Determination of Essential Fatty Acids in Captured and Farmed Tambaqui (*Colossoma macropomum*) from the Brazilian Amazonian Area

Neiva Maria de Almeida[a,]* and Maria Regina Bueno Franco[b]

[a]Universidade Federal da Paraíba, Departamento Tecnologia Rural, Centro de Formação de Tecnólogos, Campus III, Bananeiras, Paraíba, Brazil, and [b]State University of Campinas (UNICAMP), Departamento Ciência de Alimentos, Faculdade de Engenharia de Alimentos (FEA), Campinas, São Paulo, Brazil

ABSTRACT: This research aimed to quantify the methyl esters of linoleic (LA), γ-linolenic (LNA), arachidonic (AA), eicosapentaenoic (EPA), and docosahexaenoic (DHA) acids in the muscular tissue and orbital cavity of farmed tambaqui (*Colossoma macropomum*) and in those caught in the Brazilian Amazonian Area during two periods. For the farmed fish, the amounts of LA, LNA, AA, EPA, and DHA found in the muscle were 208.0, 12.4, 57.9, 5.0, and 25.1 mg/g, respectively. The amounts of these FA in the orbital cavity were 103.4, 6.6, 20.0, 4.4, and 8.1 mg/g for LA, LNA, AA, EPA, and DHA, respectively. For the fish caught during the flood period, the LA, LNA, AA, EPA, and DHA levels were 297.3, 50.7, 32.3, 3.8, and 14.1 mg/g in the muscle and in the orbital cavity were 259.7, 40.3, 10.8, 5.5, and 9.3 mg/g, respectively. For the fish captured in the dry season the levels of LA, LNA, AA, EPA, and DHA in the muscle were 262.0, 48.0, 157.6, 9.3, and 40.2 mg/g and in the orbital cavity were 102.5, 15.7, 24.6, 7.0, and 8.9 mg/g. According to their contents of AA, EPA, and DHA, tambaqui captured in the dry season can be considered as a rich source of EFA. The adipose tissue of the orbital cavity did not show sufficiently high EPA and DHA contents to classify it as a better source of FA than the muscle.

Paper no. J11253 in *JAOCS 83*, 707–711 (August 2006).

KEY WORDS: Brazilian Amazonian, Colossoma macropomum, essential fatty acids, tambaqui.

Globalization, new eating habits, the increasing ingestion of vegetable oils, and industrial expansion are factors that have resulted in a higher human intake of the long-chain PUFA (LC-PUFA) of the n-6 family in relation to those of the n-3 series, causing an increase in the n-6n-3 ratio. This has lead to the development of numerous research projects related to human diets. For instance, it has been reported that metabolic products of eicosanoids, derived from arachidonic acid (AA) (prostaglandins, thromboxans, and leukotrienes), are produced in larger quantities than those from the n-3 family, specifically those derived from eicosapentaenoic acid (EPA). The eicosanoids produced from AA are biologically active in small amounts and, in instances in which they are produced in larger amounts, play a part in the incidence of thrombosis and atheromatosis, allergic and inflammatory diseases, and cell proliferation (1,2). AA is an abundant FA present in the brain cells as in other cells of the body. The fetus receives AA from the mother to aid in the development of the brain, and during nursing the AA present in the maternal milk stimulates the development of the baby's brain. Important unsaturated FA in the brain are AA and DHA (3).

At the same time that our understanding of the role of FA is increasing, changes in eating habits are taking place. These changes have led people to search for diets containing larger amounts of the LC-PUFA from the n-3 family. The per capita ingestion of fish in Brazil is 6.4 kg/yr, an extremely low value compared with Japan (71.9 kg/yr) and Portugal (60.2 kg/yr). However, in the Amazon State, the ingestion per capita of fish is 55 kg/yr (4). In the Amazon, fish provide the main protein source for the local population (5).

The tambaqui (*Colossoma macropomum*) is native to the Amazon and Orinoco river basins and their tributaries and goes upstream to spawn. The adult fish are predominantly herbivorous, but these fish may feed on insects, snails, and occasionally on other fish (6). The sex ratio in wild-caught tambaqui was 1:1, however, the length of females was significantly longer than that of males. Fishes longer than 550 mm, were more commonly caught from September to December during the dry period stored larger cavitary fat reserves. Fishes smaller than the standard length of 550 mm stored less fat reserves and were more common during the rising of the water and flooding period. The spawning period for tambaqui extends from September to February in a total spawning synchronized with "repiquetes" (native term used to name a sudden rising of the water level). In a study by Villacorta-Correa and Saint-Paul (7), the length of tambaqui averaged 60.69 cm at sexual maturity.

