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Chemical composition of lipids from native and exotic fish in reservoirs of the state of Ceará, Brazil

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ABSTRACT. Current study analyzes the chemical composition of lipids in fish commonly found in the dams of the state of Ceará, Brazil, namely *Pterygoplichthys pardalis* (bodó), *Hoplias malabaricus* (traira), *Cichla ocellaris* (tucunaré), *Prochilodus brevis* (curimatã) and *Oreochomis niloticus* (tilapia). The animals were collected during the summer and Folch extraction procedure was used for the extraction of lipids, whilst Iupac methodology (International Union of Pure and Applied Chemistry [Iupac], 1987) was used to methylate the fatty acids. Methyl esters were analyzed by GC/MS and the different components in fish oil were identified. Palmitic acid, C16:0 (35.71-45.02%), was the saturated fatty acid with the highest percentage, while oleic acid, C18:1Δ^9 (10.62-25.29%) had the highest percentage among the unsaturated fatty acids. The chemical composition of analyzed freshwater fish lipids revealed low levels of polyunsaturated fatty acids.

Keywords: freshwater fish, chemical composition, fatty acids.

Composição química de lipídeos de peixes nativos e exóticos presentes em açudes do Ceará, Brasil

RESUMO. O objetivo deste trabalho foi analisar a composição química de lipídeos de peixes comumente encontrados em açudes do estado do Ceará: *Ancistrus sp* (bodó), *Hoplias malabaricus* (traíra), *Cichla ocellaris* (tucunaré), *Prochilodus brevis* (curimatã) e *Oreochomis niloticus* (tilápia). A coleta dos animais foi realizada durante o verão. Os lipídeos foram obtidos segundo o método de extração de Folch e os ácidos graxos foram metilados seguindo a metodologia da Iupac (União Internacional de Química Pura e Aplicada [Iupac], 1987). A análise química dos ésteres metílicos foi feita por CG/EM. Dentre os ácidos graxos saturados, o palmítico C16:0 (35.71-45.02%) apresentou o maior percentual e entre os ácidos graxos insaturados o oleico C18:1Δ^9 (10.62-25.29%) foi o mais abundante. A composição química de lipídeos dos peixes de água doce analisados revelou baixos teores de ácidos graxos poliinsaturados.

Palavras-chave: peixes de água doce, composição química, ácidos graxos.

Introduction

Aquaculture is the farming of aquatic organisms. It is not merely an efficient way to produce food but it is also an economical asset. Moreover, food production must also be characterized as environmentally and socially sustainable (Dias, Simões, & Bonecker, 2012). In fact, several health benefits are attributed to the consumption of fish, recommended as a feature in a balanced diet (Domingo, Bocio, Falco & Llobet, 2007). Research from various countries suggests an inverse relationship between fish consumption and an incidence of cardiovascular diseases, increasing attention on the significance of polyunsaturated fatty acids in human nutrition (Prato & Biandolino, 2012).

Several dams have been built in the state of Ceará, Brazil, to tackle the problem of drought in the state. Since another factor linked to drought is food scarcity, pisciculture in the dams is a strategy for the utilization of this relevant government investment. Thus the introduction of native and exotic species in the reservoirs meets an urgent and important need of the local population. However, the lipid composition of native fish has not yet been evaluated.

Pterygoplichthys pardalis, popularly known as 'bodó' or 'acari-bodó' (Ferreira, 1986), is a siluriform fish of *the* Loricariidae family, species *Ancistrus* (Simonović, Nikolić, & Grujić, 2010) which inhabits the rivers and lakes of Asia, Central and South America (Wakida-Kusunoki, Ruiz-Carus,

