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Lipids of Amazon Caimans: A source of fatty acids

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Some species of fish and other aquatic organism are important sources of protein and fatty acids that are beneficial to human health and can be industrially processed. The fatty acid profile of *Caiman crocodilus* and *Melanosuchus niger* (native to the Brazilian Amazon flooded forest) was determined in samples of a commercial cut (tail fillet) and fat (fat body and somatic fat) of these two species. There were no statistically significant differences in the total lipid content between them ($p \geq 0.05$) and both had higher levels of palmitic, stearic (saturated), and oleic (unsaturated) acids. However, omega 3 (ω-3) and omega 6 (ω-6) were not detected in the samples of the commercial cut; they were present only in the fats evaluated. Clinical studies are necessary to assess the influence of fatty acids from Amazon Caimans on human diet and the feasibility of obtaining new products such as nutraceuticals.

Key words: Black caiman, spectacled caiman, omega 3, omega 6.

INTRODUCTION

A large number of consumers have had access to food nutritional information. Therefore, there has been a growing interest in some nutrients associated with the prevention of diseases through diet, including fat consumption. Lipids are important constituents of cell membranes and play major role in metabolic processes (Martin et al., 2006). They are composed of fatty acids (FA) of different chain lengths that may be saturated (SFA) or unsaturated (UFA). The unsaturated fatty acids are classified into monounsaturated (MUFA) and polyunsaturated (PUFA) fats (Moreira et al., 2002). The term essential fatty acid (EFA) refers to polyunsaturated fatty acids that must be obtained through foods since they cannot be synthesized in the human body and are required for maintaining good health. Essential fatty acids (EFAs) are divided into two groups: (a) ω-3: which includes the alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and (b) *ω-6*: which includes cis-linoleic acid (LA) (Kaur et al., 2014). Although produced in the human body, oleic acid (ω-9) requires the presence of ω-3 and ω-6. Clinical studies have been performed on EFAs due to their special tendency to be considered as functional foods. According to Siro et al. (2008), functional foods are defined as “food that may provide health benefits beyond basic nutrition”. Despite that, some studies reported the

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western diet as “deficient” in some FA and very low levels of ω-3 PUFAs, leading to an unhealthy ω-6/ω-3 ratio without benefits to human health (Simopoulos, 2008). This ratio is important to be considered in order to prevent diabetes and the risk of cardiovascular diseases (Simopoulos, 2016; Russo, 2009).

As the availability of some EFAs depends on the diet, it is important to know, among the animal species, those that are commercially available. Fish, both saltwater and freshwater, is among the most important sources of FA. However, the amount of EFAs varies between similar species, and according to environmental variables, such as diet and habitat (Ohr, 2005). In addition to fish, the crocodilians black caiman (*Melanosuchus niger*) and spectacled caiman (*Caiman crocodillus*) are important food sources and can be used commercially by native populations (Da Silveira and Thorbjarnarson, 1999).

In the most recent global assessment of International Union for Conservation of Nature and Natural Resources (IUCN), the two species are in the category of least concern (Ross, 2000). These species belong to the family Alligatoridae and are found in South American countries. Like any kind of fauna exploitation, the commercial exploitation of caimans can be performed in a sustainable manner with animals bred in captivity or in their natural habitat. The commercial exploitation of wild crocodilian is well-known in some countries such as Australia (Seafield et al., 2014) and United States of America (Louisiana Department of Wildlife and Fisheries (DWF), 2013). In those programs, the commercial exploitation is a sustainable tool for crocodilian species and their natural habitat. In Brazil, in addition to the Amazon species, the species yacare caiman (*Caiman yacare*) and broad-snouted caiman (*Caiman latirostris*) are also commercially exploited; their meat and by-products have been frequently studied (Canto et al., 2012). Vicente Neto et al. (2007, 2006) carried out studies on *C. yacare* composition and found lipids ranging from 0.4 of 0.54% in the tail. Cernikova et al. (2015) reported lipids ranging from 4.41 to 5.90% in the same commercial cut from Nile crocodile (*Crocodilus niloticus*). Despite the available studies on commercial cuts of other crocodilians, there is no data on the FA profile of the abdominal fat cavity of black caiman and commercial cuts of spectacled caiman.

