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Full Paper

Oil in Inajá Pulp (*Maximiliana maripa*): Fatty Acid Profile and Anti-acetylcholinesterase Activity

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Abstract: The inajá (*Maximiliana maripa*) is a palm from Arecaceae family. The inajá distribution occurs from Amazon to the west-central of South America. The inajá has edible parts: the palm heart and oils obtained from pulp and seed. The aims of this study were to verify the fatty acid profile from pulp oil by GC-FID and analyzing acetylcholinesterase inhibition by inajá pulp oil. Chromatographic analysis provided eleven fatty acids. The major unsaturated fatty acids are oleic (22.32%), linoleic (4.72%) and linolenic acids (3.95%). The major saturated fatty acids are palmitic (20.76%), myristic (20.48%) and lauric acids (17.42%). The acetylcholinesterase inhibition by inajá oil pulp was over 63.76%.

Keywords: fatty acids; SFA; UFA; PUFA; anti-acetylcholinesterase

1. INTRODUCTION

The inajá palm (*Maximiliana maripa*) is from Arecaceae family. It is considered a large plant (can reach up to 20 meters in height) and its distribution occurs in flooded areas [1]. This palm is very hardy, regenerates quickly in the environments where it is burned [2, 3].

Geographically, the inajá palm is widely distributed throughout the Amazon to west-central South America. It is easily in the central and northern regions of Brazil [3]. In the state of Roraima, Brazil, which borders the north of Venezuela, there is a significant population of this palm in these areas of savannah.

Oil is extracted from *M. maripa* seed and pulp for human consumption [3 ,4]. The inajá fruit is rich in fatty acids and minerals (phosphorus and magnesium) [5], and has interest as biofuel in the process of obtaining biodiesel [6].

The aims of this study is to verify the fatty acid profile in inajá pulp collected in Roraima state, Brazil, as well as analyzing their acetylcholinesterase enzyme inhibition.

2. MATERIAL AND METHODS

2.1 Obtaining Fruit and Extracting Oil from Inajá Pulp

The fruits were obtained in Mucajaí (Roraima,

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Brazil), in Tantinho region, at 451 miles on the BR 174 highway (2º27'44" N, 60º55'10" W). Samples were taken to the Laboratory of Environmental Chemistry in the Center for Research and Post-Graduate in Science and Technology (NPPGCT) of the Federal University of Roraima, Boa Vista city, Roraima.

The pulp was removed, washed and dried for 24 hours at 50 °C in an oven with air circulation. Pulp was grounded and sieved on a 20 to 40 mesh fabric to obtain a homogeneous granulation. Lipid extraction gave Soxhlet apparatus with hexane solvent, any procedure effected in triplicate [7].

2.2 Inajá Oil Analysis by GC-FID

Were dissolved in 2.0 mL cryogenic tube approximately 12 mg of the oil sample in 100 µL of a solution of ethanol (95%) / potassium hydroxide mol L^{-1} (5%). After vortexing for 10 s, oil was hydrolyzed in a domestic microwave oven (Panasonic Piccolo), at a power of 80W for 5 minutes. After cooling, 400 µL of hydrochloric acid 20% was added a spatula tip of NaCl (about 20 mg) and 600 µL of ethyl acetate. After vortexing for 10 s rest for 5 minutes. An aliquot of 300 µL of the organic layer was removed, placed into micro centrifuge tubes, dried by evaporation, thus obtaining free fatty acids [8]. Subsequently, the free fatty acids were methylated with 100 µL of BF_3 / methanol (14%), by heating for 10 minutes in water bath at 60 °C. These samples were diluted in 400 µL of methanol and analyzed by gas chromatography.

The analyzes were performed on a HP-7820A chromatograph (Agilent) equipped with a gas flame ionization detector. As data acquisition program was used, EZChrom Elite Compact (Agilent). HP-INNOWax column 15 m x 0.25 mm x 0.20 µm with temperature gradient was used: 120 °C, 0 min, 7 ºC min^{-1} to 240 °C; injector (Split 1/50) detector at 250 °C and 260 °C. Hydrogen was used as carrier gas (3.0 mL min^{-1}), Injection volume 1µL. Identification of the peaks was performed by comparison with standards of methylated fatty acids C14-C22 FAME (Supelcocat no 18917) [8].