& Amador-Del-Angel, 2007), especially in the Brazilian and Peruvian Amazon (Simonović et al., 2010). *Hoplias malabaricus* is a characiform wolf-fish belonging to the Erythrinidae family, popularly known in Brazil as 'traíra' (Costa, Monteiro, & Brasil-Sato, 2015), widely distributed in South America (Corrêa, Karling, Takemoto, Ceccarelli, & Ueta, 2013). Further, the 'tucunaré' (*Cichla* sp.) is an ichthyophagous fish of the Amazon river basin, and a rich source of essential fatty acid: linolenic acid n-3 series and linoleic acid n-6. These fatty acids are important ω-3 and ω-6 sources (Inhamuns, Franco, & Batista, 2009). *Prochilodus brevis* (curimatã) belongs to Prochilodontidae family, a native species of the semiarid region of Brazil (Chellappa, Bueno, Chellappa, Chellappa, & Val, 2009). The fish characteristically swims against river currents to mature sexually and procreate (Gurgel, Verani, & Chellappa, 2012). However, the most economically important fish in Brazil is *Oreochromis niloticus* (Cará-Tilápia), native to Africa. It is a tropical fish with fast development into adulthood and high reproductive rate (Melo et al., 2013).

The fishes mentioned above should be included in the diet since they have low fat and high protein rates, or rather, important sources of unsaturated fats (UFA) and polyunsaturated fats (PUFA) (Marichamy, Badhul Haq, Vignesh, Shalini & Nazar, 2012). Furthermore, it is known that the oils produced by fresh or sea water fish are an important source of long-chain PUFAs, such as C18:2 (ω-6) and C18:3 (ω-3), with reduce several risk factors associated with atherosclerosis, and improvement of retina and brain development, also decreased incidence of breast cancer and rheumatoid arthritis (Ozogul & Ozogul, 2007). Therefore, oils of various fish species have been analyzed for their protective effects.

The fatty acid profile of freshwater fish is still limited to a few species and few studies have been published highlighting storage conditions. PUFA chemical composition may vary between species and little attention has been given for this aspect when selecting fish for diets (Weaver et al., 2008). Thus, when fish are suggested for a healthier diet, their PUFA profile should be taken into account.

Current study analyzes the chemical composition of lipids of the fish species bodó (Amazon river), traíra (native), tucunaré (Amazon river), curimatã (native) and Nile tilapia (Gurgel, 1990), commonly found in the dams in the state of Ceará, Brazil, and usually included in human diet.

Material and methods

Fish were collected during the dry season (summer) in Crateús, Morada Nova and Fortaleza, Ceará, Brazil. The fisherman use small boats and leave a fishing net for a few hours until capture. After removal from the fishing net, the fish are gutted and put in ice at a ratio of 1: 2 (kg ice kg fish^{-1}). When the boats come ashore, the fish are placed in plastic baskets, weighed on a scale and classified to separate those with gross changes. They are then stocked until processing.

Fish cooling was undertaken at a ratio of 1: 4 (kg ice kg fish^{-1}). Samples were taken randomly. After weighing and grading, three specimens of each species were cut and frozen, placed in styrofoam boxes and transported to the Natural Products Laboratory of the State University of Ceará (Uece), Fortaleza, State Ceará, Brazil. Fish were traíra (*Hoplias malabaricus*, Bloch), bodó (*Ancistrus* sp.), tucunaré (*Cichla ocellaris*), curimatã (*Prochilodus brevis*) and tilapia (*Oreochomis niloticus*).

Lipids were extracted following method by Folch, Lees and Stanley (1957), using 8 g meat samples and 10 g fish skin samples, in triplicate. Fish samples were mixed with chloroform and methanol solution (2:1 v v^{-1}) in an Erlenmeyer flask and homogenized for 30 min with a magnetic stirrer. A saline solution (NaCl 1.5%) was used to better separate the phases. The organic layer with the extracted material was transferred to a 100 mL volumetric flask and the volume completed with chloroform to determine total lipids and cholesterol.

Fatty acids were methylated according to Iupac method (1987), with adaptations: 500 mg of lipid, hexane (5 mL) and 0.1 M KOH in methanol (5 mL) were mixed then added to 30 cm test tubes. The test tubes were placed in a water bath at 50°C for 1 hour. Hexane (5 mL) and 5% HCl solution (15 mL) were added and the mixture transferred to a separating funnel, where the hexane phase, containing the methyl esters, was separated, dried with Na_2SO_4 and stored in a refrigerator until analysis by Gas Chromatography / Mass Spectrometry (GC/MS).