Accordingly, the aim of this study was to evaluate the FA profile of samples of the spectacled caiman and somatic and fat body of the black caiman, to contribute to scientific data for human nutrition.

MATERIALS AND METHODS

Animal sample and slaughtering procedures

Sixty animals of the species black caiman and spectacled caiman were captured and slaughtered for the purpose of this study, with official authorization granted by the Brazilian Institute for the Environment and Renewable Natural Resources - IBAMA (14498, 1-2/2009). The animals were captured in their natural environment in the Piagaçu-Purus Sustainable Development Reserve, in the municipalities of Anori, Amazonas State, Brazil. They were slaughtered in the high water level season (December) and were handled observing the current legislation and the Humane Methods of Slaughter Act (animal welfare). The samples of fat body were collected during evisceration, manually separated from the mesentery and stored in ice (0°C). After slaughter, the carcasses were submitted to cutting (commercial cuts), as described by Kluczkovski Junior et al. (2015). During the separation of the tail portions, samples of somatic fat were collected in the space between the muscles. All samples were frozen in the fish facility and were sent for analysis. The samples were classified into: (a) muscle tissue (tail fillet of both species) and (b) adipose tissue: somatic and body fat of Black caiman. Huchzermeyer (2003) previously reported the terms fat body and somatic fat.

Fatty acid profile analysis

The samples were minced using an industrial blender until homogeneous mass was obtained, and total lipids were estimated by Soxhlet. The assays were performed in triplicate according to Association of analytical communities (AOAC) (2005). For the analysis of FA, total lipids were extracted according to procedures described by Folch et al. (1957), and the preparation of FA methyl esters were carried out according to Hartman and Lago (1973). Briefly, FA were saponified with a methanolic NaOH solution and methylated under acidic conditions by adding a solution of ammonia chloride, methanol and sulphuric acid. The FA methyl esters were submitted to gas liquid chromatography on a GC-2014 chromatographer (Shimadzu Corporation, Kyoto, Japan), equipped with a flame ionization detector (FID) and a capillary column of 10% cyanopropylphenyl-90% biscyanopropylpolysixolano 105 m, 0.25 mm ID, 0.2 µm df (Restek®) in the following conditions: Injector: 260°C; Detector: 260°C; Column: 140 initial (5 min); 2.5 to 240°C (15 min)/60 min. Individual FA were expressed as percentage of the chemical components expressed on dry matter basis (DM) and the limit of detection (LOD) for the FA was 0.01%.

Statistical analysis

A non-parametric test, the Wilcoxon-Mann-Whitney U test (Bauer, 1972; Hollander and Wolfe, 1999) was used to compare the sample species instead of using the alternative Student T-test, which has to be applied on independent samples. In the FA profile analysis of *black caiman*, the normal distribution of frequencies was verified using the Shapiro-Wilk W test (Razali and Wah, 2011), and the homogeneity of variances was verified using the Fligner-Killeen test (Conover et al., 1981). All descriptive and inferential statistical tests were carried out using the R software (R Core Team, 2015).

RESULTS AND DISCUSSION

The total lipid content of black caiman and spectacled caiman, as well as data of other species of crocodilians reported by other authors, are presented in Table 1. There were no statistically significant differences in the total lipid content between the two species ($p \geq 0.05$). The lipid content in the spectacled caiman (0.02 g%) was lower than that in the black caiman (0.6 g%). This can be explained by considering that the samples were obtained from wild animals whose availability of food varies

Table 1. Fat acid profile in commercial cut (tail) of *Black caiman* and *Spectacled caiman* and other crocodilian species.

