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EPA and DHA quantification in two species of freshwater fish from Central Amazonia

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Abstract

The levels of the fatty acids EPA and DHA were determined for two species of fish (*Hypophthalmus* sp. and *Cichla* sp.), in the muscular tissue and in the orbital cavity, in two different seasonal periods in the Brazilian Amazonian area. Relatively high amounts (oil mg/g) for freshwater fish were found for DHA in the two species. *Hypophthalmus* sp. presented a higher concentration of EPA (20 ± 3 mg/g) and DHA (18 ± 3 mg/g) in the wet seasonal period in the muscular tissue, without a significant difference between the two acids. Higher concentrations of DHA were detected in the flood period in the muscular tissue (55 ± 9 mg/g) of *Cichla* sp. but with reduced concentrations of EPA (5 ± 1 mg/g).

Keywords: Freshwater fish; Fatty acid composition; Seasonal variation; Amazon river

1. Introduction

The production of ω3 fatty acid concentrates has been attracting the attention of pharmaceutical and food industries throughout the world, due to proven benefits to human nutrition and prevention of diseases, associated with the consumption of polyunsaturated fatty acids (PUFAs).

The ω3 and ω6 PUFAs are considered essential to the growth and development of children, and they are precursors of composite hormones known as eicosanoids, involved in several metabolic processes of great importance for the human body, mainly related to cardiovascular activity (Eder, 1995; Simopoulos, 1991).

Rice (1996) and Simopoulos (1991) consider the western diet to be relatively high in ω6 PUFA and lower in ω3 PUFA, a fact that generates an unbalance in the distribution of PUFA, harming the biological functions of the organism.

It is believed that the lack of balance of the polyunsaturated fatty acids in the diet is responsible for hypertension, disorders of the immune system and inflammation, depression and certain disturbances of neurological functions (Kinsella, 1986).

An increase in seafood consumption, especially fish, has been suggested as an alternative to elevate the ω3 PUFA level in the western diet. Another alternative that is being well accepted by the population is the use of food supplements based on sea oils, with a high ω3 PUFA concentration (Ackman, Ratnayake, & Macpherson, 1989; Shahidi & Wanasundra, 1998; Sidhu, 2003).

Quantification of the desired ω3 fatty acids is necessary to know the amount of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) ingested and the actual level presented in the tissue, in order to start with known amounts, so that the formulation of diets becomes more precise. In addition, quantification allows for the technological adaptation of several industrial processes, seeking

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to preserve the nutritional value of the product and prevent the oxidation of polyunsaturated fatty acids.

Studies accomplished by Yazawa (1994) in Japan, showed that, in the existent fat around the eyeball in bluish back fish, such as the tuna and the bonito, there was a high DHA concentration. Maruyama (1994) obtained oil rich in DHA, extracted from the tuna eyeball. Saito and Ishihara (1996) and Saito et al. (1995), researching EPA and DHA in the orbital fat from eyes of bonito (*Euthynnus pelamis*), and of two mackerel species (*Auxis rocheri* and *Auxis thazard*), found proportions of 9.3 ± 1.2% of EPA and 28.3 ± 2.2% of DHA in bonito while, in the *A. rocheri*, 8.4 ± 2.3% of EPA and 23.2 ± 6.5 of DHA were found and in *A. thazard*, 9.4 ± 0.5% of EPA and 26.4 ± 1.2% of DHA.

The amount of longer-chain ω3 PUFAs differs among species and can be influenced by a number of factors. The fatty acid composition of fish tissue can be affected by diet, size, age, reproductive cycle, salinity, temperature, season and geographical location (Henderson & Tocher, 1987; Luzia, Sampaio, Castellucci, & Torres, 2003; Shirai, Suzuki, Tokairin, Ehara, & Wada, 2002; Shirai, Suzuki, Tokairin, & Wada, 2001).

The mapará (*Hypophthalmus* sp.) and tucunaré (*Cichla* sp.) are planktophagic catfish and carnivorous fish, respectively, native to the Amazonian area. The mapará is destined for exportation but the tucunaré has a high acceptance on the local market.

In general, the heads of several species processed in cold stores in the Amazonas State are discarded as residues. Besides, in the Amazon area there are hundreds of species of fish with little or no commercial value for human consumption, which could be sources of ω3 fatty acids.

The objective of this work was the quantification of the polyunsaturated fatty acids with long chains of the ω3 series, EPA and DHA, in the muscular tissue and in the orbital fat of the eyeball in mapará (*Hypophthalmus* sp.) and tucunaré (*Cichla* sp.) in different seasonal periods, in order to find the best source of ω3 fatty acids during the year for diet counterbalancing and industrial use of the polyunsaturated fatty acids.

