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RESEARCH ARTICLE

Antioxidant Activity and Bioactive Compounds of Babassu (*Orbignya phalerata*) Virgin Oil Obtained by Different Methods of Extraction

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Abstract:

Background:

The investigation of new sources of raw materials and the knowledge of the composition of the food is fundamental for the evaluation of their potential and the availability of nutrients for the consumer population.

Objective:

This work aimed to deepen the knowledge about the crude oil of babassu fruit obtained by two different methods of extraction, cold pressing and extraction by cooking the fruit almond.

Method:

Total phenolic compounds contents and antioxidant activity were determined by ferric reducing antioxidant potential assay and 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity assay. By liquid chromatography, the content of different bioactive compounds was determined. Data was submitted to Analysis of Variance (ANOVA) and compared by f test ($p < 0.05$).

Results:

The results showed that for most of the bioactive compounds there was no difference between the two types of babassu oil. For those compounds where the oils differed, the virgin oil had about three times the content of the extra-virgin oil. In addition, the antioxidant activity was higher for the oil extracted by cooking of the babassu mass, ranging from approximately 2.5 times higher up to 19.2 times higher than the antioxidant activity of the babassu oil extracted by pressing.

Conclusion:

The process of extraction by cooking the almond mass can incorporate a larger number of bioactive components and improve the antioxidant activity of the virgin babassu oil. However, the extraction method does not influence the content of tocopherols of distinct types of babassu oil.

Keywords: *Orbignya phalerata*, Phenolic compounds, Tocopherols, FRAP, DPPH, Liquid chromatography.

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1. INTRODUCTION

Vegetable oils are those obtained from parts of plants, such as seeds, nuts, flowers and fruit pulp, and vegetables and legumes mainly formed by lipids. They are the most produced

and consumed oils in the world [1] and represent significant importance in the population's diet. They are sources of saturated and unsaturated fatty acids, liposoluble vitamins and antioxidants [2, 3].

Vegetable oils are versatile raw materials for the food, pharmaceutical and chemical industries, as they have different compositions and technological uses [3, 4]. For example, oils from oilseeds are rich in polyunsaturated fatty acids and vitamins [3, 5, 6], fruit pulp oils such as oil palm and olive oil

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have a high amount of antioxidant compounds [7 - 9] and palm kernel oil has a large amount of saturated fatty acids [10, 11]. These different compositions allow vegetable oils to be applied to various products. In the food industry, they are used to cook or fry [12,13], as ingredients of different food products, creams and margarine [14 - 16], bakery products [17], ice cream [18], and chocolates [19]. In the pharmaceutical industry these oils are used in cosmetics [20, 21] and therapeutic formulations [22, 23].

Thus, the world demand for oils and fats is increasing, so several researchers are engaged in finding new sources of vegetable oils with high nutritional quality, industrial importance and pharmaceutical activity [24 - 31]. Brazil has great biodiversity and therefore an infinity of plants potentially producing oil.

Babassu (*Orbignya phalerata*) is a palm commonly found in the North and Northeast regions of Brazil [32]. Babassu is a valuable resource in these regions both in economic and nutritional terms [33]. This importance is related to the exploitation of the culture [34], which is extractive and exploited by low-income people, as well as the substantial number of products and by-products that can be originated using this fruit. Babassu oil is the main product, usually used in the diet of the population of these regions [10, 32, 34] or for the production of cosmetics and hygiene and cleaning products [35, 36], and, also for the production of biofuels [36 - 38], being the by-products destined for other uses, such as the use of the defatted cake for animal feed [34, 39, 40].

According to Carazza *et al.* (2012), there are basically two forms of processing to obtain babassu oil, both handmade in domestic or cooperative form. One process is performed by cold mechanical pressing and, in the other process, by cooking the mass of babassu crushed almonds. Extra-virgin babassu oil is obtained by cold pressing whole, healthy almonds without yellowing or superficial physical damage, followed by decantation, filtration and packaging. The virgin babassu oil is obtained by crushing previously selected and toasted almonds, followed by baking the mass to obtain the oil, which is separated by decanting, filtered and reheated for total removal of the water resulting from the cooking step and then packaged.

