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# Fatty Acids, Physical-Chemical Properties, Minerals, Total Phenols and Anti-Acetylcholinesterase of *Abiu* Seed Oil

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The aim of this work is to perform analysis of the oil of the seed of *abiu* (*Pouteria caimito*) the presence of minerals (ICP-OES), fatty acids (GC-FID), physical-chemical properties ($^1$H NMR) and acetylcholinesterase activity (AChE). Thus, the following results were obtained for the majority fatty acids saturated: palmitic acid (27.3%), and unsaturated: oleic acid (43.1%), linoleic acid (8.6%), $\alpha$-linolenic acid (0.4%), and $\gamma$-linolenic acid (0.4%). The physical-chemical properties of this oil were: iodine index (42.72 mg $I_2$ $g^{-1}$), saponification index (230.87 mg KOH $g^{-1}$), acid number (1.50 mg KOH $g^{-1}$) and the average molecular weight (710.54 g $mol^{-1}$). For minerals: phosphorus (8.62 mg $100g^{-1}$), calcium (3.50 mg $100g^{-1}$), sodium (3.40 mg $100g^{-1}$), potassium (2.62 mg $100g^{-1}$), magnesium (2.29 mg $100g^{-1}$), sulfur (1.54 mg 100 $g^{-1}$). The oil of the *abiu* seed had 68.40% inhibition on AChE and was therefore considered a potent inhibitor and the oil of *abiu* presents 35.47 mg EAG. 100 $g^{-1}$ in its composition. The *abiu* is a tree that belongs to the Sapoteaceae family, originating in the Amazon region near the Andean slopes of Peru and the western Brazilian Amazon, easily found in the wild. The *abiu* is closely tropical or subtropical, adapts to the hot and humid climate, and presents better development conditions when it is located near the equator.

## 1. Introduction

The Amazon is the largest tropical forest and the largest genetic reserve on the planet. Its area covers several countries in South America, and in Brazil its area extends to six Brazilian states (Pará, Amazonas, Acre, Amapá, Rondônia and Roraima) and part of three states (Maranhão, Tocantins and Mato Grosso), which represents about half of the national territory, thus, there are a large variety of plant species, among these many are fruit, namely the *abiu* (*Pouteria caimito*) (MMA, 2002; Almeida et al., 2009; Bogusz Junior et al., 2012). The *abiu* is a fruit species of the Sapotaceae family of origin in Alto Solimões, Brazil, near the border with Peru, it is cultivated throughout Amazon and has popular names *abiu*, *abiurana*, *caimito*, *caimo*, *maduraverde* (Spanish) and egg fruit (Ferreira and Ribeiro, 2006; Virgolin, 2015). The distribution of *abiu* takes place in tropical or subtropical regions, very well adapted in humid hot regions and the different types of soil of Brazil, but its better development occurs in clayey soils and rich in organic matter (Lorenzi, 2006). The *abiu* presents fruits of yellow color, with a small greenish area, its pulp is gelatinous and, slightly whitish, sweet flavor and presents, on average, four black seeds (Figure 1). This fruit can be consumed *in natura* or in the form of products like sweets, liqueurs, jellies, ice cream, etc (Ferreira and Ribeiro, 2006; Virgolin, 2015). The objective of this work is to analyze the oil of the *Pouteria caimito* seeds in relation to the presence of

minerals by ICP-OES, fatty acid profile by GC-FID, physical-chemical properties by $^{1}$H NMR, total phenolic compounds and bioactivity on acetylcholinesterase.

(a) (b) (c) (d)

*Figure 1: Leaves and flowers (a), Tree (b), closed fruits (c) and opened and closed abiu fruits (d). Pictures by Ana Marcia D. C. da Costa.*

## 2. Materials and methods

### 2.1 Sample preparation

The *abiu* ripe fruits were collected in urban area of the Boa Vista city, Roraima state, Brazil. Samples were taken to the Environmental Chemistry Laboratory of the PPGQ/UFRR. The seeds were dried in an oven with circulating air at 50 °C for 72 h. Then, the plant materials were milled, sieved and was extracted from hexane in a Soxhlet extractor, to obtain the crude oil. Solvent was removed on a rotoevaporator, the extracts were placed in amber bottles under nitrogen atmosphere until further analysis (Santos et al., 2015).