FA (%)	**Lipids %[1]**							
	C. crocodilus	***M. niger***	***p value***	***A. mississipiensis*[2]**	***C. latirostris*[3]**	***C. niloticus*[4]**	***C. crocodillus yacare*[5]**	
							Captivity	**Wild**
Fat % (DM[6])	0.02±0.00^{a}	0.60±0.02^{a}	0.0722	1.2±0.1	16.9±9.8	1.8±0.3	3.20	19.16
Lauric (12:0)	ND[7]	0.04±0.02^{a}	0.0636	0.4	0.08	ND	NI[8]	NI
Myristic (14:0)	ND	ND	NA[9]	1.6	2.31	0.3 ± 0.1	NI	NI
Myristoleic (14:1)	ND	0.03±0.01^{a}	0.0594	0.9	0.3	ND	NI	NI
Pentadecylic (15:0)	ND	ND	NA	1.1	0.55	0.1 ± 0.0	NI	NI
Palmític (16:0)	0.12±0.03^{a}	1.41±0.06^{a}	0.1000	17.5	21.85	20.2±0.1	NI	NI
Palmitoleic (16:1)	0.01±0.01^{a}	0.56±0.03^{a}	0.0765	5.3	2.72	3.1±0.3	3.93	5.9
Margaric (17:0)	ND	ND	NA	0.3	1.07	0,1±0,0	NI	NI
Heptadecanoic (17:1cis 10)	ND	0.05±0.01^{a}	0.0594	NI	0.82	ND	NI	NI
Stearic (18:0)	0.06±0.02^{a}	0.58±0.03^{a}	0.1000	7.7	15.36	7.9±0.4	14.31	9.61
Vaccenic (18:1cis7)	ND	ND	NA	NI	NI	2.6±0.2	NI	NI
Oleic (18:1cis9)	0.05±0.02^{a}	1.42±0.11^{a}	0.1000	28.8	34.92	27.3±2.1	NI	NI
Linoleic (18:2 *n*-6)	0.02±0.01^{a}	0.29±0.01^{a}	0.0765	16.1	8.4	29.6±0.3	8.34	12.15
α-Linolenic (18:3 *n*-3)	ND	ND	NA	5.5	3.32	1.6±0.1	0.95	3.18
γ-Linolenic (18:3 *n*-6)	ND	ND	NA	NI	NI	0.2±0.1	0.42	0.58
Elaidic (18:1t9)	<0.01^{a}	0.03±0.01^{a}	0.0594	NI	NI	0.1±0.0	NI	NI
Arachidic (20:0)	<0.01^{a}	0.01±0.01^{a}	0.5050	0.3	NI	0.3 ±0.0	NI	NI
Eicosenoíc (20:1cis11)	<0.01^{a}	0.06±0.02^{a}	0.0636	NI	0.07	0.2 ± 0.1	NI	NI
Eicosadienoíc (20:2cis11,14)	<0.01^{a}	0.02±0.01^{a}	0.1876	NI	0.17	0.3±0.1	NI	NI
Eicosapentaenoic (20:5 *n*-3)	<0.01^{a}	0.03±0.01^{a}	0.0594	NI	0.76	0.2 ± 0.1	0.36	0.21
Eicosatrienoic (20:3)	0.04±0.01^{a}	0.02±0.01^{a}	0.1642	0.2	NI	042 ± 0.1	NI	NI
Arachidonic (20:4 *n*-6)	ND	ND	NA	2.9	4.34	4.2 ± 0.1	7.2	6.24
Docosapentaenoic (22:5 n-3)	ND	ND	NA	1.6	NI	0.5 ± 0.4	NI	NI
Docosahexaenoic (22:6 *n*-3)	ND	ND	NA	2.3	0.57	1.1 ± 0.3	0.69	1.69

[1] Values presented as: mean±standard deviation; numbers followed by the same letter are not significantly different; [2] Staton et al. (1990): sample of muscle tissue of captivity animals fed with mixed oils; [3] Cossu et al. (2007): intramuscular captivity animals; [4] Osthoff et al. (2010) samples of muscle of animals from captivity; [5] Vicente Neto et al. (2010) samples of muscle of animals from captivity and natural habitat; [6] Dry matter; [7] Not Detected; [8] Not Informed; [9] Not Applicable

over time (seasonal). At the time of sampling (high water level season), the animals may have less availability of food, as previously reported by Da Silveira and Magnusson (1999). Romanelli and Schmidt (1999) reported average fat ranging from 22 to 52% in caiman meat products, such as meat flour from caiman's viscera. These values show that the flour contains fat body, found in the coelomic cavity within the viscera and shows accumulation of fat within the abdominal cavity. On the other hand, Paulino et al. (2011) found lipid content ranging from 6.27 to 11.47% in different formulations prepared to make hamburgers with yacare meat using meat residue resulting from the deboning of the feet, back, and tail of this alligator species. Romanelli et al. (2002) developed a product similar to canned meat using yacare meat from muscles of the trunk. The average lipid content of this product was 5.5%. The authors also developed a smoked meat product (raw cured meat from the tail) with an average lipid content of 5.36%. A lipid content of 12.8% was found in broad-snouted caiman meat preserved in oil (due to the addition of oil); whereas, contents of 2.4% were obtained in this meat preserved in onions and 1.4% when preserved in different seasonings and spices (Azevedo et al., 2009).