Babassu oil is composed mainly of saturated fatty acids (80-91%), such as lauric acid (43-50%), myristic acid (15-18%), palmitic acid (6-10%), capric acid (4-6%), caprylic acid (0-5%) and stearic acid (3-5%); the remainder is unsaturated fatty acids (9-20%), in which oleic acid (12-19%) and linoleic acid (1-3%) are present [10, 38, 41]. This composition is like that of palm and coconut oil and has already been reported in the literature [42 - 44].

Currently, babassu oil is less used in the food industry, its major use is in the personal care industry, in the manufacture of surfactants, soaps and cosmetics in general. This is mainly because babassu oil has a high capacity to form soaps and possesses high emollient power [45]. To obtain information that may stimulate other applications of this native Brazilian raw material, the objective of this study was to investigate the content of bioactive compounds and the antioxidant activity of different types of crude babassu oil obtained employing the

two most used forms of extraction.

2. MATERIAL AND METHODS

2.1. Samples

The samples of babassu oil were obtained directly from producers in the states of Tocantins and Maranhão, Brazil. Extra-Virgin Babassu Oil (EVBO) was obtained by cold pressing whole, healthy almonds without yellowing or superficial physical damage, followed by decantation, filtration and packaging. The Virgin Babassu Oil (VBO) was obtained by crushing previously selected and toasted almonds, followed by baking the mass to obtain the oil, which was separated by decanting, filtered and reheated for total removal of the water resulting from the cooking step and then packaged.

2.2. Chemical Reagents

During the experiments, the following solvents and reagents were used: methanol with purity > 99% (Ecibra, Santo Amaro, São Paulo, Brazil) hexane with purity 98.5% (Synth, Diadema, São Paulo, Brazil), ferric chloride hexahydrate with purity 97% (Sigma-Aldrich, Sant Louis, USA) and ferrous sulfate heptahydrate with purity \geq 99% (Sigma-Aldrich, Sant Louis, USA). Acetonitrile, methanol, hexane, isopropanol and acetic acid (Sigma-Aldrich, Sant Louis, USA) (both of whit purity \geq 99.9%). Deionized water (deionizer Marte Científica, Santa Rita do Sapucaí, Minas Gerais, Brazil) and ultrapure water (Ultra water purifier MS2000, Gehaka, São Paulo, Bazil).

Folin-Ciocalteu reagents, DPPH radical solution (2,2_-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-Tris (2-pyridyl) -s-triazine), all from Sigma-Aldrich (Sant Louis, USA) were used for the analysis of antioxidant activity.

The analytical standards for the chromatographic analyzes were: gallic whit purity \geq 99.9%, caffeic acid whit purity \geq 98%, catechin whit purity \geq 99%, quercetin with purity \geq 99%, rutin with purity \geq 96%, α -tocopherol with purity \geq 96%, β -tocopherol, γ -tocopherol with purity \geq 96% and δ -tocopherol with purity \geq 96%, lycopene with purity \geq 95%, β -carotene with purity \geq 97% and α -carotene with purity \geq 95%, all from Sigma-Aldrich (Sant Louis, USA).

2.3. Methods

2.3.1. Antioxidant Activity and Bioactive Compound Content Assays

The analyses of antioxidant capacity and phenolic compound content of crude babassu oils (EVBO and VBO) were carried out from the methanolic extracts, as explained below, of the samples. The quantification of tocopherols and carotenoids was performed directly for the samples of the oils.

2.3.1.1. Obtention of the Methanolic Extracts of Crude Babassu Oil Samples

The extracts were obtained according to the procedure described by Montedoro *et al.* [46]. Approximately 5.0 g of each type of oil was mixed with 1.0 mL of methanol/water solution (80:20 v/v) and vortexed for 2 min. This mixture was

centrifuged at 1080g for 10 min and the methanolic portion was collected. These steps were repeated 3 more times and the supernatants were combined to form the extract.

