J Am Oil Chem Soc (2009) 86:717–722
DOI 10.1007/s11746-009-1416-1

ORIGINAL PAPER

Determination of Essential Fatty Acids in Captured and Farmed Matrinxã (*Brycon cephalus*) from the Brazilian Amazonian Area

Neiva Maria Almeida · Natália Soares Janzantti · Maria Regina Bueno Franco

Received: 23 July 2008 / Revised: 26 May 2009 / Accepted: 29 May 2009 / Published online: 13 June 2009

Abstract The aim of this research was to quantify the methyl esters of linoleic (LA), α-linolenic (LNA), arachidonic (AA), eicosapentaenoic (EPA), and docosahexaenoic (DHA) acids in the muscular tissue and orbital cavity of farmed Matrinxã (*Brycon cephalus*) and in those caught in the Brazilian Amazonian Area during two periods. For the farmed fish, the amounts (mg/g of fat) of LA, LNA, AA, EPA, and DHA found in the muscle were 197.6, 75.7, 165.0, 4.1, and 30.0 mg/g of fat, respectively. The amounts of these FA in the orbital cavity were 152.6, 9.1, 249.4, 3.6, and 22.3 mg/g of fat for LA, LNA, AA, EPA, and DHA, respectively. For the fish caught during the wet period, the LA, LNA, AA, EPA, and DHA found in the muscle were 438.2, 118.3, 42.7, 5.2, and 10.3 mg/g of fat, in the orbital cavity were found 489.1, 18.6, 18.1, 6.2, and 18.7 mg/g of fat, respectively. In the dry season, the amounts (mg/g of fat) of LA, LNA, AA, EPA, and DHA in the muscle were 193.1, 40.0, 43.4, 8.1, and 61.3, while the found in the orbital cavity were 152.9, 28.4, 5.1, 4.9, 19.6 mg/g of fat. According to their contents of EPA, and DHA, matrinxã captured in the dry season can be considered as a rich source of EFA.

N. M. Almeida (✉)
Departamento de Gestão e Tecnologia Agroindustrial,
Centro de Ciências Humanas Sociais e Agrárias,
UFPB, DGTA, Campus III, 58000-000 Bananeiras, PB, Brazil
e-mail: neivaa@yahoo.com; neivad@iastate.edu

N. S. Janzantti · M. R. B. Franco
Departamento de Ciência de Alimentos,
Faculdade de Engenharia de Alimentos,
UNICAMP, C.P. 6121, 13081-970 Campinas, SP, Brazil

Keywords Brazilian Amazonian Area · *Brycon cephalus* · Essential fatty acids · Matrinxã

Introduction

After research investigating the Greenland Eskimo diet revealed a low incidence of cardiovascular diseases and reduced platelet aggregation [1], interest in the ingestion of fish and fish oils increased. In this context, numerous studies on seafood fatty acid compositions have been carried out [2–6].

Currently, the western diet has a high ingestion of ω6 fatty acids and a low ingestion of the ω3 fatty acids, due to the high consumption of vegetable oils, rich in ω6 fatty acids. The ideal ω6/ω3 fatty acid ratio to obtain a healthy diet ranges from 1:1 to 2:1, a remarkably different value from that found in the western diet, which varies from 20:1 to 30:1 [7–9].

An alternative way of increasing the ingestion of polyunsaturated fatty acids (PUFA) from the ω3 family (ω3 PUFA) is to increase the ingestion of seafood. The ω3 PUFA content varies from species to species and may be influenced by a number of factors. Various species of marine fish are rich in ω3PUFA, such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, while freshwater fish may show high levels of 18 carbon atom PUFA and considerable amounts of EPA and DHA [10].

