Journal of Food and Nutrition Research, 2016, Vol. 4, No. 10, 699-702
Available online at http://pubs.sciepub.com/jfnr/4/10/10

DOI:10.12691/jfnr-4-10-10

Fatty Acid Profile of the Amazon Caiman Protein Concentrate

Augusto Kluczkovski Júnior[1,*], Alicia de Francisco[1], Ariane M. Kluczkovski[2], Ronis Da Silveira R.[2], Fábio Markendorf[3]

[1]Federal University of Santa Catarina, Rod, Admar Gonzaga, 1346, 8034-001, Florianopolis, SC, Brazil
[2]Federal University of Amazonas, Av. Gen. Rodrigo OtávioJordão Ramos, 6200, 69067-005, Manaus, AM, Brazil
[3]DVISA- Health Department, Com. Paulo Lasmar, 69049-110, Manaus, AM, Brazil
*Corresponding author: augustokjr@hotmail.com

Abstract 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 *Melanosuchus niger* (native to the Brazilian Amazon flooded forest) was determined in samples of a protein concentrate (PC) there was obtained from processing residues. The PC was prepared from cooked muscle portions and NaCl (1.5%) using an adiabatic process. Saturated fatty acids such as stearic acid (0.59%) and palmitic acid (1.43%) were present. The levels of the unsaturated fatty acids omega-6 (ω-6) and omega-3 (ω-3) linolenic acids were 0.32% and 0.15%, respectively. In conclusion, Black caiman PC seems to provide essential fatty acids for human nutrition. 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.

Keywords: *melanosuchus niger, omega 3, omega 6, flour*

Cite This Article: Augusto Kluczkovski Júnior, Alicia de Francisco, Ariane M. Kluczkovski, Ronis Da Silveira R., and Fábio Markendorf, "Fatty Acid Profile of the Amazon Caiman Protein Concentrate." *Journal of Food and Nutrition Research*, vol. 4, no. 10 (2016): 699-702. doi: 10.12691/jfnr-4-10-10.

1. Introduction

Fish and fishery products are important source of protein and micronutrients for good health [1]. In this context, the Amazon region is an important source of raw material with commercial exploitation such as fish and exotic meat, especially for by-products. 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 [2]. The protein concentrate (PC) as a by-product from fish processing that has emerged as an alternative to the use of mechanically separated meat (MSM) [3]. The PC from fish has been ingredient for protein enrichment of other products such as pasta and biscuits [4,5]. In the Amazon region, the fish PC is well known as "fish flour" and obtained from a natural drying process of residues or the whole fish. The *Acari-Bodo* (*Liposarcus pardalis*) flour, the *piracuí*, is the most typical fish flour from the Amazon region [6] and the protein content is around 70% [7]. Beyond that, other aquatic animal species, like caimans, seems to be a nutritive source to obtain PC with higher protein content and lipids. Kluczkovski Junior et al. [8] reported the yield of 57.02% in the carcass of Black caiman (*Melanosuchus niger*) from natural habitat and their residues can be considered for other products. In the most recent global assessment of the International Union for Conservation of Nature and Natural Resources (IUCN) the specie is in the category of least concern [9] and like any kind of fauna exploitation, the commercial exploitation of caimans are performed in a sustainable manner with animals bred in captivity or in their natural habitat. Therefore, the PC from crocodilian can be a lipid source for human nutrition as meat flour from viscera of yacare caiman (*Caiman yacare)* with 22-52% of lipids [10]. There has been a growing interest in some nutrients associated with the prevention of diseases through diet, including fatty acid (FA) consumption [11]. Beyond the saturated fat acids (SFA) some unsaturated fat acids (PUFA) such as omega 3 and 6 are present in several Brazilian fish species [12]. The essential fatty acid (EFA), for example, cannot be synthesized in the human body and are required for maintaining good health. The docosahexaenoic acid (DHA), for example, is an important source from fish oil in clinical studies with benefits for human health [13,14]. The ratio omega-6/omega-3 in food is important to be considered in order to prevent diabetes and cardiovascular risk [15]. Previous studies reported the western diet as "deficient" in some FA and very low levels of omega-3 [16]. In this context, the aim of this study was to obtain the Black caiman PC in order to use crocodilian residues and evaluate the FA profile of the product as a source of omega 3 and omega 6, to contribute to scientific data for human nutrition.