2. Materials and methods

2.1. Preparation of sampling

Mapará (*Hypophthalmus* sp.) and tucunaré (*Cichla* sp.) fish originating from the Amazon region, were collected from 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 drought period (July–December) and the flood period (January–June) (Table 1). Some decisive variables for total lipid composition, such as feeding habits and habitat were observed. The weight and total length of each individual were obtained. The dorsal muscles and the fatty tissue from the orbital cavity of the eyeball were dissected out, freeze-dried and frozen at −18 °C under N_2.

Table 1
Moisture and total lipid contents of mapará (*Hypophthalmus* sp.) and tucunaré (*Cichla* sp.) in different seasonal periods

Seasonal period*	Species	
	Mapará	Tucunaré
Flood		
Weight of the lot (g)	539 ± 61	357 ± 171
Moisture (%)	64 ± 3	78 ± 1
Total lipids (%)	19 ± 2	0.8 ± 0.2
Drought		
Weight of the lot (g)	320 ± 26	495 ± 34
Moisture (%)	65 ± 1	76 ± 1
Total lipids (%)	15.0 ± 1.0	2.1 ± 0.2

[a]Means and standard deviations of duplicate analyses (five batches).
* Seasonal period refers to the wet period (January–June) and to the drought period (July–December) of the Amazonian basin.

2.2. Moisture and total lipid determination

The moisture content of the fish muscle was determined by freeze-drying, according to Pitombo (1989). The Bligh and Dyer (1959) method was used for the determination of total lipids in the muscle tissue, and in the orbital cavity.

2.3. Derivation of fatty acid methyl esters

Derivatization of the fatty acids was accomplished according to Joseph and Ackman (1992).

2.4. Gas chromatographic analysis

Separation of the methyl esters was by gas chromatography, using a VARIAN Mod. 3300 gas chromatograph equipped with a flame ionization detector and a fused silica DB-WAX capillary column (30 m × 0.25 mm i.d.) (J&W Scientific, California, USA). The operation 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 make up gas at 30 ml/min, hydrogen and synthetic air at 30 ml/min and 300 ml/min for the detector; split injection at 1:100 ratio. All the stages, from the transesterification to the final injection, were accomplished under nitrogen. Retention times and peak area percentages were automatically computed by a Varian 4290 integrator.

2.5. Quantification of the polyunsaturated fatty acids EPA and DHA

The important ω3 polyunsaturated fatty acids (EPA and DHA), were quantified in the oil (mg/g) in the total lipid fraction, using the internal standard method. Methyl esters of fatty acids from Sigma with 99% purity were used (Fig. 1).

The tricosanoic acid (23:0) was used as the internal standard, according to the methodology proposed by Joseph and Ackman (1992). The following equation was applied:

$$\text{EPA or DHA (mg/g)} = [(A_X \times W_{IS} \times CF_X)/(A_{IS} \times W_S \times 1.04)] \times 1000$$

where A_X is the EPA or DHA area, A_{IS}, internal standard area, CF_X, theoretical correction factor for EPA and DHA, W_{IS}, weight of the internal standard added to the sample, W_S, weight of the sample, 1.04 is the necessary factor to express the result as mg of fatty acid oil/g (starting with methyl esters).

2.6. Recovery tests and analytical precision

These were done to evaluate the efficiency of the methylation methodology. Despite being well established, quantitatively, it was applied for the first time in the Laboratory of Food Analyses of FEA/UNICAMP, so a recovery test was done, in which standards of EPA, DHA and cod liver oil (CLO) fatty acids were employed. After determining the EPA and DHA (mg/g) contents in the CLO, known amounts of the standards of EPA and DHA fatty acids were added separately, in triplicate, to CLO, and methylation carried out.

The precision of the analysis was evaluated from the results obtained from five consecutive methylations of the same sample, with added internal standard.

2.7. Statistical analysis

The data were submitted to an analysis of variance (ANOVA) by the widespread linear model procedure (PROC GLM – Ducan's test at a level of 5% of significance), with one factor (season or parts of the body) and two levels (drought/wet period or muscle/eye), for EPA and DHA. The statistical package used was from SAS (Cary, NC).

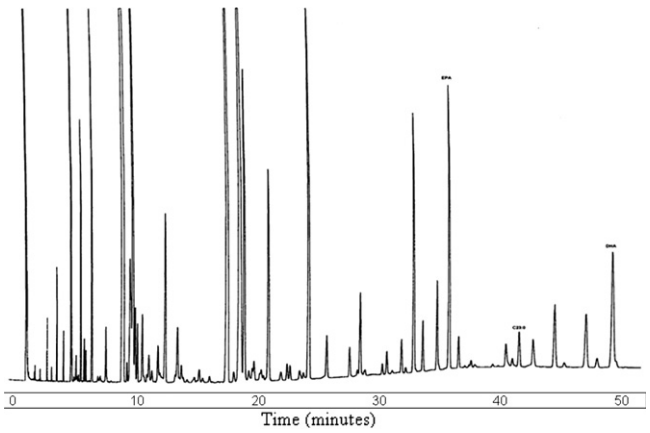

Fig. 1. Methyl esters chromatogram of mapará (*Hypophthalmus* sp.) fatty acids using the internal standard 23:0 to quantified EPA and DHA in DB-WAX 20 M column (170 °C for 16 min, programmed to increase at 2 °C/min up to 210 °C with a final holding time of 25 min).