Matrinxã (*Brycon cephalus*) belongs to the Order Characiformes, which includes most of the Brazilian freshwater fish. Although fish of this Order are restricted to South America and Africa, some species have reached Central America in recent years. In Brazil, this Order comprises approximately 1,300 fish species, which are distributed into 16 Families [11]. Matrinxã is a migratory

species, locally known as the "piracema" species. In the natural environment, it breeds at the beginning of the wet season, between December and February [12, 13]. According to Villacorta-Correa, [14], in 1 year in the wild this species can reach 550–750 g. It is excellent for farming because it shows good tolerance to manipulation and fast growth; in addition, it can be farmed in extensive or semi-intensive systems, in monoculture or polyculture farming [15].

In Brazil, research involving the quantification of EPA and DHA in fish is limited, and the results are usually shown in relative percentage data, using only the simple normalization method. In recent studies, Brazilian researchers quantified EPA and DHA in freshwater fish, pointing out the importance of adequate quantification of fatty acids, allowing for application to several nutritional and technological industrial processes [16, 17].

The aim of this research was to quantify the essential fatty acids in dorsal muscles and in the adipose tissue of the orbital cavity of matrinxã. Samples were obtained from semi-intensive farming and also from those captured in the Brazilian Amazonian Area in two different seasons.

Materials and Methods

Sampling: three batches, each consisting of four fish, were obtained from three fish farms and from the Amazon river near Manaus, Amazonas State, Brazil, in the sub-regions of Janauacá (3°23′S, 60°16′W), Paciência (3°22′S, 60°12′W), and Aruanã (3°16′S, 60°12′W) during two different seasons: the wet season (January–June) and the dry season (July–December). The muscle and the fatty tissue from the orbital cavity were dissected out, freeze-dried, frozen at −18 °C under N_2, and then transported by air to the Laboratory of Food Analysis/FEA/UNICAMP (Campinas, São Paulo, Brazil) for extraction and analysis of the lipids. Each batch was considered as a sample and analyzed in duplicate.

Moisture and Lipid Determinations

The moisture content of the fish muscle and adipose tissue was determined by freeze-drying, according to Pitombo [18]. The Bligh and Dyer [19] method was used for the determination of total lipids in the muscle tissue and in the adipose tissue.

Gas Chromatographic Analysis

Derivatization of the fatty acids was accomplished according to Joseph and Ackman [20]. Separation of the methyl esters was performed through gas chromatography, using a VARIAN model 3300 gas chromatograph equipped with a flame ionization detector and a 20-M fused-silica DB-WAX capillary column (30 m × 0.25 mm i.d) (J&W Scientific, USA). The operational parameters were as follows: detector temperature, 280 °C; injection port temperature, 250 °C; column temperature, 170 °C for 16 min, programmed to increase at 2 °C/min up to 210 °C, with a final holding time of 25 min; carrier gas, hydrogen at 0.8 mL/min, linear velocity of 38 cm/s, with an oxygen filter coupled to the line; nitrogen was used as the makeup gas at 30 mL/min, hydrogen and synthetic air at 30 mL/min and 300 mL/min for the detector; split injection at 1:50 ratio. All stages, from the transesterification to the final injection, were carried out under nitrogen.

Identification and Quantification of the Fatty Acids

For identification, the retention times of the fatty acids were compared to those of standard methyl esters (Sigma, St. Louis, MO). Equivalent chain-length values (ECL) were used [21, 22], as well as a coupled system of a gas chromatograph–mass spectrometer (Shimadzu QP 5000) and fragmentation by 70 eV electron impact. Retention times and peak area percentages were automatically computed by a Varian 4290 integrator. Quantification (in mg/g of fat) was carried out against a C23:0 internal standard from Sigma (USA), as described by Joseph and Ackman [20].

Statistical Analysis

The data were submitted to an ANOVA test and to Tukey's multiple comparison test at the level of 5% of probability, using the SAS for Windows software [23].

Results and Discussion

Table 1 shows the results for moisture and total lipid content in the muscle of matrinxã; it also provides the length and weight of the fish in each condition under investigation in this study. The results presented are the mean of the four batches from the farms and caught in wet and dry seasons from the Brazilian Amazonian Area.

According to the Ackman classification [24], the individuals from farms and those caught during the wet season can be considered as highly fatty, with 9.4 and 9.6% of total lipids, respectively, while those caught during the dry season can be considered as moderately fatty, with 4.5% of total lipids.