Several studies have been published on the characterization of crocodilian fat. Huchzermeyer (2003) reported two types of fats in crocodilians: (a) somatic fat, fat stored in the somatic cells with a small nuclei (in the thorax mediastinum, under the peritoneum and between muscles, the inner (caudofemoralis) and external (ilioischiocaudalis) muscles) and (b) fat body (within the coelomic cavity). The fat body content of crocodilians appears to vary according to age, gender, season, food availability, and animal origin (captive or wild) (Huchzermeyer, 2003). Other studies have reported that the fat content of animals bred in captivity under controlled environmental conditions and controlled food access is different from that of free-living caimans, which are exposed to larger seasonal variation. Concerning the lipid content of commercial cuts of other crocodilian species, some previous work reported levels about 4.39% in *Caiman* sp. (Cossu et al., 2007); 8.8% in *C. niloticus* (Hoffman et al., 2000); 1.9% in *Crocodilus porosus* (RIIRDC, 2007); and 1.5% in *Alligator mississipiensis* (Moody et al., 1980).

The analytical results of FA were not detectable (below the limit of detection (LOD) of <0.01) for most of the spectacled caiman samples assessed. The FA detected were: palmitic acid (0.12%), palmitoleic acid (0.01%), stearic acid (0.06%), oleic acid (0.05%), and eicosatrienoic acid (0.04%). SFA were present in larger amounts than those of PUFA and MUFA in the two species evaluated, with higher contents of palmitic acid (1.41%) and stearic acid (12.58%) in the black caiman. Vicente Neto et al. (2010) also found high content of stearic acid in the yacare caiman, both in captive and in wildlife animals, 9.61 and 14.31%, respectively. The ω-3 fatty acid (ALA and DHA) contents were below the LOD in the species evaluated; however, an EPA content of 0.03% was found in the black caiman only. As for the ω-9 FA family, the oleic acid was predominant, but there were no statistical significant differences in its content between the black caiman and spectacled caiman, 0.05 and 1.42% (p = 0.1000), respectively. It is worth to highlight the importance of this EFA in human nutrition as well as its successful clinical application in the prevention of cardiovascular diseases (Wang et al., 2006). In another species of amazonian fish with commercial exploitation, "pirarucu" or arapaima (*Arapaima sp.),* SFA content of 1.76% and 0.18% of PUFA was found (Scherr et al., 2014). In the captive-bred animals of the species *A. mississipienssis, C. latirotris,* and *C. niloticus,* studied by other authors, there were several FA present in undetectable amounts. Vicente Neto et al. (2010) determined the major FA present in the meat of *C. yacare*, specifically in neck and tail cuts. These authors found that the PUFA content was higher for animals in their natural habitat (31.0%) than for those in captivity (23.6%). Peplow et al. (1990) evaluated the FA profile in captive-bred *A. mississipienssis* and found differences in the FA contents between animals from different areas. According to these authors, a fish-based diet greatly influences the FA profile, showing higher amounts of eicosanoic acid than those found in animals fed meat diet. Staton et al. (1989) stated that *A. mississipienssis* fed diet with lower FA content and showed lower growth rate and that a diet with arachidonic acid appeared to enhance the growth rate of these animals. In another study, Mitchell et al. (1995) found high contents of oleic acid (33.0%), palmitic acid (22.5%), and linoleic acid (15.2%) in the meat of *C. porosus* and *Crocodilus johnstoni.* According to Cossu et al. (2007) there is a ω-3/ω-6 ratio (3.16) in the tail of caimans *(C. latirostris* and *C. yacare)*, near the optimum of 4 recommended by United States Department of Agriculture (USDA) (2006). In the present study, although the presence of ω-3 and ω-6 FA was detected in the commercial cut (tail fillet), their low amounts do not meet official health requirements.

