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## EXTRACTION AND CHARACTERIZATION OF FATTY ACIDS IN CARNAÚBA SEED OIL<sup>1</sup>

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**ABSTRACT** - This paper describes the composition of fatty acids in oil extracted from seeds of carnaúba (*Copernicia prunifera* (Miller) H. E. Moore), an important palm species native to Northeastern Brazil. After extracting the crude oil, the physico-chemical characteristics (density, refraction index, pH, acidity and saponification index) were registered and the chemical composition of the fatty acids was determined by gas chromatography (GC-FID). The predominance of saturated fatty acids does not make carnaúba seed oil a promising alternative for the food industry, and the small yield obtained (approx. 5%) may constitute a limiting factor for commercial exploitation, but carnaúba seed oil could potentially be used in the production of biofuels, cosmetics and detergents.

**Keywords:** *Copernicia prunifera*. Vegetable oil. Fatty acids. Gas Chromatography.

## EXTRAÇÃO E CARACTERIZAÇÃO DOS ÁCIDOS GRAXOS DO ÓLEO DE SEMENTES DA CARNAÚBA

**RESUMO** - Este trabalho descreve a composição centesimal de ácidos graxos do óleo de sementes da carnaúba (*Copernicia prunifera* (Miller) H. E. Moore), uma importante espécie de palmeira nativa do nordeste do Brasil. Após a extração do óleo bruto, as características (densidade, índice de refração, pH, acidez e índice de saponificação) foram determinadas e a composição química de ácidos graxos foi determinada por cromatografia de gás (GC-FID). A predominância de ácidos graxos saturados não sugere que o óleo de carnaúba seja uma alternativa promissora para indústria de alimentos, e o baixo rendimento obtido (aprox. 5 %) pode constituir um fator limitante para exploração comercial, mas o óleo de semente de carnaúba poderia potencialmente ser usado na produção de biocombustíveis, detergentes e cosméticos.

**Palavras-chave:** *Copernicia prunifera*. Óleo vegetal. Ácidos graxos. Cromatografia gasosa.

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## INTRODUCTION

With over 40,000 species of plants, representing 20% of the world flora, the Brazilian flora is one of the most diversified in the world. However, this vegetable diversity is far from optimally exploited, in part due to historical circumstances (OLIVEIRA et al., 2012).

A number of Brazilian plant species have been shown to possess important bioactive properties. Much research has been done worldwide to evaluate the ability of extracts from such plants to prevent chronic diseases in humans (AYRES et al., 2008; RUFINO et al., 2010; BOEING et al., 2012). For example, the pulp of green fruit of carnauba palm, boiled to remove its astringent taste was considered good and healthy, especially when eaten with milk. It is rich in bioactive compounds and antioxidant (RUFINO et al., 2011; OLIVEIRA et al., 2012).

Upon his visit to Ceará in the first half of the nineteenth century, the British botanist George Gardner described the landscape along the Jaguaribe river, with special attention to the native vegetation of which the carnaúba palm (*Copernicia prunifera* (Miller) H. E. Moore) is a conspicuous component. Gardner was impressed with the extensive carnaúba groves he beheld and made copious notes about the use of this palm by the local population as a primary means of adjustment to the environment in the form of construction materials, domestic utensils (hats, wickerwork, carpets etc.), wax and fodder for livestock (REIS et al., 2011; PEREIRA et al., 2014).

The carnaúba belongs to the class of monocotyledons. A xerophyte, it is endemic and native to Northeastern Brazil, a region with the insolation required for the species to thrive. A member of the family *Palmae*, which includes other economically important species such as the coconut, the açaí and the babassu (MEDEIROS-COSTA et al., 1996). The earliest known reference to the carnaúba is in a text from 1648 by naturalists Marcgrave and Piso intitled *Historia Naturalis Brasiliae*. Locally, the palm is known as “carnaúba” or “carnaubeira”. The former is also the name of the fruit produced by this palm (MELO et al., 2012).