### 2.2 *Abiu* Oil analysis by GC-FID

The chemical composition of *abiu* seed oil by GC in the Chromatography Laboratory from UFMG, where hydrolysis and methylation of the oil were made, in cryogenic tube (2 mL), ~12 mg of the oil sample in 100 µL of ethanol (95%)/1 mol $L^{-1}$ potassium hydroxide (5%) solution was dissolved. Vortex agitation for 10 s, the oil was hydrolyzed in domestic microwave oven (Panasonic Piccolo) at power of 80 W (Power 2) for 5 min. After cooling, 400 µL of hydrochloric acid (20%), one tip of NaCl spatula (~20 mg) and 600 µL of ethyl acetate were added. After vortex agitation for 10 s and standing for 5 min, an aliquot of 300 µL of the organic layer was removed, placed in microcentrifuge tubes and dried by evaporation (Adapted from W. W. Christie). The free fatty acids were methylated with 100 µL $BF_3$/methanol (14%) by heating for 10 min at 60 °C water bath. Then, they were diluted in 400 µL methanol and analyzed by GC. Aiming at the determination of fatty acids by GC, the analyzes were conducted on a HP7820A (Agilent) GC-FID. EZChrom Elite Compact (Agilent) Data Acquisition Program (Agilent). Column of 15 m x 0.22 mm x 0.20 mm (SGE) was used with temperature gradient: 80 °C, 0 min, 7 °C $min^{-1}$ up to 220 °C; injector (split of 1/50) at 250 ºC and detector at 260 ºC. Hydrogen as entrainment gas (3.0 mL $min^{-1}$) and injection volume of 1 µL. Peak identification was done by comparison with FAME $C_{14}$-$C_{22}$ methylated fatty acid standards (Supelco cat. No. 18917).

### 2.3 Determination of total phenolic compounds

Through this test, known as the Folin-Ciocalteau assay, it is possible to quantify the total phenolic compounds in the sample according to the methodology proposed by (Singleton et al., 1999). The Folin reagent, which is a mixture of phosphotustene and phosphomolybdene salts, yellow color, will react with the phenolic compounds present in the sample, giving a blue coloration depending on the amount of phenolic compounds present in the sample. The reading is performed by ultraviolet-visible molecular spectrophotometry at 765 nm using gallic acid as a standard, preparing dilutions from a concentration of 100 mg $L^{-1}$.

### 2.4 Physicochemical Properties by $^{1}$H NMR spectra

The *abiu* oil was solubilized in 0.6 mL of deuterated chloroform, using trimethylsilane as an internal standard and its spectrum was obtained by $^{1}$H NMR (500 MHz) of 11.7 Tesla from the University of São Paulo (USP), under the following conditions: for the $^{1}$H NMR the following acquisition parameters were used: pulse: 30°, relaxation time: 1s, acquisition time: 3,276 s, scanning width: 10,000 Hz, Line width: 0.152 Hz, 128 replicates were accumulated for each free induced decay with a total time of 13.21 s. To analyze the $^{1}$H NMR spectrum and signal integrations, SpinWork 4.2.0 free software was used. To determine the physicochemical properties value by means of $^{1}$H NMR according to Reda (2004) and Reda and Carneiro (2006).

### 2.5 Individually Coupled Plasma Optical Emission Spectrometry (ICP-OES)

The samples were digested using concentrated nitric acid and 30% hydrogen peroxide, with microwave oven heating. The identification and quantification of the minerals were performed using the ICP-OES brand Spectro, model Arco, of the University of São Paulo, under the following conditions: power applied 1400 W, RF generator frequency 27.12 MHz, plasma gas flow 12 L $min^{-1}$, auxiliary gas flow 1 L $min^{-1}$, nebulization gas flow 0.85 L $min^{-1}$, sample 0.85 L $min^{-1}$, pump speed 30 RPM.

### 2.6 Anti-acetylcholinesterase 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 (Frank and Gupta, 2005; Ellman et al., 1961).