2.3.1.2. Antioxidant Activity Assays

The total phenolic compounds of the samples [47 - 48] were determined using the Folin-Ciocalteu Reagent (FCR) and solutions of gallic acid with different concentrations for the construction of the analytical curve, ranging from 0.01 to 0.1 mg/mL. 0.2 mL aliquots of the methanolic extracts were mixed at 0.2 mL of FCR. After 4 min, 1.6 mL of 5% (m/v) calcium carbonate aqueous solution was added. The mixture remained 20 min in thermostated bath at 40°C and then the assays were monitored on a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The absorbance was measured at 750 nm against a blank which was methanol. The total content of phenolic compounds in the extracts was expressed in mg of gallic acid equivalent per 100 g of babassu oil.

The antiradical activity of the oils was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical following the procedure described by Franco *et al.* (2014). Aliquots of 0.1 mL of methanolic solution of the babassu oil extracts (4 different concentrations) were mixed with 3.9 mL of the 60 µM DPPH solution. These mixtures were held in the dark for 30 min and then the absorbance was measured at 515 nm against a blank which was methanol. The percentage of inhibition of the radical was calculated according to Equation 1 and these results were used to obtain a curve relating the percentage of inhibition of the radical *versus* the concentration of the extract. Through linear regression, the IC₅₀ was calculated which is the value that estimates the antioxidant concentration required to inhibit 50% of the DPPH radical.

$$\% \text{ Inhibit DPPH} = \frac{Abs_{DPPH} - Abs_{Sample}}{Abs_{DPPH}} \times 100 \quad (1)$$

Where:

Abs_{DPPH} is the measured absorbance for the solution containing only the radical DPPH (UA);

Abs_{Sample} is the absorbance measured after the reaction between the extracts of the samples and the radical DPPH (UA).

Antioxidant activity using the ferric reducing method (FRAP) was evaluated as described by Benzie & Strain [49], with some modifications. Aliquots of 0.9 mL of the methanolic extracts of babassu oils, plus 0.27 mL of distilled water and 2.7 mL of the FRAP reagent were homogenized and incubated at 37° C for 30 minutes. After this period a reading at 595 nm was carried. To obtain the analytical curve, the same procedure was repeated by replacing the aliquots of the extract by ferrous sulfate solutions with different concentrations ranging from 0.15 mM to 5 mM. Thus, the results were expressed in mmol of ferrous sulphate/g of babassu oil.

2.3.1.3. Contents of Bioactive Compounds

2.3.1.3.1. Phenolic Compounds

The presence of different phenolic acids and flavonoids,

namely gallic acid, caffeic acid, catechin, quercetin and rutin, were investigated. For the identification and quantification of these bioactive compounds, the methanolic extracts of the EVBO and VBO samples were obtained as described previously in item 2.3.1.1. These extracts were purified by the procedure also described by Montedoro *et al.* [46]. The methanolic extract was rotoevaporated at 30° C until complete evaporation of the solvent, and then resuspended in 1.0 mL of acetonitrile. This solution was washed three times with 1.0 mL of hexane and the acetonitrile layer was separated and evaporated at 30° C. The resulting residue was dissolved in 1.0 mL of chromatographic grade methanol and filtered through a 0.45 µm pore syringe filter.

The analyzes were performed using a liquid chromatograph (Shimadzu, Kyoto, Japan), equipped with a quaternary pump system, degasser, injection valve with 20 µL sampling loop, column furnace and diode arrangement detector. The phenolic compounds were separated on C18 reverse phase analytical column (0.25 m x 4.6 mm d.i. x 5µm particle size) (Supelco Analytical, Bellefonte, USA).

The operating parameters of the chromatograph were established as described by Azevedo *et al.* [50]. The mobile phase consisted of a mixture of two solvents, acidified water (98: 2 v/v acetic acid water) (Solvent A) and methanol (Solvent B). Elution occurred in a gradient ranging from 100% A to 50% A and 50% B in 5 min. Passing to 35% A and 65% B in 7 min and remaining in this proportion up to 10 min. Between 10 and 12 min returning the condition 50% A and 50% B and passing to 100% A in 15 min, thus remaining up to 18 min for column stabilization and preparation for a new run. The mobile phase flow was adjusted to 1.0 mL/min and the column furnace temperature remained at 40 ° C throughout the run.

Identification of the phenolic compounds was performed by comparing the peak retention time of the samples with the peak retention time of gallic acid, caffeic acid, catechin, quercetin and rutin and by the characteristic wavelength of each substance. Chromatograms were processed at 280 nm for gallic acid and catechin, 330 nm for caffeic acid and 360 nm for quercetin and rutin [50].