Another important factor to be considered is that the seasonal variation influences the lipid composition of Amazonian fish species. Almeida et al. (2008) studied the FA profile in the muscle, orbital cavity, and abdominal cavity of "tambaqui" or black pacu (*Colossoma macropomum*), wild and in captivity, in different times of the year. The authors concluded that the FA profile of the free-living fish is more adequate for human consumption and that the animals caught during the dry season had higher amounts of PUFA.

Table 2 shows the FA profile in the fat body and somatic fat in *M. niger* samples. With the exception of the margaric acid and elaidic acid ($p \leq 0.0403$), all others showed normality in their frequency distribution ($p \geq 0.0502$). There was homogeneity of variance between the FA groups ($p \geq 0.0538$). However, when comparing the FAs, statistical significant differences were observed only in the contents of stearic acid, arachidic acid, and eicosanoic acid ($p \leq 0.0440$). Among the EFAs, the DHA content found in the fat tissues evaluated was of 0.88% (fat body) and 0.75% (somatic fat). Nevertheless, the amounts found the in the samples evaluated are not significant when compared with those reported by Osthoff et al. (2010), who found an average content of 9.4% in wild animals (*C. niloticus*). When comparing this DHA value with those of beef and chicken, the most widely eaten meats in Brazil, it is clear that the values found in *M. niger* are higher since Daley et al. (2010) reported a

Table 2. Fat acid profile in lipids samples from *Melanosuchus niger.*

FA (%)	**Lipids (%)**[1]			***C. niloticus***[2]
	Fat body	**Somatic**	***p value***	
Lauric (12:0)	0.13 ± 0.03^{a}	0.11 ± 0.05^{a}	0.4732	0.11 ±0.0
Myristic (14:0)	2.06 ± 0.31^{a}	1.56 ± 0.49^{a}	0.1026	3.9 ±0.3
Myristoleic (14:1)	0.49 ± 0.07^{a}	0.39 ± 0.12^{a}	0.1365	0.1 ±0.0
Pentadecyclic (15:0)	1.78 ± 0.39^{a}	1.30 ± 0.37^{a}	0.1044	0.3 ±0.1
Cis-10-pentadecanoic (15:1)	0.09 ± 0.01^{a}	0.09 ± 0.02^{a}	0.5556	NI[3]
Palmitic (16:0)	9.71 ± 5.08^{a}	4.41 ± 4.28^{a}	0.1411	25.6 ±1.6
Palmitoleic (16:1)	5.22 ± 0.44^{a}	4.98 ± 1.31^{a}	0.7107	6.2 ±0.3
Margaric (17:0)	0.92 ± 0.78^{a}	0.82 ± 0.57^{a}	0.4127	0.5 ±0.1
Heptadecenoic (17:1)	0.57 ± 0.09^{a}	0.49 ± 0.12^{a}	0.2653	ND[4]
Stearic (18:0)	5.00 ± 0.62^{a}	3.73 ± 0.95^{b}	*0.0440	4.7 ±1.0
Elaidic (18:1n9trans)	0.39 ± 0.12^{a}	0.33 ± 0.16^{a}	0.2857	0.1 ±0.1
Oleic (18:1cis9)	8.62 ± 0.72^{a}	7.43 ± 1.50^{a}	0.1592	28.0 ±1.8
Linoleic (18:2 *n*-6)	2.83 ± 0.52^{a}	2.58 ± 0.86^{a}	0.6030	6.5 ±2.6
Araquidic (20:0)	0.31 ± 0.05^{a}	0.21 ± 0.06^{b}	* 0.0261	0.3 ±0.0
γ-Linolenic (18:3cis3 *n*-6)	0.16 ± 0.03^{a}	0.15 ± 0.05^{a}	0.7141	0.2 ±0.0
Linolenic (18:3 *n*-3)	1.86 ± 0.47^{a}	1.68 ± 0.63^{a}	0.6375	2.0 ±0.4
Eicosenoíc (20:1cis11)	0.60 ± 0.16^{a}	0.39 ± 0.09^{a}	0.0590	0.5 ±0.2
Heneicosanoic (21:0)	0.13 ± 0.02^{a}	0.08 ± 0.02^{b}	* 0.0123	NI
Eicosadienoic (20:2)	0.28 ± 0.03^{a}	0.21 ± 0.04^{b}	* 0.0290	1.9 ±2.2
Behenico (22:0)	0.20 ± 0.04^{a}	0.14 ± 0.05^{a}	0.0541	0.1 ±0.0
Eicosatrienoic (20:3)	0.37 ± 0.03^{a}	0.31 ± 0.07^{a}	0.1170	0.3 ±0.2
Arachidonic (22:1)	$0.07\pm.0.01^{a}$	0.05 ± 0.02^{a}	0.1372	0.8 ±0.3
Docosahexaenoic (22:6 *n*-6)	0.88 ± 0.8^{a}	0.75 ± 0.24^{a}	0.3114	9.4 ±1.9