On the other hand, the amount of research related to the quantification of FA in Brazilian products is still small. The results are usually expressed as relative percentage data, using only simple normalization methods. Recently Brazilian researchers have quantified the amounts of EPA and DHA in freshwater fish, showing the relevance of the correct quantification of FA (8,9).

The quantification of EPA and DHA in the adipose tissue of the orbital cavity, depending on the results, should stimulate the use of this tissue as a source of LC-PUFA and also generate interest in this tissue on an industrial level.

*To whom correspondence should be addressed at UFPB, Dep. Tecnologia Rural, Centro de Formação de Tecnólogos, Campus III, CEP 58000-000, Bananeiras, Paraíba, Brazil. E-mail: neivaa@yahoo.com or neiva@cft.ufpb.br

This work aimed to quantify the EFA, in the dorsal muscles and the adipose tissue of the orbital cavity, of tambaqui obtained from semi-intensive farming and from those captured in the Brazilian Amazonian Area in two different seasons.

MATERIALS AND METHODS

Sampling. Three batches, each consisting of three fish, were obtained from three fish farms and from the Amazon River near Manaus, State of Amazonas, Brazil, in the subregions 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 for extraction and analysis. Each batch was considered as a sample and analyzed in duplicate.

Moisture and lipid determinations. The moisture content of the fish muscle was determined by freeze-drying, according to Pitombo (10). The Bligh and Dyer (11) method was used for the determination of total lipids (TL) in the muscle tissue and in the adipose tissue of the orbital cavity.

GC analysis. Derivatization of the FA was accomplished according to Joseph and Ackman (12). Methyl esters were separated by GC, using a Varian model 3300 gas chromatograph fitted with an FID and a fused-silica DB-WAX capillary column (30 m × 0.25 mm i.d) (J&W Scientific, Folsom, CA). 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 and 300 mL/min, respectively for the detector; split injection at 1:50 ratio. All the stages, from the transesterification to the final injection, were accomplished under nitrogen. The retention times and peak areas were automatically computed by a Varian 4290 integrator.

FA quantification. For the identification of the FA, the retention times were compared with those of standard methyl esters (Sigma, St. Louis, MO). Equivalent chain-length values were used (13,14), as well as a coupled system of a GC–MS (Shimadzu QP 5000) and fragmentation by electron impact (70 eV). The retention times and peak areas were automatically computed by a Varian 4290 integrator. Quantification (in mg/g of TL) was accomplished against a C23:0 internal standard from Sigma, as described by Joseph and Ackman (12).

Statistical analysis. The data were submitted to the ANOVA test and to Tukey's multiple comparison test at the 5% probability level, using the SAS for Windows software (15).

RESULTS AND DISCUSSION

Table 1 shows the results for the moisture and TL contents in the muscle and also the length and weight of the individual tambaqui fish. The results were expressed as the mean of three batches of each sample (farmed, caught in dry season, caught in wet season). Ackman (16) classified fish into four categories according to their lipid content: very low fat (<2% fat), low fat (2–4% fat), medium fat (4–8% fat), and high fat (>8% fat). According to Ackman's classification (16), the fish from the semi-intensive farms can be considered to contain intermediate fat levels, whereas wild-caught fish were of low fat content.

The fat content of the farmed tambaqui was higher than that of the tambaqui caught in the natural environment in both seasons. The TL content (4.8%) of the farmed tambaqui was greater than that found by Arbeláez-Rojas *et al*. (17) for semi-intensive farming (2.4%) but lower than that found by Almeida (18) (7.6%). These results suggest that farming procedures may influence the fat content of the species. In our research, the values for the TL contents in the muscle were very similar for both seasons. This may be due to the variation in the level of the water that takes place during the hydrological cycle in the Amazon from year to year.

For the quantitative determination of PUFA, the use of a theoretical correction factor (Fcx) has been recommended by several researchers (12,19,20). The relative theoretical response factor is the response factor found according to the theories proposed by Ackman and Sipos (21), and whose value is based on the proportional weight of the active carbons in the FA molecule.

The empirical correction factors for LA, LNA, AA, EPA, and DHA with respect to methyl tricosanoate as internal standard were (mean ± SD) 0.97 ± 0.27, 1.03 ± 0.13, 1.00 ± 0.12, 1.06 ± 0.14, and 0.96 ± 0.01, respectively, while the Fcx were 1.02 for LA, 1.01 for LNA, 0.99 for AA, 0.98 for EPA, and 0.97 for DHA (22). Inhamuns (8) and Visentainer *et al*. (9) found, respectively, empirical correction factors of 1.02 and 0.99 for EPA and 0.94 and 0.98 for DHA.