The quantification of the phenolic compounds was done through external standardization. The analytical curves were constructed by injecting solutions of the standards with concentrations ranging from 0.2 x 10⁻³ to 0.2 µg/mL and the phenolic amounts present in the EVBO and VBO were calculated using the equations of the lines.

2.3.1.3.2. Tocopherols and Carotenoids

The presence of non-esterified tocopherols and carotenoids was investigated. For identification and quantification of these compounds, 0.05 g of each of the oils were weighed and 950 µl of HPLC grade hexane was added. The mixture was run on a vortex type stirrer (Labnet International Inc., Edison, New Jersey, EUA) for 30 seconds and centrifuged in the microcentrifuge MiniStar (VWR Colletion, Vienna, Austria) at 1080g for 5 min. The supernatant was collected and filtered through a 0.45 µm pore diameter syringe filter [51].

The analyzes were performed using a Shimadzu liquid

chromatograph (Kyoto, Japan), equipped with a quaternary pump system, degasser, injection valve with 20 μ L sampling loop, column furnace and diode arrangement detectors and fluorescence. The analytes were separated on Zorbax-SIL normal phase analytical column (0.25 m x 4.6 mm d.i. x 5 μ m particle size) (Supelco Analytical, Bellefonte, USA).

The mobile phase consisted of a mixture of hexane: isopropanol (99:1, v/v), elution being isocratic. The mobile phase flow was adjusted to 1.0 mL/min and the column furnace temperature remained at 25 °C throughout the run [52].

The identification of the compounds was performed by comparing the sample retention time with the peak retention time of the carotenoid standards (lycopene, β -carotene and α -carotene) and tocopherols (α , β , γ and δ - tocopherols) and also by the characteristic wavelength of each substance. Chromatograms were processed at 290 nm (excitation) and 330 nm (emission) at the fluorescence detector for the tocopherols; and, between 390 and 700 nm, in scan mode, in the diode arrangement detector for carotenoids [53, 54].

The quantification of tocopherols was done through external standardization. The analytical curves were constructed by injecting solutions of the standards with concentrations ranging from 0.01 to 1.5 μ g/mL. The amounts of these compounds present in EVBO and VBO were calculated using the equations of the lines obtained for each curve. Curves were not constructed for carotenoids because they were not detected in the samples.

2.3.2. Statistical Analysis

The data obtained in this study, both for the antioxidant activity and for the content of bioactive compounds, was submitted to analysis of variance (ANOVA) using the SAS program, Studio version.

The antioxidant activity and contents of the different phenolic compounds and tocopherols were expressed as mean \pm standard deviation and were compared using the f test ($p < 0.05$).

3. RESULTS

3.1. Antioxidant Activity

The determination of the antioxidant capacity is based

mainly on two mechanisms of reaction, the transfer of a hydrogen atom and / or the transfer of an electron. In addition to the mechanism, the objective is to determine the protective effect of the material against free radicals, which differ in the initiator radical, reaction kinetics and side reactions [55]. Thus in investigating the total antioxidant capacity of a substance, it is important that at least one test of each mechanism is used. Thus, the samples were tested for the DPPH radioactivity and ferric reducing antioxidant potential assay (FRAP). In the DPPH assay both mechanisms are involved and in the FRAP assay the transfer of a hydrogen atom is involved [56 - 58].

The results for the determinations of the antioxidant activity of the different babassu oils studied (EVBO and VBO) can be observed in Table 1. Regardless of the test used, the antioxidant activity was higher for the oil extracted by cooking the babassu mass, ranging from \approx 9.3 times higher up to 19.7 times higher than for the antioxidant activity of the extra virgin babassu oil.

Results presented as means \pm standard deviations of the analyzes of each type of babassu oil. Results followed by different letters in the same line are significantly different by the f test at 5% probability.

3.2. Contents of Bioactive Compounds

Chromatographic conditions were suitable for separating the different phenolic compounds, gallic acid, caffeic acid, catechin, quercetin and rutin (Fig. 1) and tocopherols (α , β , γ , and δ -tocopherol) (Fig. 2) present in the babassu oil samples.

