchez-Machado, D. I., Lopez-Cervantes, J., Lopez-Hernandez, J., & Paseiro-Losada, P. (2004). Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chemistry, 85*, 439–444.

Sant'Ana, L. S., & Mancini-Filho, J. (2000). Influence of the addition of antioxidants in vivo on the fatty acid composition of fish filets. *Food Chemistry, 68*, 175–178.

Silvers, K. M., & Scott, K. M. (2002). Fish consumption and self-reported physical and mental health status. *Public Health Nutrition, 5*, 427–431.

Steffens, W. (1997). Effects of variation in essential fatty acids in fish feeds on nutritive value of freshwater fish for humans. *Aquaculture, 151*, 97–119.

Sushchik, N. N., Gladyshev, M. I., Moskvichova, A. V., Makhutova, O. N., & Kalachova, G. S. (2003). Comparison of fatty acid composition in major lipid classes of the dominant benthic invertebrates of the Yenisei river. *Comparative Biochemistry and Physiology, B134*, 111–122.

Tarley, C. R. T., Visentainer, J. V., Matsushita, M., & de Souza, N. E. (2004). Proximate composition, cholesterol and fatty acids profile of canned sardines (*Sardinella brasiliensis*) in soybean oil and tomato sauce. *Food Chemistry, 88*, 1–6.

Torstensen, B. E., Froyland, L., Ornsrud, R., & Lie, O. (2004). Tailoring of a cardioprotective muscle fatty acid composition of Atlantic salmon (*Salmo salar*) fed vegetable oils. *Food Chemistry, 87*, 567–580.

Vujkovic, G., Karlovic, D., Vujkovic, I., Vorosbaranyi, I., & Jovanovic, B. (1999). Composition of muscle tissue lipids of silver carp and bighead carp. *Journal of the American Oil Chemists Society, 76*, 475–480.