3. Results and discussion

3.1. Moisture and total lipids

The values for moisture and total lipids found, in both fish, in the different seasonal periods of the Amazonian basin, are presented in Table 1.

Mapará is a fish which feeds on plankton and is classified as a high fat fish, according to Ackman's (1989) classification. Tucunaré presented a mean moisture content of (76% ± 1 to 78% ± 1) and a total lipids content in the range from 0.8% ± 0.2 to 2.1% ± 0.2 in both seasonal periods; thus this fish is considered as a low fat species (Ackman, 1989). In the wet season, the lipid content was equal or inferior to 1%, probably due to the high loss of energy of the consumed fish species in its search for food dispersed throughout the flooded river delta and to the reproductive period.

3.2. Response factor for EPA and DHA

With respect to tricosanoic acid, EPA presented an experimental correction factor of 1.02 ± 0.03 and DHA 0.94 ± 0.02. These values were close to the theoretical correction factors proposed by Joseph and Ackman (1992), which were 0.99 for EPA and 0.97 for DHA. Shantha and Ackman (1990) obtained a theoretical correction factor of 0.99 for EPA and DHA in relation to this acid.

In the present work, a saturated fatty acid (20:0) was used to verify the response of the detector in relation to the 23:0 internal standard. In this case, the experimental factor obtained was 1.008 ± 0.005, while the theoretical factor was 1.006. These values demonstrated that the conditions of analysis were optimized, and therefore the theoretical correction factors were adopted for the calculations, in mg/g, of EPA and DHA in relation to the internal standard used.

3.3. Recovery and analytical precision

The percentage recovery for EPA was 99.1 ± 0.01% and for DHA, 99.4 ± 0.01%, indicating high accuracy. The values obtained for the coefficients of variation were 2.8% and 3.0%, respectively, indicating high precision. Joseph and Ackman (1992), in their collaborative study, reported coefficients of variation of 5.9% and 5.3% for EPA and DHA, respectively, involving samples of a "menhaden" deodorized oil.

3.4. Quantification of EPA and DHA fatty acids

The quantification values obtained (mg/g of oil) for EPA (20:5ω3) and DHA (22:6ω3) in the muscular tissue

and in the fat from the orbital cavity of the eyeball in different seasonal periods, are presented in Table 2.

Considering the fatty acid patterns for freshwater fish, the mapará presented a great potential for the ω3 fatty acids, EPA and DHA, with a quantitative balance between these two acids. In the flood period, the muscle presented a larger amount of these fatty acids (average of 38 mg/g); however, in the drought there was not a significant difference at the 5% level in the concentration presented in the muscle or in the orbital fat of the eyeball. There was a significant difference at the 5% level between these fatty acids in the muscle between the seasonal periods, with a larger concentration detected in the flood period; there was no significant difference in the values found in the orbital fat of the eyeball between the seasonal periods.

Carvalho (1979), studied a type of Amazonian mapará, verified that its feed was almost exclusively zooplankton and aquatic insects during the whole year, a fact that certainly contributes to the high content of ω3 PUFAs found in this study during the two seasonal periods, because EPA and DHA are natural constituents of plankton and aquatic insects (Henderson & Tocher, 1987). In the flood period, a time for fattening of the mapará, 90% of its diet is zooplankton, the remaining 10% being insects and algae. During the drought, when it begins its reproductive cycle, there is a diversification of its diet, with algae, insects, fish eggs and vegetable leftovers besides zooplankton. This larger zooplankton participation in the feed of the mapará during the flood period can justify the larger concentration of EPA and DHA found in the muscle during this seasonal period.

Guler, Aktumsek, Citil, Arslan, and Torlak (2007) determined the seasonal variations in total fatty acid composition and ω3/ω6 fatty acids ratio of muscle of zander (*Sander lucioperca*), of commercial importance in the largest freshwater lake in Turkey. The authors reported that the fatty acid composition and ω3/ω6 fatty acids ratio in the muscle were significantly influenced by spawning and season.

The influences of season (summer and winter) on the total lipid, fatty acid and cholesterol contents of five popular Brazilian fish species (marine species and fresh water species) from the south to west of Brazil were studied by Luzia et al. (2003). The sardine (*Sardinella* sp.), the croaker (*Micropogonias furnieri*), the tilápia (*Oreochromis* sp.) and the curimbatá (*Prochilodus* sp.) were not influenced by seasonality in terms of their total saturated or unsaturated acid contents. The shrimp (*Xiphopenaeus kroyeri*) was the only species influenced by seasonality, showing higher saturated and unsaturated fatty acid contents during the summer.