The results for the quantification of the bioactive compounds, phenolics, tocopherols and carotenoids in the different babassu oils (EVBO and VBO) can be observed in Table 2. For most of the compounds, there was no difference between the types of oil. In cases where the oils differed, VBO presented content about three times higher than EVBO, except for gallic acid, where this ratio was reversed. With regard to catechin, it can be observed that for the EVBO samples the presented values are low and with high variability. Thus, the presence of this compound can be confirmed, but it is not possible to determine in what quantities.

Results presented as means \pm standard deviations of the analyzes of each type of babassu oil. Results followed by different letters in the same line are significantly different by the test f at 5% probability.

Table 1. Antioxidant activity of babassu crude oils obtained by different extraction methods.

Antioxidant Assay	Type of Babassu Oil	
	EVBO	VBO
Total Phenolic (FCR) (mg gallic acid / g oil)	1.1 ^a \pm 0.1	22 ^b \pm 2
Ferric Reducing Test (FRAP) (mM ferrous sulfate / g oil)	0.3 ^a \pm 0.3	2.8 ^b \pm 0.2
DPPH radical assay (IC ₅₀) (mg / mL)	2349 ^a \pm 57	121.5 ^b \pm 0.8

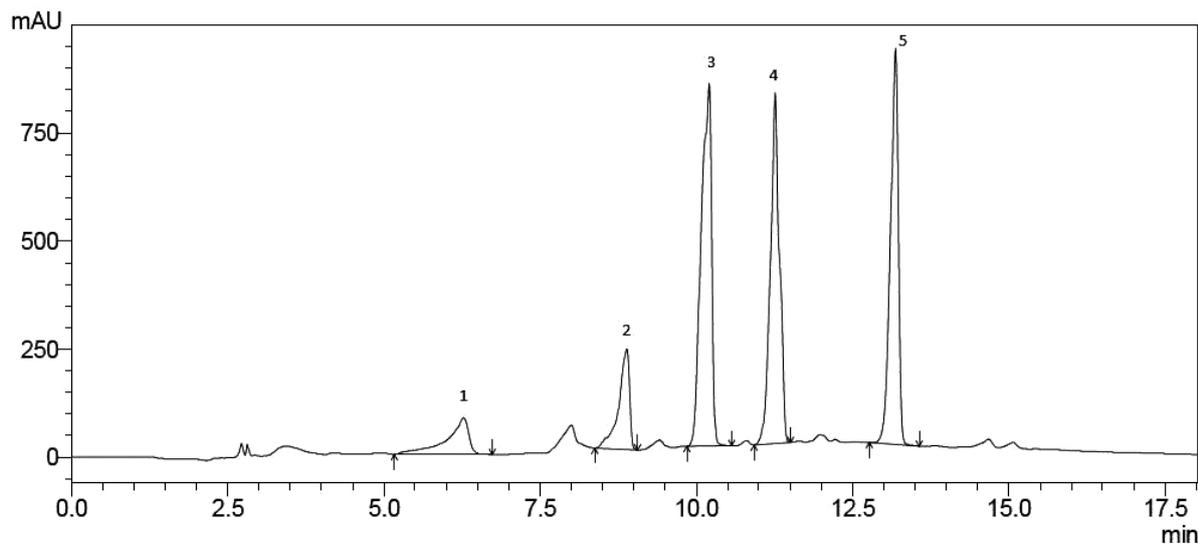


Fig. (1). Chromatogram characteristic of babassu oil samples. Separation of different phenolic compounds: 1) Gallic acid; 2) Catechin; 3) Caffeic acid; 4) Rutin; 5) Quercitina.