## 3. Results and Discussion

### 3.1 The yield of the *abiu* seeds oil and profile of fatty acids by GC-FID

The yield of the *abiu* seeds oil was calculated from the ratio of the arithmetic mean of oil mass extracted from the three samples by the arithmetic mean of seeds mass of the fruit. Obtaining a yield of 14.01%.
In Table 1 a fatty acid profile of the above mentioned oil of *abiu.* Fatty acids are of great importance in the human diet, especially unsaturated fatty acids such as linolenic, linoleic and oleic acids (omega 3, 6 and 9, respectively). Oleic acid (or omega 9) is the majority (43.1%) among all fatty acids (Table 1), and is the most common among vegetable oils, such as olive oil (Guillén and Ruiz, 2003). Omega 9 is beneficial for several diseases: cancer, autoimmune diseases, rheumatic, anti-inflammatory, antidiabetic, among many others (Sales-Campos et al., 2013; Lou-Bonafonte et al., 2012; Carrillo et al., 2012; Pauwels, 2011; Bermudez et al. 2011; Vassiliou et al., 2009; Sales et al., 2009; Colomer and Menéndez, 2008; Menendez and Lupu, 2006). Tha concentration of γ-linolenic acid (ω-6) is a polyunsaturated fatty acid of the omega-6 family, found in human milk and in various seed oils used as a dietary supplement (Sergeant et al., 2016). The content of ω-6 was of 0.4% and your isomer α-linolenic acid, ω-3, (0.4%). The ω-6 helps in the nervous function and the prevention of diseases in the nervous cortex, in people suffering from diabetes, in addition to being beneficial in the case of pathologies such as "dry eye" (Guiné and Henriques, 2011) and ω-3 a polyunsaturated fatty acid shown in many clinical studies to attenuate inflammatory responses (Hou et al., 2016). The ratio ω-6/ω-3 in the *abiu* seed oil was calculated with the following result: 21.75% the ratio between ω-6 and ω-3. According to Martin (2006), the ratios of 2:1 to 3:1 have been recommended by some authors.

*Table 1: Profile of fatty acids in abiu seed oil.*

| Common Name | Composition | RT(min) | % |
|---|---|---|---|
| Lauric acid | C12:0 | 5.1 | 0.3 |
| Myristic acid | C14:0 | 7.8 | 0.8 |
| Palmitic acid Margaric acid | C16:0 | 10.4 | 27.3 |
| Margaric acid | C17:0 | 1.6 | 0.2 |
| Stearic acid | C18:0 | 12.8 | 6.2 |
| Oleic acid (ω-9) | C18:1 | 13.0 | 43.1 |
| Linoleic acid (ω-6) | C18:2 | 13.5 | 8.6 |
| γ-Linolenic acid (GLA, ω-6) | C18:3 | 13.9 | 0.4 |
| α-Linolenic acid (ALA, ω-3) | C18:3 | 14.2 | 0.4 |
| Eicosadienoic acid | C20:2 | 15.1 | 0.7 |
| Behenic acid | C22:0 | 17.2 | 0.2 |
| Others | - | - | 11.8 |
| Σ Saturated (SFA) | - | - | 35 |
| Σ Unsaturated (UFA) | - | - | 53.2 |
| Σ Monounsaturated (MUFA) | - | - | 43.1 |
| Σ Polyunsaturated (PUFA) | - | - | 10.1 |
| Ratio ω-6⁄ω-3 | - | - | 21.75 |

* Retention time

### 3.2 Total phenolic compounds

The calibration curve obtained from the gallic acid pattern gave an equation of the line of y = 0.0193 x + 0.0459 $r^2$ = 0.996. The oil of the *abiu* seed, obtains concentration of phenolic composts in its composition of 35.46 mg EAG 100$g^{-1}$. Rufino et al. (2010) suggest that the classification of total phenol content varies as follows in fresh materials: low (<100 mg EAG 100 $g^{-1}$), medium (100-500 mg EAG 100 $g^{-1}$) and high (> 500 mg EAG 100 $g^{-1}$). The oil of the *abiu* seed can be considered as an extract of low teor of total phenol, because presented the result below of 100 mg EAG 100 $g^{-1}$.

### 3.3 Physicochemical Properties by $^1$H NMR spectrum

With the use of the values of the chemical shifts can calculate important information on the physicochemical and chemical properties as iodine value (IV), saponification index (SI), acidity index (AI), molecular mass (MM) and the relation of oleophilic/aliphatic hydrogen $R_{O,A}$ (Table 2), without the need to use reagents.

*Table 2. Physicochemical and chemical characterization of abiu oil.*

| Physical-Chemical Parameters | IV (mg $I_2$ $g^{-1}$) | SI (mg KOH $g^{-1}$) | AI (mg KOH $g^{-1}$) | MM (g $Mol^{-1}$) | $R_{O,A}$ |
|---|---|---|---|---|---|
| *Abiu* | 42.72 | 230.87 | 1.51 | 710.54 | 0.34 |

The IV indicates the degree of establishment of the fatty acids present in vegetable oils, thus, the greater the degree of saturation of an oil, the use becomes improper for human consumption (Reda and Carneiro, 2006). The oil of the abiu seed had an IV of 42.72 mg $I_2$ 100 $g^{-1}$. Through Table 3, the value found for the iodine content of the *abiu* seed oil is lower than the oil of the Crimson Sweet watermelon seeds studied by Ávila (2012). The oils studied had a saturation content lower than the unsaturation content.