[1] Values presented as: mean±standard deviation; numbers followed by the same letter are not significantly different; *FA with higher amount among the evaluated samples; [2] Osthoff et al. (2010) samples of adipose fat from wild animals (*C. niloticus*); [3] Not Informed; [4] Not detected

DHA content in beef of 0.20%. On the other hand, the DHA content in chicken breast was reported as 0.04% (Mirghelenj et al., 2009). Therefore, the results obtained in the present study are relevant since DHA is essential in the diet and cannot be synthesized in the human body and, like the ALA and EPA acids, it is considered as a functional substance. Nevertheless, although the DHA has been more frequently studied in cold water fish, such as salmon and anchovy (Oksuz and Özyılmaz, 2010), its presence in tropical fish suggests the need for further studies on its use in food industries.

The fat body showed higher concentration of palmitic acid (9.71%) than that of somatic fat and muscle tissue, followed by oleic acid (8.62%). This value can be explained because the steatotheca (abdominal fat body) is located in the mesenteric fold close to the abdominal wall and its amount varies depending on the nutritional status, while its shape varies in different species. Fat cells have wide nuclei able to pick up stored fat quickly. Osthoff et al. (2014) studied different adipose tissues of *C. niloticus* and found no statistically significant differences between the stheatoteca and the abdominal tissue. They reported that males have higher SFA content (44.4%) than that of PUFA and MUFA. Somatic fat had higher content of oleic acid (7.43%) than that of other FA, followed by palmitoleic acid (4.98%). Similarly, Almeida and Franco (2007) found SFAs in another fish species, wild "matrinxã" (*Brycon cephalus*). On the other hand, the PUFA content in wild animals was higher than that of captive-bred animals, including DHA. Castelo (1981) studied the FA composition in the species "piracatinga" or red-bellied pacu (*Colossoma bidens*) and "pacu-caranha" or pacu (*Colossoma mitrei*) and found that the oleic acid was predominant in the two species with 44.48 and 48.71%, respectively. These authors stated that the content of fat within the abdominal cavity is dependent on seasonal food consumption, which can even change the fat color from light yellow to dark yellow. Like the "tambaqui" or black pacu, these species are omnivorous and feed on various fruits and vegetables, which can explain why these fats are more similar to vegetable oils than are to saltwater fish oil. Almeida and Franco (2006) also reported that palmitic acid and oleic acid are the most predominant FAs in freshwater fish, which is in accordance with the results of FA content obtained in the present study. Additionally, the intake of

FAs through amazonian fish consumption has clinical benefits. Souza et al. (2002) investigated the addition of “tambaqui” fat in laboratory animals and concluded that it is a good dietary source of lipids and can substitute beef fat producing effects similar to those of soybean oil when risk factors for atherosclerotic are considered.

Conclusion

The variability of FAs found in the lipid profile of crocodilians studied is significant from nutritional and commercial point of view because it suggests the possibility of obtaining high calorie products. It was found that the average content of lipids in the fats and commercial cut studied strongly contributes to the total caloric value of the caiman meat, making it a nutritionally attractive product. On the other hand, in future works, it is important to collect the samples in the low water season to evaluate if there are changes in the FA profile. Considering that some PUFAs with clinical significance were present in the samples investigated, it would be important to assess their effect on consumers’ diet, as the FA absorption in humans varies according to the microbiome. Therefore, an *in vivo* study on the properties of Amazon caiman’s fat and its antioxidant activity is suggested to investigate its possible biotechnological use as nutraceutical, such as the fish oils that have already been manufactured by the pharmaceutical companies.

Conflict of interest

The authors have not declared any conflict of interest.

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