Despite its astringency, the fruit is a potential and appreciated source of fodder for cattle. The seeds contain extractable oil and, if roasted, may be used as a replacement for traditional coffee beans (MELO et al., 2012; GOMES et al., 2013).

The wax which coats the leaves of the carnauba palm is composed of acids and alcohols (CARVALHO; GOMES, 2008). In the past, crude carnaúba wax was an important commodity for export and was used locally in the manufacture of candles. Nowadays, the wax is refined industrially, classified and employed in a number of specific contexts, in many cases without a perfect substitute (ARAGÃO FILHO; ARAGÃO, 2013; PEREIRA et al., 2014).

The lack of knowledge about plant species native to Brazil is reflected in the overall undervaluation of the country's heritage of biodiversity. In addition, the currently adopted agriculture model of monoculture of exotic species is producing severe impacts on the environment, endangering native species (OLIVEIRA et al., 2012; PEREIRA et al., 2014). It is therefore desirable to expand current knowledge of the biology and properties of the carnaúba, a rich and expressive palm species native to Northeastern Brazil (ARAÚJO et al., 2014). The purpose of the present study was to identify the fatty acids contained in oil extracted from carnaúba seeds using a simple standard laboratory procedure and determine the physicochemical properties of the oil.

## MATERIAL AND METHODS

Berries of carnaúba were obtained from the community at Sítio Várzea do Cobra in the rural zone of Limoeiro do Norte (a town in Ceará, Northeastern Brazil) between December 2012 and January 2013. Intact and healthy seeds were immersed in a chloride solution (100 ppm), then rinsed in tap water, air-dried in the shade, packaged in plastic bags and stored in a conventional refrigerator at  $7 \pm 2$  °C. The seeds were stocked under refrigeration until the moment of oil extraction.

The berries were cut in half with a stainless steel knife and the pericarp was discarded. The seeds were ground, first in a common blender (Waring®), then in a homogenizer (Biofoco®, model BF2 MMH 27000), and stored in dark plastic jars in a conventional refrigerator.

The oil was extracted with a Soxhlet extractor using n-hexane as solvent (TIGLEA et al., 2008). Fifteen extractions were performed based on 5g aliquots. The flask residue was extracted with an oven to 85 °C, holding for about one hour. Then it was cooled to room temperature in a desiccator until constant weight. The density of the oil was determined at 25 °C using a 10 mL pycnometer and a 0.0001g analytical scale (Bel Engineering®). The measurements were performed in duplicate on subsequent days.

The refraction index of the extracted oil was determined with a refractometer (A. Krüss Optronic®) corresponding to a temperature of 40 °C. The pH values were measured with a pH meter (Hanna Instruments®, model pH 20).

To determine the acidity index, 2g aliquots of oil and two drops of phenolphthalein were added to 20 mL of neutral ether-alcohol solution (2:1). Finally, the solution was titrated with a standard solution of 0.1 N sodium hydroxide (TIGLEA et al., 2008).

The saponification index was determined as follows: a 4.0 g aliquot was solubilized in 50 mL of a 4% alcoholic potassium hydroxide solution (m/v) (ARAÚJO, 2011). The mixture was then transferred

to a 250 mL volumetric flask connected to a condenser and a heating plate and boiled over low heat for an hour. Once the flask had cooled off, the condenser was disconnected and washed with distilled water to collect the residue. Phenolphthalein (1 mL) was added and the solution was titrated with 0.5 N hydrochloric acid until the pink color had disappeared. The blank was prepared in the same way, following the guidelines of the Adolfo Lutz Institute (TIGLEA et al., 2008).