2.3 Acetylcholinesterase Inhibition Assay

Aliquots of a working solution (25 µL) (sample in DMSO 10 mg mL^{-1}) were added to microplate wells and positive and negative controls were also prepared. To the first five wells of a column (positive control) 25 µL of an eserine solution prepared at 10 mg mL^{-1} (31 mM; 2.7 mM in the whole reaction mixture 275 µL) in Tris/HCl at pH 8.0) was added. Then, 25 µL of acetylthiocholine iodide (ATChI, Sigma A5751) 15 mM; the reaction mixture, 125 µL of 5',5-dithio-bis (2- nitrobenzoate) (DTNB, Sigma D8130) (3 mM) and 50 µL of Tris/HCl (50 mM, pH 8) containing 0.1% (m/v) bovine serum albumin was added to each well. Absorbance was measured at 405 nm every 1 min for 8 times. Then 25 µL (0.226 U mL^{-1}) of Electric eel AChE (type VI-S) provided by Sigma (C3389-500UN) in Tris/HCl was added to each well. Absorbance was measured at 405 nm by 10 times [9,10].

3. RESULTS AND DISCUSSION

3.1 Fatty Acids Determination in Inajá Pulp

The GC-FID analysis provided 11 fatty acids (94.01%), as shown below in the chromatogram of Figure 1 and in Table 1.

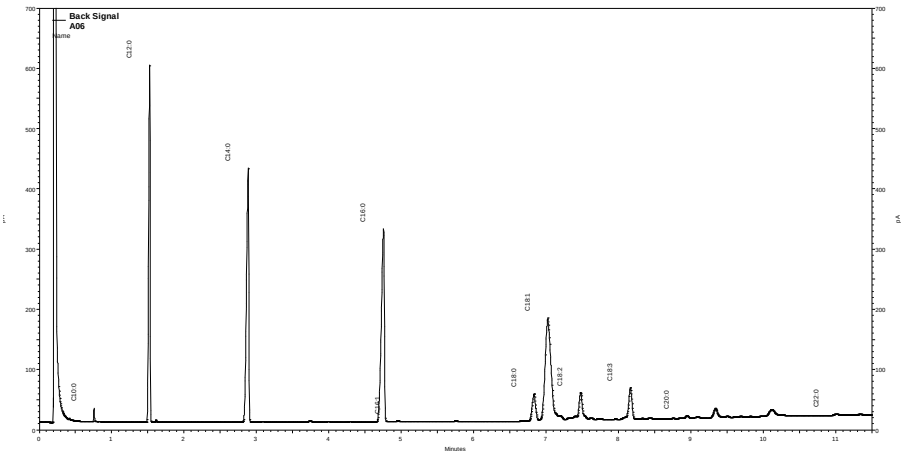

Figure 1. Inajá pulp oil chromatogram.

Note that the unsaturated fatty acids, UFA, predominate in the inajá pulp oil studies of Roraima are oleic, linoleic and linolenic acids (Table 1). The oleic acid which is found in greater proportion in the studied sample (22.32%).

Table 1. Fatty acids in inajá pulp oil.

	Sample		Compared with the literature		
Fatty acid	**Retencion Time (min)**	**Amount (%)**	**Inajá [11]**	**Tucumã [12]**	**Palma Yagua [13]**
Capric acid (C10:0)	0.761	0.38	-	1.0	-
Lauric acid (C12:0)	1.563	17.42	4.6	0.1	0.5
Myristic acid (C14:0)	2.862	20.48	10.7	0.2	0.3
Palmitic acid (C16:0)	4.742	20.76	25.1	22.6	22.2
Palmitoleic acid (C16:1)	4.903	0.24	0.3	0.4	-
Stearic acid (C18:0)	6.867	3.40	1.6	3.0	2.6
Oleic acid (ω-9) (C18:1)	7.071	22.32	39.2	64.7	64.0
Linoleic acid (ω-6) (C18:2)	7.562	4.72	12.9	4.7	2.6
Linolenic acid (ω-3) (C18:3)	8.197	3.95	1.5	3.6	7.3
Arachidic acid (C20:0)	8.977	0.34	1.3	0.2	0.2
Behenic acid (22:0)	11.040	-	-	-	-
Saturated		62.78	43.3	27.1	25.8
Monounsaturated		22.56	39.5	65.1	64.0
Polyunsaturated		8.67	14.4	8.3	9.9