2. Material and Methods

2.1. Raw Material

The animals were captured and slaughtered, for the purpose of this study, in their natural environment (Sustainable Development Reserve of Piagaçu-Purus, in Beruri and Codajás, AM, Brazil) in the high water level season. The slaughtering procedures were handled observing the current legislation and the Humane Methods of Slaughter Act (animal welfare). The official authorization was granted by the Brazilian Institute for the Environment and Renewable Natural Resources - IBAMA (14498-1/2008). The PC was prepared from residues (meat) portions cooked in 100°C water/20 min, adapted from Kluczkovski and Kluczkovski [17]. Sodium chloride (1.5%) was added, and the material was placed in an adiabatic heating system; the water was evaporated under continuous stirring at speed of approximately 40 rpm (65-70 °C for 4h). The product was vacuum packed in metallized film and stored at room temperature (±25°C). The production is presented in Figure 1.

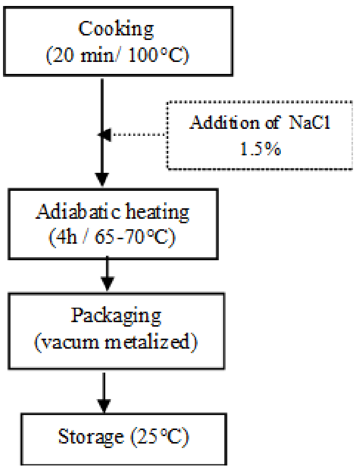

Figure 1. Flowchart of the Black caiman protein concentrate production

2.2. 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 American Oil Chemists` Society [18]. For the analysis of FA, total lipids were extracted and the preparation of FA methyl esters were carried out. 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-2014chromatographer (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%.

2.3. Statistical analysis

The samples analyzed in triplicate were compared using analysis of variance and the Student's t-test (descriptive statistics) with error rate classification to obtain standard deviation of significance level of 5% ($p \leq 0.05$). All descriptive and inferential statistical tests were carried out using the R software [19].

3. Results and Discussion

The drying process seems to affect the level of total lipid [20]. Kluczkovski-Junior et al. [21] evaluated carcass from Black caiman commercial cuts and tail reported level of fat of 0.60%. This can be explained by considering that the samples were obtained from wild animals whose availability of food varies over time (seasonal). At the time of sampling (high water level season), the animals may have less availability of food.

According Table 1, in the Black caiman PC the lipids decreased after 4h/65-70° C of drying to 0.54%. This could be explained by the raw material from residues (meat) with low lipid concentration. In addition, Table 1 presents data of a Pantanal alligator flour with 12.11% of lipids, according to Fernandes *et al.* *[22]*. Their product is composed from bones, meat and spices ground into a flour. The meat was obtained from deboned carcass *in natura* with 3.94% of lipids. It could explain their higher level of lipids, as in our study only meat residues were used. On the other hand, Paulino *et al.* [23] 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.

Table 1. Fatty acid profile in Black caiman protein concentrate

Parameters	Protein Concentrate[a]	
	Black caiman	Pantanal alligator
Fat	0.54	12.11
Fatty acid profile[b]		
Lauric (12:0)	0.34[c] (0.02 ±0.01)	1.75[d]
Meristic (14:0)	4.05 (0.24±0.15)	NI[e]
Tridecanoic (13:0)	0.51 (0.03±0.15)	NI
Meristoleic (14:1)	3.21 (0.05±0.10)	NI
Pentadecanoic (15:0)	3.21 (0.19±0.01)	NI
Palmitic (16:0)	24.15 (1.43±0.05)	22.16
Palmitoleic (16:1)	9.46 (0.56±0.01)	NI
Margaric (17:0)	2.70 (0.16±0.01)	NI
Heptadecenoic (17:1)	0.68 (0.04±0.01)	NI
Stearic (18:0)	9.97 (0.59±0.10)	18.69
Elaidic (18:1n-9)	0.17 (0.01±0.02)	36.81
Oleic (18:1cis9)	25.68 (1.52±0.05)	NI
Linoleic (18:2 cis9, 12 ω-6)	5.40 (0.32±0.01)	5.90
γ-Linolenic (18:3cis3 ω-6)	0.5 (0.03±0.10)	NI
Linolenic (18:3n3)	2.52 (0.15±0.10)	NI
Araquidic (20:0)	<0.01	NI
Eicosenoic (20:1cis11)	0.68 (0.04±0.10)	NI
Eicosatrienoic (20:3cis11,14,17)	0.5 (0.03±0.05)	NI
Eicosapentanoic (20:5n3)	0.68 (0.04±0.15)	2.96
Heneicosanoic (21:0)	<0.01	NI
Behenic (22:0)	0.5 (0.03±0.05)	NI
Docosadienoic (22:2)	<0.01	NI
Docosahexanoic (22:6n3)	1.35 (0.08±0.05)	7.84