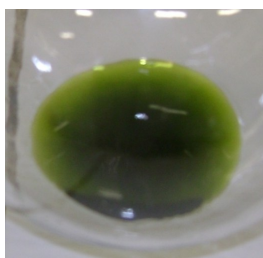
The percent composition of fatty acids in the crude oil extracted from the carnaúba seeds was determined by gas chromatography and flame ionization detection (GC-FID, Thermo®, model: Focus®), using a 30 m x 0.25 mm x 0.25 µm carbowax column. Nitrogen (N<sub>2</sub>) was used as carrier gas at a flow rate of 1.0 mL.min<sup>-1</sup>. The injection method was splitless, and the temperature of the injector and the de-

tector was 250 °C and 280 °C, respectively. The temperature was raised from 70 °C to 240 °C at a rate of 5 °C.min<sup>-1</sup>, then maintained at 240 °C for 10 minutes (KHAN, 2013).

## RESULTS AND DISCUSSION

On the average, the carnaúba seeds yielded  $4.88 \pm 0.19\%$  oil. This figure is substantially lower than the rare figures available in the literature. Such a low yield may constitute a limiting factor for the exploitation of the oil by the food and biofuel industries.

Figure 1 shows the crude oil extracted from the carnaúba seeds. The green color indicates the likely presence of chlorophyll.



**Figure 1.** Green coloring of crude oil extracted from seeds of carnaúba (*Copernicia prunifera* (Miller) H. E. Moore).

A summary of the physico-chemical properties of the crude oil extracted from the carnaúba

seeds it is showed in Table 1.

**Table 1.** Physico-chemical properties of crude oil extracted from seeds of *Copernicia prunifera*.

Physico-chemical properties	Average values*
Density (g.cm <sup>-3</sup> )	0.9422
Refractive index (at 40 °C)	$1.4534 \pm 0.0003^*$
pH (at 25 °C)	$3.46 \pm 0.14^*$
Acidity index (% oleic acid)	$0.68 \pm 0.04^*$
Saponification index (mg KOH.g <sup>-1</sup> )	$124.39 \pm 1.99^*$

\* Analysis in triplicate

The absolute density of the extracted carnaúba oil was  $0.9422 \text{ g.cm}^{-3}$  at 25 °C. This figure is close to figures published for other crude vegetable oils, such as dendê oil ( $0.9118 \text{ g.cm}^{-3}$ ), babassu oil ( $0.9153 \text{ g.cm}^{-3}$ ) and castor oil ( $0.9578 \text{ g.cm}^{-3}$ ) (GUNSTONE et al., 2002).

The refractive index was  $1.4592 \pm 0.0003$  at 25 °C. To determine the index at 40 °C (the temperature specified by regulatory agencies), the value was recalculated to  $1.4534 \pm 0.0003$ . This is slightly above the range (1.448-1.450) determined by Brazilian Agency of Sanitary Surveillance (BRASIL, 2005). The refractive index of crude palm kernel oil from Malaysian standard is in the range from 1.4500 to 1.4518 at 40 °C (GUNSTONE et al., 2002). The pH value of the extracted oil was 3.46 when measured at 25 °C.

The acidity index was  $1.374 \pm 0.075 \text{ mg NaOH/g oil}$ ; or, if expressed as oleic acid percentage,  $0.68 \pm 0.04\%$ . It is defined as the weight of NaOH in

mg needed to neutralize the organic acids present in 1g of fat and it is a measure of the free fatty acids (FFA) present in the oil. FFA are a source of flavors and aromas. For example, the acid value of some vegetable oils: maize (0.22 %), soybean (0.60 % and virgin olive oil (6.6%) (GUNSTONE et al., 2002). No information was found in the literature regarding the acidity index of oil extracted from carnaúba seeds.

The saponification index was  $124.39 \pm 1.99 \text{ mg KOH/g oil}$ . As with the acidity index, no information was found in the literature to which our findings could be compared. The value found was below the reference range for soybean oil (180-200 mg KOH/g oil) (BRASIL, 2005), indicating that the average fatty acid molecular weight is greater in carnaúba seed oil than in soybean oil (ARAÚJO, 2011).

A comparison of the percentage composition of fatty acids in the carnaúba seed oil and babassu oil is demonstrated in Table 2.