TABLE 1
Total Lipid and Moisture Contents, Length, and Weight for Farmed and Wild Tambaqui Caught in the Brazilian Amazonian in Two Seasons[a]

Sample	Total lipids (%)	Moisture (%)	Length (cm)	Weight (kg)
Farmed	4.8 ± 0.8	72 ± 2	41 ± 2	2.5 ± 0.6
Wild-				
Flood season	2.5 ± 0.3	77 ± 1	43 ± 3	3.4 ± 0.6
Dry season	2.8 ± 0.8	74 ± 3	45 ± 3	3.2 ± 0.5

[a]Each value is the mean of duplicates with the respective estimate of SD.

In this study methyl eicosanoate (20:0) was used to verify the response of the detector to a saturated FA in relation to the internal standard of methyl tricosanoate (23:0). The empirical correction factor found was 1.01 ± 0.03, the theoretical response factor being 1.006. This agreement between the two values showed that the chemical and instrumental parameters were optimized and therefore the Fcx could be adopted as recommended by Joseph and Ackman (12) for the quantification of FA.

There is a possibility of error when quantifying n-3 PUFA in marine fish oil as their ethyl esters and using 23:0 as the internal standard. Under certain chromatographic conditions, the internal standard peak area may merge with the 21:5n-3 ethyl ester peak area (23).

In a previous study with tambaqui, 61 FA were detected in the TL (24). The contents of LA, LNA, AA, EPA, and DHA (mg/g) in the TL are shown in Table 2.

Fish caught in the wet season showed greater amounts of LA and LNA in both tissues, whereas fish caught in the dry period showed greater amounts of AA, EPA, and DHA in the muscle.

The LNA content found in the muscle of farmed tambaqui was higher than that in the muscle tissue of farmed tilapia (*Oreochromis niloticus*) fed on commercial feed (9).

A higher amount of LA was found in the muscle and in the adipose tissue of the orbital cavity of the fish caught during the wet season; however, a higher AA content was found in the fish caught during the dry period and in both tissues. The amount of AA in the muscle was even higher than that found in the dorsal muscle of *Merluccius hubbsi* captured in Rio de la Plata (25).

Tambaqui caught in the dry season showed the highest EPA and DHA contents. For the adipose tissue of the orbital cavity, the highest EPA content was 7.0 mg/g, found in fish caught in the dry season. However, the highest value for DHA (9.3 mg/g) in this tissue was found in fish caught in the flood season. Previous research with blue dorsal fish such as the tuna and bonito revealed that high levels of DHA could occur in the adipose tissue of their orbital cavities (26–28).

The higher EPA and DHA values found in the muscle of tambaqui caught in the dry season might be explained by the fact that the tambaqui feed basically on plankton. Araújo Lima and Goulding (6) reported that 58% of the total food ingested by tambaqui in the dry season is zooplankton. Since EPA and DHA are natural components of zooplankton and aquatic insects (29), they might be considered as high-quality food and, thus influence the EPA and DHA content. It is important to comment on the metabolic needs for EPA and DHA of this species for spawning (6), because they are necessary for correct metabolic function.

The value of 25.1 mg/g found for the concentration of DHA in farmed tambaqui was also a surprise, being higher than that found in fish caught in the wet season (14.1 mg/g). It was speculated that fish from the natural environment would show higher concentrations of these FA, since they have a more diversified diet. Probably, the semi-intensive farming system contributed significantly to the diversification of the diet of the fishes, allowing ingestion of certain zooplankton besides the granulated feed. Araújo-Lima and Goulding (6) commented that in intensive and semi-intensive farming, zooplankton are more important as a qualitative factor than a quantitative one.

The EPA detected in the feed composition might have contributed to the higher content of EPA found in the muscles of farmed fish, in relation to those caught in the flood season. This fact allows one to suppose that the quality of fish from pisciculture might be improved by a better formulation of the feed, as already observed by Visentainer *et al*. (9), who elaborated a linseed oil-enriched feed, thus obtaining a higher content of LNA, EPA, and DHA in tilapia.

Inhamuns (8) found a mean value of 5.0 mg/g for EPA in the muscle of tucunaré (*Cichla ocelaris*) caught in the Amazon basin, which was higher than that found in the muscle of the tambaqui caught in the wet season (3.8 mg/g). The EPA content of 3.0 mg/g found in the muscle of tucunaré caught in the dry season was lower than that found in the muscle of tambaqui (9.3 mg/g) caught in the same season. The DHA content of tucunaré (muscle) caught in the flood season was 55.0 mg/g, a value higher than that in tambaqui caught in the same season. However, the content of 21.0 mg/g found in the muscle of tucunaré in the dry season was lower than the value found in tambaqui, 40.2 mg/g. In the adipose tissue of the orbital cavity of tucunaré, the EPA and DHA contents were both lower than the values found in tambaqui, considering the same tissue and the same season.