[a] Protein concentrate from: (a) Black caiman: residues cooked in an adiabatic system for 4h/65-70°C; (b)Pantanal alligator: meat+bones+spices cooked for 3h/60°C; [b] LD=0.01; [c] values expressed in percentage of total fat content; [d] values expressed in percentage of total fatty content in 100g of the sample; [e] Not informed

In general, the Black caiman PC seems to be a source of FA. Table 1 shows the FA in the Black caiman PC and it can be seen that SFA were present in larger amounts than unsaturated FA. The FA detected were stearic acid (9.97%) and palmitic acid (24.25%). Those SFA were previously reported in a PC from waste meat with bones from tilapia, by Petenucci *et al*. [24]. The authors reported amounts of 7.51% of stearic acid and 27.18% of palmitic acid. In addition Table 1 shows the amount FA reported by Fernandes *et al*. [22] for Pantanal alligator PC. Those authors found higher levels of stearic (18.69%). On the other hand most of the SFA were not detected or evaluated.

Concerning UFA, the Black caiman PC presented omega-6 (5.40%) and omega-3 (2.52%). Beyond that, the levels of omega-9 was 25.68% and DHA was 1.35%. DHA, for example is an important source for clinical benefits for humans, and intake may contribute for brain development and functions [25]. Fernandes *et al.* [22], reported omega-6 (5.90%) and DHA (7.84%) in Pantanal alligator PC and their results for these acids were higher than our findings. On the other hand, they do not reported omega-3. The differences between the crocodilian PC profiles could be explained by the animal feed composition, despite of the text of the authors do not discuss this information. According to FAO [26], in the wild most crocodile eat a wide variety of food, including invertebrates and as they grow, crocodiles eat more vertebrates, mainly fish. In captivity, crocodiles have been grown to commercial size on a number of diets but usually with far less variety than they would have in the wild. In captivity, crocodiles grow twice as fast as their wild counterparts but their faster growth seems to be the result of having more to eat rather than being given a better diet. Red meat may produce even faster growth to juvenile crocodile in captivity than fish. Beyond the animal feed sources, some vegetable sources were tested in fish feeding in captivity. For example, soybean was tested for tilapia in order to increase the levels of FA [27]. In addition, it is not possible to justify the amount of DHA reported by the authors because they did not presented the feed composition in their study. However, the FA content in the Black caiman PC is associated with the animals' diet and environmental variations (season of the year) since they are wild animals.

Although the FA levels of Black caiman PC were not high, our findings are relevant because the FA from Amazon fish have potential clinical applications. Souza *et al.* [28] investigated the application of *tambaqui (Colossoma macropomum)* fat in laboratory animals and concluded that it is a good lipid source and that it can be used as a substitute for beef fat with similar effects to those of soybean oil in terms of risk factors for atherosclerosis. The Black caiman PC showed an omega6/omega3 ratio of 1.52. The recommendations of omega-6/omega-3 ratio in diet range from 4:0 to 5:1 in different countries [29]. The authors emphasize some aquatic animals are sources of very-long-chain acids. On the other hand, their essentiality is very dependent of individual metabolism, as well their health effects. As by-products from crocodilians, the Black caiman PC can be a nutritive source for other products, such as ingredient. For example, the waste from Pantanal caiman (*Caiman yacare*) was raw material for hamburgers made from minced meat [30]. In addition, the FA from waste can be evaluated in the biotechnology field concerning the oil encapsulation for health benefits, such as other fish oils.