In a previous study, Moreira et al. [25] reported that matrinxã (*Brycon cephalus*) farmed using the cage system and the pond system, showed a total lipid content of 6.8

Table 1 Total lipid and moisture contents in the muscle, length, and weight for farmed and wild Matrinxã caught in the Brazilian Amazonian in two seasons

Origin	Total lipids (%)	Moisture (%)	Length (cm)	Weight (kg)
Farmed	9.4 ± 0.5	66.0 ± 3.5	39.0 ± 1.1	1.5 ± 0.1
Wet season	9.6 ± 0.5	71.0 ± 2.3	32.4 ± 3.1	0.8 ± 0.2
Dry season	4.5 ± 0.4	72.0 ± 3.0	36.2 ± 0.2	1.2 ± 0.6

Each value is the mean of duplicates with the respective estimate of SD

and 5.1%, respectively. The variations in fat content were probably due to handling and farming conditions.

The variation in results for the total lipid contents in the muscle of matrinxã caught during the two seasons was still within the range of the expected limits. Since the matrinxã is a "piracema" species, such fish increase their energy consumption while searching for food, which is scarce during the dry season [12, 13, 26].

For quantitative determination of PUFA, the use of the theoretical correction factor (Fcx) is recommended by several researchers [20, 27, 28]. In order to accomplish this, the chemical and instrumental parameters must be optimized in an attempt to assure that the errors from these sources are eliminated. The theoretical correction factors were 0.98 for EPA and 0.97 for DHA [29].

The oxidative instability of PUFA makes it virtually impossible to obtain and maintain high purity standards of these compounds [27]. Thus, methyl-eicosanoate (20:0) was used in order to verify the response of the detector to a saturated fatty acid, in relation to the internal standard of methyl-tricosanoate (23:0). The empirical correction factor found was 1.01 ± 0.03, while the theoretical response factor was 1.006. This agreement between the two values showed that the chemical and instrumental parameters were optimized and that the theoretical correction factors could thus be adopted, as recommended by Joseph and Ackman [20] for the quantification of fatty acids.

The contents found for LA, LNA, AA, EPA and DHA (mg/g of fat) in the total lipids are shown in Table 2.

Fish caught in the wet season revealed greater amounts of LA and LNA, while fish caught in the dry period showed greater amounts of EPA and DHA in the muscle. The amount of LA in the muscle was even higher than that found in the dorsal muscle of *Merluccius hubbsi* captured in Rio de la Plata [30]. A higher amount of AA was found in the muscle and in the adipose tissue of the farmed fish.

A higher amount of LA was found in the muscle and in the adipose tissue of the fish caught during the wet season and was higher than that found in both tissues of tambaqui (*C. macropomum*) caught in the same season [31].

The LNA content found in the muscle of farmed matrinxã was higher than that found in the muscle tissue of

Table 2 Essential fatty acids contents (mg/g of total lipids) in the muscle and adipose tissue of the for the farmed and wild Matrinxã, caught in the Brazilian Amazonian area in two seasons

Origin	LA		LNA		AA		EPA		DHA	
	Muscle	Orbital cavity	Muscle	Orbital cavity	Muscle	Orbital cavity	Muscle	Orbital cavity	Muscle	Orbital cavity
Farmed	197.6 ± 1.2^{d}	152.6 ± 2.2^{f}	75.7 ± 1.1^{h}	9.1 ± 0.8^{n}	165.0 ± 1.6^{e}	249.4 ± 2.2^{c}	4.1 ± 1.9^{pq}	3.6 ± 0.8^{q}	30.0 ± 3.1^{l}	22.3 ± 3.8^{m}
Wild										
Wet season	438.2 ± 2.4^{b}	489.1 ± 2.7^{a}	118.3 ± 2.0^{g}	18.6 ± 0.2^{b}	42.7 ± 0.5^{j}	18.1 ± 1.7^{b}	5.2 ± 1.4^{p}	6.2 ± 1.5^{p}	10.2 ± 1.6^{n}	18.7 ± 2.8^{m}
Dry season	193.1 ± 1.8^{d}	152.9 ± 1.9^{f}	40.0 ± 0.7^{i}	28.4 ± 1.4^{lm}	43.4 ± 0.8^{j}	5.1 ± 2.0^{p}	8.1 ± 1.4^{no}	4.9 ± 1.1^{pq}	61.3 ± 3.6^{i}	19.6 ± 4.9^{m}