This acid is with values close to the inajá and other fruits of the Amazon region, as shown in Table 1. To saturated fatty acids, SFA (62.78%), the most abundant in this oil are palmitic (20.76%), myristic (20.48%) and lauric acids (17.42%). There is a difference of lauric and myristic sample studies compared with inajá of literature, but the values are close to as palmitic acid, even to other oleaginous species. The same applies to the unsaturated, UFA (22.56%), note the ω-9 and ω-6 sample and literature, while the ω-3 there is a slight difference. The amount of polyunsaturated fatty acids, PUFAs, differs from inajá of literature, but it is close to other species in Table 1. According to Crowley and Fröhlich (1998) [14] differences may occur due biotic and abiotic factors that influence the composition and concentration of fatty acids.

Table 2 provides a comparison of the main constituent of olive oil, oleic acid [15]. The amount of oleic acid inajá pulp lipid is similar to that found in cocoa butter and higher than coconut oil [16, 17].

Table 2. Profile main fatty acids in edible oils and fat.

%	**ω-9**	**ω-6**	**ω-3**
Olive oil [15]	67.0-81.0	3.5 -14.0	0.3-1.2
Cacao butter [16]	26.3	41.0	0.34
Coconut oil [17]	5.0-10.0	1.0-2.5	0.2

Observed that the percentage of linoleic acid in the inajá pulp is 4.72% and 3.95% for linolenic acid, found in higher concentrations than in coconut oil [17].

Fatty acids when in equilibrium in the diet have benefits for human health by preventing heart disease, inflammation and even neurodegenerative diseases. Some fatty acids such as linoleic and linolenic acids, mammals do not synthesize and can only be obtained through diet [18, 19].

3.2 Inhibition of Acetylcholinesterase

The test results for acetylcholinesterase enzyme inhibition by inajá pulp oil is considered potent, as Table 3. This classification is given by Vinutha et al. (2007) [20] in which higher than 50% inhibitions are potent and inhibition values between 30-50% are moderate and below 30% are weak inhibitors.

Table 3. Percentage inhibition of acetylcholinesterase and its classification.

	% Inhibition	**Classification**
Inajá	63.76	Potent

This enzyme has biochemical importance in humans, but its sudden increase may develop neurodegenerative diseases, one of them is Alzheimer disease [20, 21].

The inajá pulp oil could be beneficial for society because according to the World Health Organization estimated about 115 million people develop Alzheimer's disease by 2050 [22]. Other Amazonian plants or found in the Brazilian Amazon have equal importance, namely: *Combretum laurifolium* (crude extract) [23], *Lantana camara* (essential oil) [24] and *Annona hypoglauca* (oil) [7].

4. CONCLUSION

The oil from the inajá pulp has a fatty acid profile similar to other oils or fats, so its lipid content can bring many benefits to human health. Thus, we can see a potent inhibition of acetylcholinesterase, almost 64%. The good results obtained of the fatty acid profile from inajá suggests future studies of chemical and biological prospecting for this plant species with the intention of developing bio products for human health.

5. ACKNOWLEDGEMENTS

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6. REFERENCES AND NOTES

[1] Miranda, I. P. A.; Rabelo, A.; Bueno, C. R.; Barbosa, E. M.; Ribeiro, M. N. S.; Frutos de palmeiras da Amazônia, 19th ed. Manaus: MCT/INPA, 2001.

[2] Douglas, C. D.; Krukoff, B. A.; Silveira, M.; Floristics and Economic Botany of Acre, Brazil. Amazonian Botany, 2th ed. New York: The New York Botanical Garden, 2002.

[3] Shanley, P.; Cymerys, M.; Serra, M.; Medina, G.; Frutales y plantas útiles en la vida amazónica. Productos Forestales no Madereros 20. Organización de las Naciones Unidas para la Alimentación y la Agricultura, el Centro para la Investigación Forestal Internacional y Pueblos y Plantas Internacional – FAO, CIFOR y PPI, 2012.

[4] Lorenzi, H.; Souza, H. M.; Medeiros-Costa, J. T.; Cerqueira, L. S. C.; Behr, N.; Palmeiras no Brasil: nativas e exóticas, 1th ed. Nova Odessa: Plantarum, 1996.