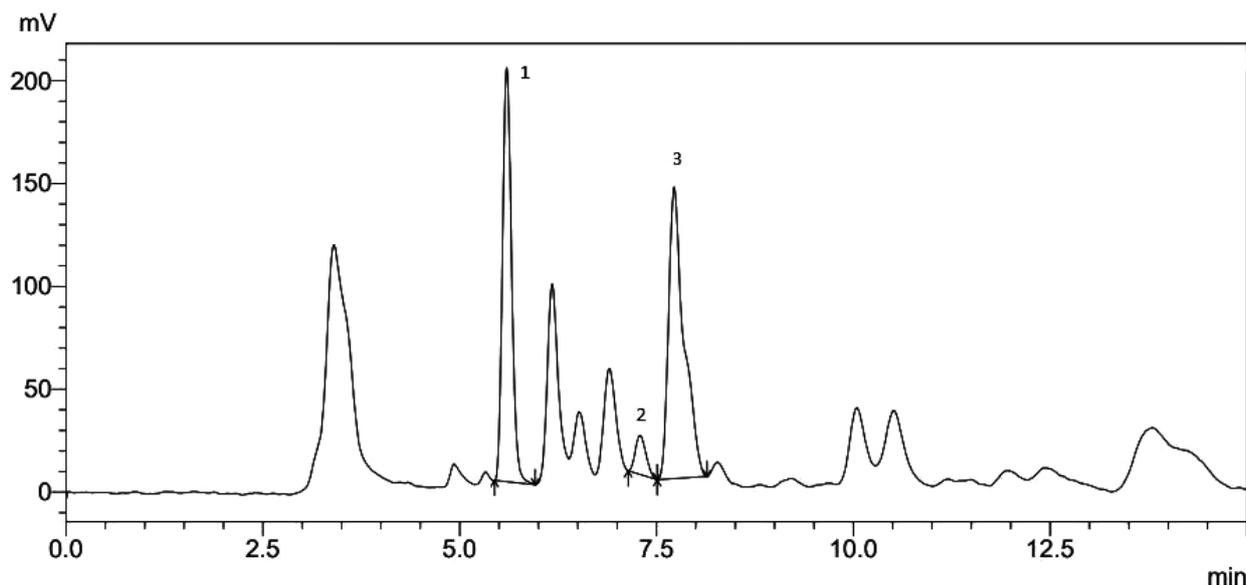


Fig. (2). Chromatogram characteristic of babassu oil samples. Separation of the different tocopherols: 1) α-tocopherol, 2) β-tocopherol and 3) γ-tocopherol.

Table 2. The content of the bioactive compounds identified in the different types of crude babassu oils.

Compounds Bioactive (mg / 100g of oil)	Type of Crude Babassu Oil	
	EVBO	VBO
Gallic acid	0.17 ^a ± 0.02	0.06 ^b ± 0.03
Coffee Acid	0.045 ^a ± 0.001	0.111 ^b ± 0.003
Catechin	0.03 ^a ± 0.02	0.72 ^b ± 0.02
Quercitin	0.21 ^a ± 0.01	0.23 ^a ± 0.01
Rutin	0.18 ^a ± 0.02	0.13 ^a ± 0.01
α-tocopherol	1.61 ^a ± 0.08	1.32 ^a ± 0.03
β-tocopherol	3.50 ^a ± 0.08	3.46 ^a ± 0.03

(Table 2) contd....

Compounds Bioactive (mg / 100g of oil)	Type of Crude Babassu Oil	
	EVBO	VBO
γ -tocopherol	1.45 ^a ± 0.07	1.61 ^a ± 0.06
δ -tocopherols	Not detected	Not detected
Carotenoids	Not detected	Not detected

4. DISCUSSION

4.1. Antioxidant Activity

Analyzes for concentrations of total phenolic compounds showed a significant difference between the types of babassu oil studied. Phenolic compounds are substances that have structures with aromatic rings and double conjugated bonds from which they exert their antioxidant action, besides being the most abundant antioxidants in the diet [59]. They are generally determined *via* reaction with the Folin-Ciocalteu reagent. However, this reagent is not only sensitive to phenolic compounds [60]. Other structures with reducing power may also influence the results, for example of conjugated aldehydes, ketones, dienes and trienes, in addition to the melanoidins.

The process of obtaining VBO has two important stages of heating. At first, the mass of crushed kernel is cooked with a little water for oil scoring, which by being less dense floats on the cake and can be separated. In the second stage, this oil collected, still has a certain amount of water and therefore goes through an evaporation process. Thus, this exposure to heat may have favored oxidation and darkening reactions and consequently raised the total phenolic values of virgin babassu oil.