Means followed by the same letters did not differ significantly at a level of 5%

farmed tilapia (*O. niloticus*) fed on commercial feed [16], and was also higher than that found in muscle tissue of tambaqui [31].

There was no significant difference ($p < 0.05$) between the muscle and the adipose tissue of the farmed fish with respect to the contents of EPA and DHA. However, the fish caught during the wet and the dry season, the two tissues differed significantly ($p < 0.05$), for both the EPA and the DHA contents.

Comparing the same tissue (muscle) between the farmed and wild fish, those caught in the dry season differed from those caught in the wet season and from the farmed ones with respect to EPA, showing the highest content of 8.1 mg/g of fat. The fish from different sources differed significantly ($p < 0.05$) with respect to the DHA content in the muscle; the highest value of 61.3 mg/g of fat was found in the fish caught during the dry season.

EPA showed a significant difference ($p < 0.05$) between the farmed fish and those caught during the wet season, when comparing the adipose tissue. In the same tissue the fish from the wet season and from the dry season did not differ significantly ($p < 0.05$) with respect to both EPA and DHA contents.

The values obtained for DHA showed a higher concentration in the muscle of fish caught during the dry season in comparison to fish caught in all other conditions. This occurred in the same period in which the matrinxã presented the lowest lipid content (Table 1). This could probably be explained by the tendency of this species to accumulate DHA for its metabolic functions, given that gonads maturation begins in this season and the diet, which consists mainly of insects and small fish, is of high quality [12, 13, 32].

Another surprise was the DHA content found in the muscle of the farmed matrinxã (30.0 mg/g of fat) as it was three times higher than that found in the fish caught during the wet season (10.3 mg/g of fat). It was expected that the fish from the natural environment would show a higher PUFA content, since they have a more diversified diet. A possible explanation is that fish farmed in a semi-intensive system may also eat insects and small fish of other species [33] in addition to granulated feed. During the wet season, their diet is strongly centered on seeds and fruits, which are rich in carbohydrates [32].

The results obtained for the contents of EPA and DHA in the muscle of farmed fish compared to the values found in those caught during the wet season, allow one to speculate that the quality of fish from farms could be further improved by using a better formulation of the administered feed, as observed by Visentainer et al. [16]. These authors studied the lipid composition of tilápia (*Oreochromis niloticus*) muscle by using the intensive system and by supplying the fish with feed enriched with increased levels of linseed oil. For the treatment containing the highest percentage of linseed oil, the contents of EPA and DHA found in the muscle were 2.5 and 26.1 mg/g of fat, respectively. These values were lower than those found in the muscle of matrinxã farmed in the semi-intensive system (EPA, 4.1 mg/g of fat and DHA, 30.0 mg/g of fat).

Tambaqui caught in the Amazon basin during both the wet and dry seasons presented mean EPA values of about 3.8 and 9.30 mg/g of fat in the muscle, respectively. This was similar to the value found in the muscle of matrinxã caught under the same conditions. However, the DHA content of 61.3 mg/g of fat found in the matrinxã caught during the dry season was higher than that found in the muscle of tambaqui captured in the same period [31].

Tucunaré (*Cichla ocelaris*) caught in the Amazon basin during the wet season presented mean EPA values of about 5.0 mg/g of fat in the muscle [17], value similar to that found in the muscle of matrinxã (5.2 mg/g of fat) caught in the same season. However, the EPA content of 3.0 mg/g found in the tucunaré caught during the dry season was lower than that found in the muscle of matrinxã captured in the same period.