**Table 2.** Composition of fatty acids in carnaúba seed oil (*Copernicia prunifera* (Miller) H. E. Moore) and babassu oil (*Orbygnia speciosa*), [Mart. ex Spreng.] expressed in percentage (w/w).

Fatty acid	Carnaúba	Babassu*
Caprylic (C8:0)	0.52	2.6 - 7.3
Capric (C10:0)	0.68	1.2 - 7.6
Lauric (C12:0)	39.42	40.0 - 55.0
Myristic (C14:0)	24.9	11.0 - 27.0
Palmitic (C16:0)	9.74	n/a
Palmitoleic (C16:1)	0.00	n/a
Stearic (C18:0)	2.98	1.8 - 7.4
Oleic (C18:1)	12.16	9.0 - 20.0
Linoleic (C18:2)	5.47	1.4 - 6.6
Linolenic (C18:3)	0.41	n/a
Eicosanoic (C20:0)	0.13	n/a
Behenic (C22:0)	0.02	n/a
Erucic (C22:1)	0.00	n/a
Lignoceric (C24:0)	0.08	n/a

\*Source: Codex (2001)

n/a = not available

The Codex (2001) using gas liquid chromatography from authentic samples coupled with mass spectrometry (GC-MS) drew the figures for babassu oil presented in Table 2. As shown in the Table 2, carnaúba seed oil and babassu oil have several important aspects in mutual.

The chromatographic analysis showed a predominance (more than 74%) of saturated fatty acids (lauric, myristic and palmitic) in addition to a certain amount (12.16%) of oleic acid, an unsaturated fatty acid. Lauric acid is generally found in palm oils at concentrations of 40-50% (BRASIL, 2005; ARAÚJO, 2011). The percentage of lauric acid found in the seed oil of carnaúba was 39.42%, so this value is very close to the value reported for seeds of other palm species such as babassu oil (range from 40.0 to 55.0%) and coconut oil (45.1 to 53.2%) (CODEX, 2001; GUNSTONE et al., 2002).

The carnauba oil contains a high concentration of saturated fatty acids such as lauric and myristic acids, which contribute to the solidification of the oil at temperatures below 18 °C (MANN et al., 1994). The density of the oil extracted in this study (0.9422 g.cm<sup>-3</sup>) is close to that of castor oil (0.9578 g.cm<sup>-3</sup>), suggesting carnaúba seeds may be employed in the production of biofuel (MELO et al., 2014).

The high acidity index (0.68 ± 0.04%) and saponification index and the presence of high-molecular-weight fatty acids suggest that crude oil from carnaúba seeds is not a particularly attractive option for human consumption. It is not rich in fatty acids of nutritional value when compared to most oils used in the food industry. Over the past years, preference has been given to oils with high concentrations of triglycerides and unsaturated fatty acids (REDA; CARNEIRO, 2007). The predominance of saturated fatty acids (more than 74%) does not make carnaúba seed oil a promising alternative for the food industry, but the high percentage of lauric acid in the crude oil suggests a potential use in the production of detergents. The fatty acid composition of seed oil

carnauba is very similar to the babassu oil, as both have a high content of lauric and myristic acids (Table 2). Therefore, the oil carnauba can be used in cosmetic products to replace babassu oil. It is known that babassu oil has emollient properties and can be used in products for skin care and hair as bar soaps, conditioners and bath oils due to its moisturizing action (LUBBE; VERPOORTE, 2011; MAPRIC, 2014).

Studies have shown that pretreatment of the skin with lauric acid increases its permeability to certain active substances. It is commonly employed in obtaining surfactants for industrial and cosmetic purposes. Features emulsifying properties which justify their use in the preparation of emulsions of the type oil / water (MAPRIC, 2014).

## CONCLUSION

The low yield of oil extracted from carnaúba seeds may constitute a limiting factor for commercial exploitation. However, the extraction technique used in this study should be taken into account. In conclusion, carnaúba seed oil displays an attractive green color and a fatty acid profile potentially exploitable by the detergent and cosmetic industries.

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