The same author reported that, for mapará (*Hypophthalmus* sp.) caught in the dry and wet periods, respectively, the EPA contents in the muscle and in the adipose tissue of the orbital cavity were higher than those found in tambaqui caught in the

TABLE 2
Essential Fatty Acid Contents (mg/g of total lipids) in the Muscle and Adipose Tissue of the Orbital Cavity for the Farmed and Wild Tambaqui, Caught in the Brazilian Amazonian Area in Two Seasons[a]

	LA		LNA		AA		EPA		DHA	
Origin	Muscle	Orbital cavity	Muscle	Orbital cavity	Muscle	Orbital cavity	Muscle	Orbital cavity	Muscle	Orbital cavity
Farmed	208.0 ± 2.8[c]	103.4 ± 1.6[e]	12.4 ± 1.6[i]	6.6 ± 0.5[l]	57.9 ± 0.6[f]	20.0 ± 2.0[h]	5.0 ± 0.8[j,l]	4.4 ± 1.4[j,l]	25.1 ± 6.7[h]	8.1 ± 3.7[j]
Wild										
Flood season	297.3 ± 1.6[a]	259.7 ± 2.4[b]	50.7 ± 1.3[f]	40.3 ± 0.6[g]	32.2 ± 0.9[g]	10.8 ± 1.4[i,j]	3.8 ± 1.1[l]	5.5 ± 1.3[j,l]	14.1 ± 4.6[i]	9.3 ± 2.0[j]
Dry season	262.0 ± 1.4[b]	102.5 ± 2.0[e]	48.0 ± 0.9[f,g]	15.7 ± 1.7[i]	157.6 ± 0.7[d]	24.6 ± 1.9[h]	9.3 ± 2.0[j]	7.0 ± 1.2[j]	40.2 ± 4.9[g]	8.9 ± 2.1[j]

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

same seasons. The DHA content in the muscle of mapará was 18.0 mg/g in the wet season, which was also higher than that found in the muscle of tambaqui. In the dry season, however, the DHA content of mapará muscle was lower than 40.2 mg/g, the value found in the muscle of tambaqui in the dry season. In the adipose tissue of the orbital cavity of mapará, the DHA content was higher than the values found in tambaqui in both seasons.

Tambaqui caught in the dry season showed higher EPA and DHA contents, although for the adipose tissue of the orbital cavity the highest DHA content was found in the fishes caught in the wet season. An inversion of the values found for EPA and DHA contents was observed in tucunaré and mapará, which showed the highest contents of these acids during the wet season. This observation occurred as a result of the biology of the species and proves (i) that the FA content may vary depending on the species and (ii) that the seasons in the Amazon are capable of influencing the FA content of the fishes.

Tambaqui showed the highest EPA and DHA contents when caught in the dry season, when the food availability was low, and when the food of this species, according to Araújo-Lima and Goulding (6), is 58% zooplankton. This fact was also observed for the mapará, whose EPA and DHA contents were highest when its diet was constituted of 90% of zooplankton, in the flood season (8).

With respect to the recommendations about the ingestion of LC-PUFA, the concentrations of EPA and DHA (in mg/100 g of muscle tissue) were calculated (hypothetically) based on the data for the farmed and wild tambaqui, 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).

Tambaqui caught in the wet season showed the lowest concentration of EPA (9.6 mg/100 g) and DHA (35.4 mg/100). The sum of EPA and DHA was higher for the farmed fishes owing to the content of DHA, which was 25.1 mg/g (Table 2) and to the TL percentage of 4.8% (Table 1).

The amounts of tambaqui muscle tissue that a person should eat daily to ensure the ingestion of 200 mg/d (30) of EPA and DHA were 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/d of muscle tissue of farmed fish, 144 g/d of the muscle from fish caught in the dry season, or 445 g/d of muscle from fish caught in the wet season.

These results show that the lowest daily ingestion value required to supply the needs of EPA and DHA of an individual was provided by the farmed tambaqui. However, these showed EPA and DHA contents below those of fish caught in the dry season and TL contents higher than that of wild fish caught in either season.

Although the daily value of farmed fish required to supply a person's needs for EPA and DHA was a little lower than that found for the wild fishes caught in the dry season, wild fish are more highly recommended since they showed a lower TL content and higher EPA and DHA contents.

Assuming that EPA and DHA are the only source of n-3 LC-PUFA, a person would have to eat 27 g/d of the muscle of mapará caught in the wet season or 43 g/d of those caught in the dry season. For tucunaré, the quantity would be 416 and 396 g/d for fish caught in the flood and dry seasons, respectively (8). Tambaqui may be considered as a better source of n-3 LC-PUFA, as compared to tucunaré caught in the same seasons.

ACKNOWLEDGMENTS

Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior and Fundação de Amparo à Pesquisa do Estado de São Paulo for their financial support.

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[Received October 6, 2005; accepted June 5, 2006]