In the adipose tissue of the tucunaré, the EPA content was 3.1 mg/g of fat for the fish caught during the wet season and 4.0 mg/g of fat for those caught during the dry season. Both of these values were lower than those found in the matrinxã caught in the same season [17].

The DHA content of the muscle of the tucunaré caught during the wet season was 55.0 mg/g of fat, a value higher than that found in the muscle of the matrinxã caught in the same period (10.3 mg/g of fat) [17]. Nonetheless, the value found in the muscle of the tucunaré caught during the dry season (21.0 mg/g of fat) was lower than that found for matrinxã.

Inhamuns [17] also studied the mapará (*Hypophthalmus* sp.) in the Amazon basin during both seasons and found an EPA content of 20.0 and 16.0 mg/g of fat in the muscle of fish caught during wet and dry seasons, respectively. These contents were higher than those observed in the muscle of the matrinxã caught in the same period of the year.

In the adipose tissue of the mapará, the EPA content was 18.0 mg/g for the fish caught during the wet season, and 13.0 mg/g of fat for those caught during the dry season. Both values were also higher than the contents found in the same tissue of the matrinxã caught in wet and dry seasons.

The DHA content in the muscle of the mapará caught during the wet season was 18.0 mg/g of fat, which is higher than the value found in the muscle of the matrinxã (10.3 mg/g of fat) for the same period [17]. However, the DHA content in the muscle of fish caught during the dry season was 15.0 mg/g of fat, which is lower than that found in the muscle of the matrinxã (61.3 mg/g of fat) during the same period.

In the adipose tissue of the mapará, the DHA content was 15.0 and 14.0 mg/g of fat for the fish caught during the wet and dry seasons, respectively. Both values were lower than those found in the same tissue of the matrinxã.

Accordingly, the matrinxã presented higher EPA and DHA contents in the muscle the fish caught during the dry season. In this case, an inversion was observed with respect to tucunaré and mapará: both presented higher EPA and DHA contents during the wet season [17]. This fact can be explained by the biology of the species. The fatty acid composition is directly related to the diet, which, in turn, depends on the species. The seasonal variation in the Amazon also showed influence on the fatty acid composition of the fish.

The recommendations concerning the ingestion of LC-PUFA, the EPA and DHA concentrations (in mg/100 g of muscle tissue) were calculated (hypothetically) based on the data for the farmed and the wild matrinxã caught in both seasons. The basis for the calculation was the percentage of TL (Table 1) and the concentration of each FA in mg/g of TL (Table 2).

Matrinxã presented the lowest EPA content (36.4 mg/100 g) for the fish caught during the dry season, while the lowest DHA content (97.9 mg/100 g) was found in the muscle of fish caught during the wet season. During the dry season, matrinxã presented the highest EPA plus DHA content because of the high level of DHA in this period. It is remarkable that this value was close to that reached by the farmed fish, due to the high level of DHA found in the muscle and the total lipid percentage.

From these findings, it was possible to calculate the daily amounts of muscle tissue that an individual should consume in order to assure an ingestion of 200 mg/day [34] of the n3 family polyunsaturated acids, EPA and DHA, in order to prevent cardiovascular and inflammatory diseases.

According to the results and assuming only EPA and DHA as sources of n3 PUFAs, a person would have to eat 64 g/day of muscle from wild matrinxã caught during the dry season. This shows that the fish from this season have the best nutritional quality.

A person would have to eat 62 g/day of muscle from the farmed matrinxã, a value similar to that of wild matrinxã captured during the dry season, but lower than the amount of 135 g/day required for the wild fish caught during the wet season. These results indicate that the matrinxã farmed using the semi-intensive system may be considered as an excellent source of essential fatty acids from the omega n3 family, as compared to wild fish caught in either season in the Central Amazon.

The amounts of tambaqui muscle tissue that a person should consume daily to ensure the ingestion of 200 mg/day, suggested by the Department of Health, [34] of EPA and DHA were also estimated. According to the results, and assuming that EPA and DHA were the only source of n-3 LC-PUFA, a person would need to eat 138 g/day of muscle tissue of farmed fish, 144 g/day of the muscle from fish caught in the dry season, or 445 g/day of muscle from fish caught in the wet season [31].