The SI is intended to indicate if the oil can be consumed by humans, since the higher the SI the greater the composition of free fatty acids, thus the greater its purpose for human consumption (Solomos, 2012; Moretto and Fett, 1998). The *abiu* seed oil presented saponification index had a low degree of deterioration (Table 3), whose value was a little higher than the one compared to the watermelon (Table 3). The fact that the *abiu* seed oil presents a higher saponification index than the literature comparison can be explained because this physicochemical parameter is inversely proportional to the molecular mass (Moretto and Fett, 1998).

For Reda and Carneiro (2005), the value of the $R_{O,A}$ ratio should be greater than or equal to 0.66 indicates that the vegetable oil is suitable for human consumption. This parameter needs to be calculated to obtain the acid value of a vegetable oil. The oil of the *abiu* seed presented this value is 0.34. From Table 3, the value found for the $R_{O,A}$ that of the seed oil of the *abiu* the value is lower than the value found for the Crimson Sweet watermelon oil from Ávila (2012) and higher than the value found for the oil of *andiroba* from Farias (2013). The value found indicates that the oil of the *abiu* seed cannot be destined for human consumption.

### 3.4 Quantification and Identification of Minerals Constituents by ICP-OES

The increasing order of minerals in *abiu* oil is as follows: P, Ca, Na, K, Mg and S. Made analysis of the oil of *abiu*, was detected the high concentration of the major minerals, in which according to Wills et al. (1998) is necessary as plant nutrients for its maintenance and its development. In addition to serving as nutrition for plants, minerals can be used in food and feed (Fioniri, 2008; Cozzolino, 2007; Cozzolino, 1997).

The minerals constituents in the *abiu* oil were quantified, being all of them the major one the phosphorus with concentration of 86.19 ± 0.73 mg 100 $g^{-1}$. Phosphorus is a major component of the ATP molecule, with essential role in photosynthesis, respiration, sugar metabolism, cellular respiration, and information genetics, being important in plant growth Marschner (1995).

The next important element is calcium, with 3.50 ± 0.21 mg 100 $g^{-1}$. Calcium is important, because it is involved in addition to the processes of bone formation, in the transport of cell membranes, activation or release of enzymes and transmission of nerve impulses Bueno and Czepielewski (2007) and close to Ca, is Na with concentration of 3.40 ± 0.74 mg 100 $g^{-1}$. Sodium is important in the balance and functioning of muscles and contraction of blood vessels Alveranga (2011).

Potassium is at a concentration of 2.62 ± 0.53 mg 100 $g^{-1}$and the magnesium concentration is 2.29 ± 0.67 mg 100 $g^{-1}$, with magnesium being important against heart disease, promoting the functioning of the heart besides participating in more than 300 enzymatic systems Alveranga (2010). Of all of them, the element found in lower concentration is the sulfur with concentrations of 1.54 ± 0.09 mg 100 $g^{-1}$.

### 3.5 Anti-acetylcholinesterase assay

The oil of the *abiu* seed had 68.40% inhibition on Acetylcholinesterase (AChE) and was therefore considered a potent inhibitor according to Vinutha et al. (2007), because it is above 50% inhibition of the enzyme, and for

weak inhibitors, below 30%, and moderate, between 30-50%. AChE is of great importance in the human body, is an enzyme responsible for the transmission of impulses in cholinergic synapses and thus hydrolyze the neurotransmitter acetylcholine acetate and choline (Čolović et al., 2013). Change the pathway that can generate neurodegenerative diseases, the most common and is increasing alarmingly in the world is Alzheimer's disease (AD). According to the World Health Organization (WHO, 2012) the Alzheimer's disease will develop in more than 115 million people by 2050. Some authors, such as Santos (2016) and Trevisan and Macedo (2003) made bioprospecting of plants from the Amazon region and parts of Brazil, respectively.

## 4. Conclusion

The oil yield of the *abiu* seeds was about 14.01% and in its chemical composition indicates a higher quantity of UFA (52.10%), oleic acid (43.1%) being the major of all identified fatty acids. The SFA was 47% and its major acid was palmitic (27.3%). The presence of unsaturated as the majority can be confirmed by the attributions provided by the Infrared spectrum, which are characteristic. As for mineral composition in the *abiu* oil, it was observed that the majorities were: P (8.62 ± 0.07) mg $100g^{-1}$; Ca (3.50 ± 0.21) mg $100g^{-1}$; Na (3.40 ± 0.74) mg $100g^{-1}$; K (2.62 ± 0.53) mg $100g^{-1}$; Mg (2.29 ± 0.67) mg $100g^{-1}$ and S (1.54 ± 0.09) mg $100g^{-1}$. Moreover, the physicochemical properties of the oil of the *abiu* seeds presented good results in their indexes. Presented in its bioactivity a potent of acetylcholinesterase, about 68%.

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