Chemical analysis by GC/MS of methyl esters was developed on a Shimadzu Q P-5050 instrument with a fused silica capillary column DB-1ms with dimethylpolysiloxane (30 m x 0.25 mm id x 0.25 mm); carrier gas: He (1 mL min^{-1}) at constant linear velocity mode (47.4 cm s^{-1}). Injection temperature: 250°C and detector temperature: 230°C. The column temperature program ranged between 35 and 180°C at 4°C min^{-1}; between 180 and 280°C at 17°C min^{-1}; at 280°C for 10 min. Mass spectrum was obtained by electron impact at 70 eV.

The compounds were identified by the retention times (IK) comparing with those in the National Institute of Standards and Technology database (NIST: 147,198 compounds - USA) and by visual comparison of the mass spectra, following Adams (2001) catalog.

Results and discussion

Table 1 shows lipid percentage contents (g 100 g^{-1}) of meat and skin from different fish species ranging between 1.2 and 2.6%. Total fatty acid methyl esters percentages ranged between 90.43 and 94.83%.

Table 1. Lipid content of different fish species in State Ceará dams.

Fish (Place of origin)	(% Lipids)
Traira (Brazil)	2.06
Tilapia (Africa)	1.37
Curimatã (Brazil)	1.34
Tucunaré (Brazil)	1.08
Bodó (Brazil)	1.02

In their studies of fish in the Amazon region, Inhamuns et al. (2009) quantified the meat lipids of the peacock bass species, between 0.8 and 2.1 g 100 g^{-1} during the rainy and dry season, respectively. The average variation found in the lipid fraction in typical freshwater fish, such as the tilapia, was 1.33-3.19% (Oliveira et al., 2008). Low fat was evident in freshwater fish species.

Table 2 shows fatty acid percentages identified in different fish species. The experimental retention indices (KI) of compounds were obtained by a linear regression equation, using gas chromatogram retention times (in min) and the literature KI (Kovat´s index) of main fatty acids.

GC/MS analysis identified the lipid components of fish oils: SFA percentages of the main components were 62.74 (Bodó), 61.65 (Traira), 60.72 (Tucunaré), 64.18 (Curimatã) and 58.71 (Tilapia). SFA with highest percentages were C16:0 (35.42 - 45.02) and C18:0 (14.94 - 7.88). UFA percentage were 37.26 (Bodo), 38.35 (Traira), 39.28 (Tucunaré), 35.82 (Curimatã) and 41.59 (Tilapia); UFA´s highest percentage was C18:1 Δ^{9} (25.29 - 10.21) and C16:1 Δ^{9}, with the results ranging from 4.11 to 7.60.

PUFA consists of C20:2 $\Delta^{11,14}$ (ω-6), with 0.62%, found only in Bodó, C18:2 $\Delta^{9,12}$ (ω-6), C20:3 $\Delta^{8,11,14}$ (ω-6), C22:3 $\Delta^{10,13,16}$ (ω-6) in Curimatã, with percentages 1.57, 0.36 and 0.69%, respectively. According to Connor (1997), MUFA and PUFA ω-3, ω-6 and ω-9, are considered essential and demonstrate curative and preventive effects on cardiovascular, neurological diseases in children, and for the prevention of cancer and glycemic control. Inhamuns and Franco (2008) report that fatty acids belonging to the family AGI ω-3 and ω-6 are associated with the synthesis of eicosanoids, such as prostaglandins, thromboxanes, and leukotrienes.

Intake of ω-3 rich oils decreases triglycerides and LDL levels, whereas the incorporation of ω-3 EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) in the muscle tissue of tilapia fed with diets supplemented with different levels of head silage shrimp *Litopenaeus vannamei* significantly increased EPA and DHA absorption levels in the fillet (Costa et al., 2011).

Table 2. Percentage composition of fatty acids identified in freshwater fish species in dams of the state of Ceará, Brazil.