4. Conclusion

The Black caiman PC contained omega 3 and 6. They can be considered as important nutritional components with clinical benefits to human health in future *in vivo* studies. The PC can be used as an ingredient for new food products such as dehydrated soups or an emulsifier. Further studies are necessary in order to determine digestibility, shelf life, and other functional properties of the products obtained for future applications.

Acknowledgement

This work was supported by the Amazonas State Research and Technology Support Foundation (FAPEAM).

References

[1] Palmeira, K.R., Marsico, E., Monteiro, M.L., Lemos, M., Junior, C.A.C. Ready-to-eat products elaborated with mechanically separated fish meat from waste processing: challenges and chemical quality. *Cyta. J. Food*, 14. 227-238. 2016.

[2] Da Silveira, R., Thorbjarnanarson, J. Conservation implications of commercial hunting of black and spectacled caiman in the Mamirauá Sustainable Development Reserve, Brazil. *Conservat. Biol*., 88:103-109. 1999.

[3] Rebouças, M.C., Rodrigues, M.C.P., Castro, R.J.S., Vieira, J.M.M. Characterization of fish protein concentrate obtained from the Nile tilapia filleting residues. *Semina*, 33. 697-704. 2012.

[4] Goes, E., Souza, M.L.R, Michka, J., Graton, M., Kimura K. S., Lara, .J. A. F., Delbem, A. C. B., & Gasparino, E. Fresh pasta enrichment with protein concentrate of tilapia: nutritional and sensory characteristic. *Food Sci. Technol*. 36(1). 76-82. 2016.

[5] Mohamed, G.F, Sulieman, A.M., Soliman, N.G., Bassiuny, S.S. Fortification of Biscuits with Fish Protein Concentrate. *World Journal of Dairy & Food Sciences*, 9. 242-249. 2014.

[6] Lourenço, L.F.H., Santos, D.C., Ribeiro, S.C.A., Almeida H., Araujo, E.A.F. Study of adsorption isotherm and microbiological quality of fish meal type "piracuí" of Acari- Bodo (*Liposarcus pardalis*). *Procedia - Food Science*, 1. 455-462. 2011.

[7] Nunes, E.S.C.L, Bittencourt, R. H. F. P. M., Silva, M. C., Marsico, E.T., Franco, R.M. Microbiological and physico-chemical qualities of dried-salted shrimp ("aviú") and of "piracuí" type fish meal sold in retail markets in Belém, Pará – Brazil. *Rev Inst Adolfo Lutz*, 72. 47-54. 2013.

[8] Kluczkovski Júnior, A., Kluczkovski, A. M., Moroni, F. T., Markendorf, F., Inhamuns, A. Carcass Yield and composition of *Melanosuchus niger*. *Intern. J. of Fisheries and Aquaculture*, 7. 47-53. 2015.

[9] Ross, J.P. (2000) *Melanosuchus niger*. The IUCN Red List of Threatened Species 2000.

[10] Romanelli, P.F., Schmidt, J. Estudo do aproveitamento das vísceras do jacaré do Pantanal (*Caiman crocodylus yacare*) em farinha de carne. *Ciênc.Tecnol.de Alimentos*, 23. 131-139. 2003.

[11] Bourre, J.M. Where to find omega-3 fatty acids and how feeding animals with diet enriched in omega-3 fatty acids to increase nutritional value of derived products for human: what is actually useful? *J Nut. Health Aging*, 9. 232-42. 2005.

[12] Scherr, C., Gagliardi, A.C. M., Miname, M.H., Santos, R.D. Fatty Acid and Cholesterol Concentrations in Usually Consumed Fish in Brazil. *Arq Bras. Cardiol*. 104. 152-158. 2015.

[13] Toupchian, O., Sotoudeh, G., Mansoori, A., Nasli-Esfahani, E., Djalali, M., Keshavarz, S. A., Koohdani, F. Effects of DHA-enriched fish oil on monocyte/macrophage activation marker sCD163, asymmetric dimethyl arginine, and insulin resistance in type 2 diabetic patients. *J. Clin. Lipid.* 10. 798-807. 2016.

[14] Meldrum S.J., D'Vaz, N., Casadio, Y., Dunstan, J.A., Niels Krogsgaard-Larsen, N., Simmer, K., Prescott, S.L. Determinants of DHA levels in early infancy: Differential effects of breast milk and direct fish oil supplementation. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* 86. 233-239. 2012.