[5] Bezerra, V. S.; Ferreira, L. A. M.; Pereira, S. S. C.; Carim, M. J. V. O inajá (*Maximiliana maripa* (Aubl.) Drude) como potencial alimentar e oleaginoso. In: Congresso Brasileiro de Plantas Oleaginosas, Óleos, Gorduras e Biodiesel, 3., 2006, Varginha. **Artigos**.... Varginha: UFLA, 2006. p. 301-305.

[6] Mota, R. V.; França, L. F. *Rev. Cient. UFPA* **2007**, *6*. [Link].

[7] Santos, R. C.; Melo Filho, A. A.; Chagas, E. A.; Takahaski, J. A.; Ferraz, V. P.; Costa, A. K.; Melo, A. C. G. R.; Montero, I. F.; Ribeiro, P. R. E. *Afr. J. Biotechnol.* **2015**, *14*, 30. [CrossRef]

[8] Christie W. W. Gas Chromatography and Lipids: A Practical Guide. Ayr: The Oil Press, 1989.

[9] Frank, B.; Gupta, S. *Ann. Clin. Psychiatry* **2005**, *17*, 269. [PubMed] [CrossRef]

[10] Ellman, G. L.; Courtney, K. D.; Andres Jr., V.; Featherstone, R. M. *Biochem. Pharmacol*. **1961**, *7*, 88. [CrossRef]

[11] Santos, M. F. G.; Marmesat, S.; Brito, E. S.; Alves, R. E.; Dobarganes, N. C. *Grasas Aceites*. **2013**, *64*, 328. [CrossRef]

[12] Mambrin, M. C. T.; Barrera-Arellano, D. *Grasas Aceites* **1997**, *48*, 154. [CrossRef]

[13] Cordero, J.; Alemán, W.; Torrellas, F.; Ruiz, R.; Nouel, G.; Sousa, N. M.; Espejo, M.; Sánchez, R.; Molina, E. *Bioagro* **2009**, *21*, 49.

[14] Crowley, J. G.; Fröhlich, A.; Factors affecting the composition and use of Camelina. Dublin: Teagasc, 1998.

[15] Yúfera, E. P., Química Agrícola III Alimentos, 1th ed. Granada: Editorial Alhambra, 1979.

[16] Lipp, M.; Anklam, E. *Food Chem.* **1998**, *62*, 73. [CrossRef]

[17] Moigradean, D.; Poiana, M.-A.; Alda, L.-M.; Gogoasa, I. *J. Agroaliment. Proc. Technol.* **2013**, *19*, 459.

[18] Simopoulos, A. P. *Am. J. Clin. Nutr.* **1999**, *70* (suppl), 560S.

[19] Tan, L. C.; Methawasin, K.; Tan, E.-K.; Tan, J. H.; Au, W.-L.; Yuan, J.-M.; Koh, W.-P. *J. Neurol. Neurosurg. Psychiatry* **2016**, *87*, 86. [CrossRef]

[20] Vinutha B.; Prashanth D.; Salma K.; Sreeja S. L.; Pratiti D.; Padmaja R.; Radhika S.; Amit A.; Venkateshwarlu K.; Deepak M. *J. Ethnopharmacol*. **2007**, *109*, 359. [PubMed] [CrossRef]

[21] Trevisan, M. T. S.; Macedo, F. V. V.; Meent, M. V.; Rhee, I. K.; Verpoorte, R. *Quím. Nova* **2003**, *26*, 301. [CrossRef]

[22] WHO, World Health Organization (2012). World Health Organization and Alzheimer's Disease International. Dementia: a public health priority. 2012, pp. 112. [Link]

[23] Fernández, I. M.; Silva, F. S.; Costa, H. N. R.; Melo Filho, A. A.; Santos, R. C.; Costa, G. V.; Lima, C. A. C. *Orbital: Electron. J. Chem.* **2014**, *6*, 233.

[24] Santos, R. C.; Melo Filho, A. A.; Chagas, E. A.; Takahashi, J. A.; Ferraz, V. P.; Fernández, I. M.; Ribeiro, P. R. E.; Melo, A. C. G. R.; Holanda, L. C. *J. Med. Plants Res.* **2015**, *9*, 922. [CrossRef]