The tucunaré showed a surprisingly high content of DHA. The amounts found in the lipids of the muscular tissue (55 ± 9 mg/g), in the flood period, were higher than the values of all the other samples. This high concentration detected in the season when the tucunaré presented the lowest amount of lipids in its chemical composition (Table 1), evidenced a probable tendency of this species to accumulate DHA for its metabolic functions during this period (reproduction time). During the drought, the behaviour was similar but with values for DHA on average, 50% smaller (21 ± 1 mg/g). The amounts of EPA verified in this species were not expressive; however, in the drought period, the amount of EPA found in the fat of the orbital cavity was significantly greater than that found in the muscle. A different behaviour was observed in the flood period. Possibly, in the tucunaré, there is a high amount of D4-desaturase, the enzyme responsible for the conversion of EPA–DHA through the introduction of a sixth double bond (Simopoulos, 1991).

Studying the lipid composition of fresh water fish in the north of Europe, Ahlgren, Blomqvist, Boberg, and Gustafsson (1994) found values of 20.3 mg/g of DHA and 5.7 mg/g of EPA, in "sea trout" (*Psalm trutta* L.) of lacustrine origin. Values close to these were found for the tucunaré in the drought time and for DHA in the mapará in the flood period. Analyzing the composition of the fatty acid tissue of the "pike" (*Exos lucius* L.), these authors detected high concentrations of EPA and DHA, 19.9 mg/g and 49.9 mg/g, respectively. Such results were similar to those found in this work for DHA in the tucunaré, in the muscular tissue during the flood period. This fact is due, probably, to the larger capacity that freshwater fish have

Table 2
Polyunsaturated fatty acids EPA and DHA (mg/g of oil) in the muscle and in the orbital cavity of the eyeball of mapará (*Hypophthalmus* sp.) and tucunaré (*Cichla* sp.) in different seasonal periods in Amazonia

Specie	Seasonal period[a]							
	Flood period				Drought period			
	Muscle[d]		Eyes[e]		Muscle		Eyes	
	EPA[b]	DHA[b]	EPA[c]	DHA[c]	EPA[b]	DHA[b]	EPA[b]	DHA[b]
Mapará	20 ± 3	18 ± 3	14 ± 1	15 ± 3	16 ± 4	15 ± 4	13 ± 3	14 ± 3
Tucunaré	5 ± 1	55 ± 9	3.1 ± 0.5	22 ± 5	3 ± 1	21 ± 1	4 ± 1	17 ± 3

EPA – 5, 8, 11, 14, 17 – eicosapentaenoic; DHA – 4, 7, 10, 13, 16, 19 – docosahexaenoic.

[a] Seasonal period refers to wet period (January–July) and drought period (July–December).

[b] Mean and standard deviation of five batches in duplicate.

[c] Mean and standard deviation of four batches in duplicate.

[d] Muscle – extracted lipids from fillet.

[e] Eyes – extracted lipids of fat from orbital cavity of eyeball.

to elongate and desaturate the short chain fatty acids synthesized by algae or plants, transforming them into long chain fatty acids of the ω3 family, EPA and DHA, and also converting feed of low to high nutritional value (Henderson & Tocher, 1987).

Wang, Miller, Perren, and Addis (1990) analyzed freshwater fish originating from cold water (Superior Lake, USA), and they found concentrations that varied from 1.0 mg to 12.0 mg/g for EPA and from 1.0 mg to 18.0 mg/g for DHA. The authors considered the analyzed fish to be excellent sources of EPA and DHA. Considering that they are cold water species, such results were expected, even for freshwater fish. However, the tucunaré and the mapará are tropical species and they reached similar or higher concentrations, demonstrating that environmental and biological factors can influence the concentrations of the polyunsaturated fatty acids.

Nutritionists believe that the ratio ω6:ω3 should be 5:1 and that the addition of ω3 PUFAs to food could improve the nutritional picture and help in the prevention of diseases. The amount of EPA and DHA suggested for daily ingestion in the range 200–1000 mg (Muggli, 1997; Rice, 1996; Simopoulos, 1991). Therefore, based on the results obtained in this work, the tucunaré and the mapará can be considered as rich sources of essential fatty acids of the ω3 and ω6 series, with a high potential in the muscle tissue during the flood period of the Amazonian basin (from January to June). They could be used in balanced diets when the objective is to increase the ingestion of PUFAs.

Considering the quality of the orbital fat of the eyeball, the fish studied presented amounts of EPA and DHA in an acceptable range which justifies the industrial use of the polyunsaturated fatty acids.

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