The oxidation of fatty acids leads to the formation of compounds such as aldehydes, ketones, dienes and conjugated trienes, among others [61]. And melanoidins are heterogeneous brown pigments that have aromatic rings, are formed by reactions of non-enzymatic browning [62, 63]. These pigments are produced during the Maillard reaction. This reaction occurs between the carbonyl groups of reducing sugars and the amino groups of amino acids, peptides or proteins, and is catalyzed by heating [64, 65]. The presence of proteins and carbohydrates in the babassu cake [66] together with the heating during the VBO extraction process contribute to the formation of these compounds.

Regarding antioxidant capacity, both types of babassu oils can be considered low antioxidant power. Ferreira *et al.* (2011) evaluating the antioxidant capacity of different oils, including babassu oil, obtained comparable results. Essential oils such as oregano and thyme, which have high antioxidant capacity, have values between IC50 3.9 and 1.1 mg/mL [67]. When compared with excellent antioxidant substances, such as ascorbic acid and BHT [68, 69], babassu oil presents activity up to 11.11×10^5 times lower. This may be related to the plant's own anatomy. The babassu kernel is protected within the fruit by at least three layers, epicarp, mesocarp, and endocarp [32, 34, 70] that form a protective physical barrier for the constituent substances of this almond, so the plant need not produce other compounds for this purpose.

4.2. Contents of Bioactive Compounds

The differences presented by EVBO and VBO oils, both for the antioxidant activity discussed above and the content of phenolic compounds may be related to the higher release of these bioactive substances from the food matrix when it is heated. The presence of water in the cooking step increases the solubility of flavonoids and phenolic acids, consequently increasing their extraction during the process. With the evaporation of water these components remain in the extracted oil. Equivalent results were demonstrated by Serevinatne *et al.* (2008) in a study about the influence of the method of coconut oil extraction on the content of phenolic compounds of this product [71].

Generally flavonoids, among them, catechin, quercetin and rutin, are the predominant class among the phenolics present in plant foods. However, the presence of phenolic acids, such as gallic and caffeic acids, is considered beneficial since these compounds are considered multipurpose bioactive. These acids, in addition to the known antioxidant effect, may still have antimutagenic, anticarcinogenic, anti-inflammatory and antimicrobial action [59, 72].

The tocopherols are part of the compounds with vitamin E activity and are the main antioxidants naturally present in vegetable oils [51]. The biological activity varies among the isomers, with an increased vitamin activity associated with greater methylation and heightened activity associated with methylation at the fifth carbon, vitamin activity: $\alpha > \beta > \gamma > \delta$ [6, 73, 74]. According to the Specified Oil Standard [73] babassu oil has low or undetectable levels of these compounds. The results presented in this study show low levels of total tocopherols ($\cong 6.5$ mg/100 g of olive oil), and not all of them are found. This fact may be related to the fatty acid composition of babassu oil. Since babassu oil is mostly composed of saturated fatty acids and is therefore more stable, a high concentration of these natural antioxidants is not expected. Other highly saturated vegetable oils, such as coconut oil and palm kernel oil, also have low or undetectable levels of tocopherols [73]. On the other hand, in more unsaturated vegetable oils have highly levels of tocopherols, such as palm ($\cong 19$ mg/100 g oil) [75], soybean oil ($\cong 40$ mg/100g oil) [76], canola ($\cong 65$ mg/100g oil) [77], sunflower ($\cong 80$ mg/100g oil) [51] and olive oil (200-450 mg/100g olive oil) [78].

CONCLUSION

The results obtained demonstrate that the extraction method can influence the characteristics and composition of crude babassu oil. Extraction by cooking the crushed kernel cake is capable of incorporating a larger amount of bioactive compounds. This process improves the antioxidant capacity of virgin oil (VBO). Despite this, regardless of the method chosen for its production, babassu oil cannot be considered a potential source of antioxidants.

LIST OF ABBREVIATIONS

DPPH	= 2,2-diphenyl-1-picrylhydrazyl
EVBO	= Extra-Virgin Babassu Oil
FCR	= Folin-Ciocalteu Reagent
FRAP	= Ferric Reducing Antioxidant Potential
HPLC	= High Performance Liquid Chromatography
VBO	= Virgin Babassu Oil

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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