Aiming at a comparative discussion and considering only the EPA and DHA as the source of n3 PUFAs, a person would have to eat 27 g/day of the muscle of mapará caught during the wet season or 43 g/day of the same fish caught during the dry season [17]. With respect to the amount of tucunaré muscle needed to supply the daily ingestion of 200 mg/day of EPA and DHA, 416 or 396 g/day would be required of the fish caught in the wet and dry seasons, respectively. When compared to other species of the Central Amazon, such as the tambaqui and tucunaré, matrinxã may be considered as a better source of ω3 PUFA.

Conclusions

Fish caught during the wet season presented the highest contents of LA in the muscle and in the adipose tissue, while the fish from farms presented the highest contents of AA in the muscle and in the adipose tissue.

Fish caught during the dry season showed the highest contents of EPA and DHA in the muscle and are better suited for ingestion. Farmed and wild matrinxã caught during the wet season showed no distinction regarding their EPA content. However, the wild fish showed a higher content of EPA in the adipose tissue during the same period. Fish from farms are more suitable for ingestion than those caught in wet seasons.

The adipose tissue did not present sufficient amounts of EPA and DHA to distinguish it from the muscle as a better source of essential fatty acids.

The seasonal character of the Amazon influenced the LNA, EPA and DHA contents of the species under study.

Farmed and wild matrinxã are both suited for balanced diets when the aim is to increase the ingestion of EPA and DHA in order to reduce the ω6 to ω3 ratio, which is considered too high in western countries.

According to the amount of matrinxã muscle needed to supply the amount of 200 mg/day of EPA and DHA, matrinxã is a better source of ω3 PUFA when compared to other species of the Central Amazon.

Acknowledgments The authors are grateful to Capes and Fapesp for their financial support.