[15] Simopoulos, A. An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. *Nutrients*, 8. 128. 2016.

[16] Simopoulos, A. The omega-6/omega-3 fatty acid ratio, genetic variation, and cardiovascular disease. *Asia Pac. J. Clin. Nutr*., 17. 131-134. 2008.

[17] Kluczkovski, A., Kluczkovski Junior, A. Aflatoxin in Fish Flour from the Amazon Region. Ariane M. Kluczkovski and Augusto Kluczkovski Junior. Aflatoxin in Fish Flour from the Amazon Region, Aflatoxins - Recent Advances and Future Prospects, Prof. Mehdi Razzaghi-Abyaneh (Ed.), InTech, 197-206. 2013.

[18] AOCS. American Oil Chemists` Society. Official Methods and Recommended Practices of the American Oil Chemits` Society. 5th ed. Champaign, USA, (Method Ce 1F 96). 1998.

[19] R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2015.

[20] Selmi, S., Bouriga, N., Cherif, M;, Toujani, M., Trabelsi, M. Effects of drying process on biochemical and microbiological quality of silverside (fish) Atherina lagunae. Int. J. of food Sci. and technology. *45 (6)*. 1161-1168. 2010.

[21] Kluczkovski Júnior, A., De Francisco, A., Kluczkovski, A. M., Beirão, L., Barbosa, H. Lipids of Amazon Caimans: a source of fatty acids. *African J. of Biotechn*. 15. 1559-1565. 2016.

[22] Fernandes, V.R. T., Souza, M. L. R., Gasparino, E., Coutinho, M. E., Visentainer, J. V., Goes, E. S. R. Several techniques for the preparation of flour from carcasses of the Pantanal alligator (*Caiman crocodilus yacare*). *Food Sci. Technol.*, 35. 487-492. 2015.

[23] Paulino, F.O., Silva T.J.P., Franco, R. M., Marsico, E.T., Canto, A.C.V.C., Vieira, J.P., Amaral AP, Pereira AAS (). Processing and quality characteristics of hamburger of Pantanal alligator meat (*Caiman crocodillus yacare*). *Rev. Bras. Cienc. Vet*. 18. 129-132. 2011.

[24] Petenucci, M. E., Stevanato, F. B., Morais, D. R., Santos, L. P., Souza, N. E., Visentainer, J. V. Composição e estabilidade lipídica da farinha de espinhaço de tilapia. *Ciênc. Agrotecnologia*, 34. 1279-1284. 2010.

[25] Lauritzen, L., Brambilla, P., Mazzochi, A., Harslof, L.B.S., Ciapolino, V., Agostini, C. DHA Effects in Brain Development and Function. *Nutrients*, 8. 3-17. 2016.

[26] FAO. Food and agriculture and Organization of the United Nations (1989). FAO: Conservation guide 22. The management of crocodiles in captivity.

[27] Tonial, I. B., Bravo, C. E. C., Souza, N. E., Matsushita, M., Furuya, W. M., Visentainer, J. V. Qualidade nutricional dos lipídios de tilápias (*Oreochromis niloticus*) alimentadas com ração suplementada com óleo de soja. *Alim. Nutr*., 22. 103-112. 2011.

[28] Souza, R. V., Santos, P. C. F., Bambirra, E. A., Vieira, E. C., Alvarez-Leite, J. I. Nutritional Characteristics of Amazonian Fish Fat (*Colossoma macropomum*) and its effect on lipid metabolism of rats fed hypercholesterolemic diets. *Ciênc. Tecnol. Aliment*. 22. 88-93. 2002.

[29] Martin, C.A., Almeida, V.V., Ruiz, M.R., Visentainer, J.E.L., Matshushita. M., Souza, N.E., Visentainer J.V. Omega-3 and omega-6 polyunsaturated fatty acids: importance and occurrence in foods. *Rev. Nutr.*, 19, 761-770. 2006.

[30] Fernandes V.R.T., Franco, M.L.R.S., Gasparino, E., Tanamati, A., Coutinho, M.E., Bielawsk,i K.. Hamburgers from pantanal caiman meat (*Caiman yacare*) subjected to different smoking technique. *Arq. Bras. Med. Vet. Zootec*. 65. 927-933. 2013.