FA Methyl esters	Chemical notation	Kovats Index	Bodo catfish	Traira wolffish	Tucunare (native)	Curimata (native)	Tilapia
Myristic	(C14:0)	1768	1.94	1.07	2.96	3.11	4.42
Pentadecanoic	(C15:0)	1869	4.50	0.98	1.43	2.00	1.62
Hexadecenoic (ω-7)	(C16:1 Δ^{7})	1899	0.94	0.82	0.39	1.13	ND
Palmitoleic (ω-9)	(C16:1 Δ^{9})	1932	4.09	4.67	7.60	10.06	11.04
Palmitic	(C16:0)	1977	35.71	42.56	36.49	45.02	40.34
Iso-heptadecanoic	(C17:0)	1983	1.08	1.15	0.42	0.97	2.13
Heptadecenoic (ω-9)	(C17:1 Δ^{8})	2015	0.81	0.65	1.11	0.83	1.84
Margaric	(C17:0)	2077	3.41	1.72	2.78	2.83	2.02
Iso-octadecanoic	(C18:0)	1718	0.88	ND	ND	ND	ND
Linoleic (ω-6)	(C18:2 $\Delta^{9,12}$)	2094	ND	ND	ND	1.57	0.19
Oleic (ω-9)	(C18:1 Δ^{9})	2107	24.87	25.29	21.10	10.62	17.79
Octadecenoic (ω-7)	(C18:1 Δ^{11})	2115	4.02	5.35	6.35	8.39	9.59
Stearic	(C18:0)	2170	15.22	14.17	14.94	9.10	7.88
Nonadecenoic (ω-9)	(C19:1 Δ^{10})	2209	ND	ND	0.53	ND	ND
Nonadecenoic (ω-7)	(C19:1 Δ^{12})	2598	0.37	ND	0.47	ND	ND
Nonadecanoic	(C19:0)	2359	ND	ND	0.86	0.40	ND
Eicosatrienoic (ω-3)	(C20:3 $\Delta^{8,11,14}$)	2302	ND	ND	ND	0.69	ND
Eicosadienoic (ω-6)	(C20:2 $\Delta^{11,14}$)	2092	0.62	ND	ND	ND	ND
Eicosenoic (ω-6)	(C20:1 Δ^{11})	2045	1.54	1.57	1.73	2.17	1.14
Eicosanoic	(C20:0)	2567	ND	ND	0.38	ND	ND
Docosatrienoic (t-3)	(C22:3 $\Delta^{10,13,16}$)	2249	ND	ND	ND	0.36	ND
Docosanoic	(C22:0)	2236	ND	ND	0.46	0.40	ND
Total UFA			37.26	38.35	39.28	35.82	41.59
Total SFA			62.74	61.65	60.72	64.18	58.41

SFA – Saturated Fatty Acids; UFA – Unsaturated Fatty Acids; ND – not determined.

High levels of palmitoleic acid C16:1Δ^9 (ω-6) are characteristic of freshwater fish. Research indicates that ω-6 increases HDL and reduces risks in Type II diabetes. Ramos Filho, Ramos, Hiane and Souza (2010) demonstrated that high levels of palmitic, palmitoleic and oleic acids have been found in fish from natural rivers of Brazil. As mentioned above, curimatã provides linoleic acid content - C18:2 $\Delta^{9,12}$ (ω-6) - 1.57%. According to Nishiyama et al. (2014), C18:2 fatty acids are essential because they are the precursors for the synthesis of several PUFA, such as C20:2 (ω-6), C20:5 (ω-3) and C22:6 (ω- 3) acids.

Analyses of fish samples in current study demonstrated higher contents of C-16 and C-18 fatty acids similar to other fishes, reported by Jabeen and Chaudhry (2011). SFA, UFA and C-17 fatty acids were also present, the latter being a ω-9 type FA.

Ozogul, Ozogul and Alagoz (2007) reported that fatty acids from freshwater fish were more saturated. Luzia, Sampaio, Castellucci, and Torres (2003) registered a predominance of palmitic acid in Tilapia (*Oreochromis spp*.) with 35.9% and in Curimatã (*Prochilodus* spp.) with 28.9%. The prevalence of these acids seems to characterize freshwater fish.

Conclusion

The freshwater fishes from Ceará State reservoirs are basically formed by native species, species from Amazon River and Nile tilapia, which is very well adapted to Brazilian weather conditions. SFA with the highest percentage were palmitic acid, stearic acid and, to a lesser extent, margaric acid. Among UFA oleic acid presented highest percentage. Other fatty acids followed with lower but significant percentage such palmitoleic and octadecenoic acids (W-7). In general, the chemical composition of lipids from freshwater fish in tropical region, including the state of Ceará, provided low PUFA levels.

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