References

1. Dyerberg J, Bang HO (1979) Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. Lancet 2:433–435

2. Conor WE (2000) Importance of n-3 fatty acids in health and disease. Am J Clin Nutr 71(1):171S–175S
3. Hunter BJ, Roberts DCK (2000) Potential impact of the fat composition of farmed fish on human health. Nutr Res 20:1047–1058
4. Rose DP, Connolly JM (1999) Omega-3 fatty acids as cancer chemopreventive agents. Pharmacol Ther 83:217–244
5. Crawford MA, Bloom M, Broadhurst CL, Schmidt WF, Cunnane SC, Galli C, Gehbremeskel K, Linseisen F, Lloyd-Smith J, Parkington J (1999) Evidence for the unique function of docosahexaenoic acid during the evolution of the modern hominid brain. Lipids 35:S39–S74
6. Simopoulos AP (1991) Omega-3 Fatty Acids in Health and disease and in growth and development. Am J Clin Nutr 54:438–463
7. Simopoulus AP (2002) The importance of the ratio omega6/omega3 essential fatty acids. Biomed Pharmacother 56:365–379
8. Simopoulus AP (2000) Commentary on the workshop statement. Prostaglandins Leukot Essent Fatty Acids 63:123–124
9. Simopoulos AP, Leaf A, Salem N (1999) Essentiality and recommended dietary intakes for omega-6 and omega-3 fatty acids. Ann Nutr Metab 43:127–130
10. 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
11. Ferreira EJG, Zuanom JAS, Santos GM (1998) Peixes Comerciais do Médio Amazonas*:* Região de Santarém, Pará. Ed. MMA/IBAMA, p 211
12. Zaniboni EF, Carvalho JL, Villacorta-Correa MA, Rezende EK (1988) Caracterização morfológica do matrinxã, *Brycon cephalus* (Günther, 1869) (Teleostei: Characidae). Rev Bras Biol 48:41–50
13. Zaniboni EF, Rezende EK (1988) Anatomia de Gônadas, escala de maturidade e tipo de desova do matrinxã, *Brycon cephalus* (Günther, 1869) (Teleostei: Characidae). Rev Bras Biol 48:833–844
14. Villacorta-Correa MA (1987) Crescimento do matrinxã, *Brycon cephalus* (Gunter, 1869) (Teleostei, Characidae) no baixo rio Negro, seus afluentes e no baixo rio Solimões. PhD Thesis, Instituto Nacional de Pesquisa da Amazônia, Universidade Federal do Amazonas
15. Graef EW (1995) As espécies de peixes com potencial para criação no Amazonas. In: Val AL and Honczary A (eds) Criando Peixe na Amazônia,19th edn Instituto Nacional de Pesquisas da Amazônia, Manaus, pp 29–43
16. Visentainer JV, Souza NE, Matsushita M, Hayashi C, Franco MRB (2005) Influence of diets enriched with waxseed oil on the α-linolenic, eicosapentaenoic and docosahexaenoic fatty acid in Nile tilapia (*Oreochromis niloticus*). Food Chem 90:557–560
17. Inhamuns AJ (2000) Composição de ácidos graxos de peixes da Região Amazônica. PhD Thesis, Universidade Estadual de Campinas, Campinas, p 128
18. Pitombo RNM (1989) A liofilização como técnica de conservação de material de pesquisa. Ciên Cult 41:427–431
19. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917
20. Joseph JD, Ackman RG (1992) Capillary column gas chromatographic method for analysis of encapsulated fish oils and fish oil ethyl esters: collaborative study. J AOAC Int 75:488–506
21. Stransky K, Jursík T, Vitek A (1997) Standard equivalent chain length values of monoenic and polyenic (methylene interrupted) fatty acids. J High Resolut Chromatogr 20:143–158
22. Thompson RH (1996) Simplifying fatty acid analyses in multicomponent foods with a standard set of isothermal GLC conditions couplet with ECL determinations. J Chromatogr Sci 34:495–504
23. SAS Statistical Analytical System (1996) SAS Institute INC., SAS Campus drive, Cary, Version 6.0
24. Ackman RG (1996) Nutritional composition of fats in seafood. Prog Food Nutr Sci 13:161–241
25. Moreira AB, Visentainer JV, Souza NE, Matsushita M (2001) Fatty acids profile and cholesterol contents of three Brazilian *Brycon* freshwater fishes. J Food Compost Anal 14:565–574
26. Junk WJ (1984) Ecology, fisheries and fish culture in Amazonia. In: Sioli H, Junk W (eds) The Amazon—Limnology and landscape ecology of a mighty tropical river and its basin. Junk, Dordrecht, pp 443–475
27. Shantha N, Ackma G (1990) Nervonic acid versus tricosanoic acid as internal standards in quantitative gas chromatographic analysis the fish oil longer-chain n-3 polyunsaturated acid methyl esters. J Chromatogr B 533:1–10
28. Bannon CD, Craske JD, Hilliker AE (1986) Analysis of fatty acid methyl esters with high accuracy and reliability. Validation of theorical relative response factors of unsaturated esters in the flame ionization detector. JAOCS 63:105–110
29. Craske JD, Bannon CD (1988) Letter to the editor. JAOCS 65:1190–1191
30. Méndez E, González RM (1997) Seasonal changes in the chemical and lipid composition of fillets of the Southwest Atlantic hake (*Merluccius hubbsi*). Food Chem 59:213–217
31. Almeida NM, Franco MRB (2006) Determination of essential fatty acids in captured and farmed tambaqui (*Colossoma macropomum*) from the Brazilian Amazonian Area. JAOCS 83:707–711
32. Goulding M (1980) The fishes and the forest, exploration in Amazonian natural history. University of California Press, Berkeley, p 280
33. Pereira-Filho M (1995) Alternativas para a alimentação de peixes em cativeiro, in Criando Peixe na Amazônia In: Val AL and Honczary A (eds) 19th edn INPA, Manaus, pp 75–82, 149p
34. Department of Health (1994) Report on Health and Social Subjects No. 46. Nutritional aspects of cardiovascular disease. HMSO, London