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FOOD TECHNOLOGY

Characterization of munguba oil obtained by ultrasound

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ABSTRACT: *This study analyzed the use of the ultrasound-assisted method as an alternative to obtain munguba oil. The extraction provided a 47.70 % yield of an oil with appropriate quality, evaluated by assessing the %FFA as oleic acid, iodine, peroxide, and saponification values, in addition to the refractive index and density. The oil presented thermal stability up to 300 °C, was constituted mainly by palmitic acid (C16:0), and presented a total phenolic content of 55.02 ± 1.872 μgEAG g^{-1}. Results suggest that the ultrasound-assisted method has the potential to obtain vegetable oils without compromising their characteristics and quality, as well as optimize extraction time, solvent volume, and operational costs. Moreover, munguba oil presents itself as a suitable and sustainable alternative as an adjuvant in food products, pharmaceuticals, cosmetics, and biofuels.*
Key words: *extraction, Pachira aquatica Aublet, ultrasound, vegetable oil.*

Caracterização do óleo de munguba obtido por ultrassom

RESUMO: *O estudo analisou a aplicação do método de extração assistida por ultrassom como método alternativo para a obtenção do óleo de munguba. A extração do óleo resultou num rendimento de 47.70 % com qualidade adequada avaliada pela %AGL como índices de ácido oleico, iodo, refração, peróxido, saponificação e densidade. O óleo apresentou estabilidade térmica até 300 °C sendo constituído majoritariamente do ácido palmítico (C16:0) e expressou um teor de fenóis totais de 55.02 ± 1.872 μg/EAGg^{-1}. Os resultados sugerem que o método de extração assistida por ultrassom apresenta potencial para obtenção de óleos vegetais sem alterar suas características e qualidade, além de possibilitar a otimização do tempo, volume de solvente e custo operacional. Ainda, o óleo de munguba mostra-se como alternativa sustentável na aplicação como adjuvante em alimentos, medicamentos, cosméticos e biocombustíveis.*
Palavras-chave: *extração, Pachira aquatica Aublet, ultrassom, óleo vegetal.*

INTRODUCTION

Munguba (*Pachira aquatica* Aublet (Malvaceae)) (THE PLANT LIST, 2019) is a fruitful species that can be found from southern Mexico to northern Brazil (DOURADO et al., 2015). The fruits are similar to the ones from the cocoa tree (*Theobroma cacao* L) and have high amounts of lipids, proteins, and carbohydrates (LORENÇON et al., 2016). They can be consumed raw, roasted, cooked or toasted (JORGE & LUZIA, 2012). Inside the fruit, there are several seeds containing high amounts of oil constituted mainly by palmitic and oleic acids (SILVA et al., 2015; RODRIGUES et al., 2019).

Vegetable oils have been studied as alternative sources in the development of biofuel and cosmetic, pharmaceutical, and food products (JORGE & LUZIA, 2012; LORENÇON et al., 2016; OLIVEIRA et al., 2019). The quality of these oils and their bioactive compounds depend on several factors, such as raw materials origin, storage conditions, utilized extraction solvent, and, especially, the extraction process applied (PEREIRA et al., 2017).

Alternative oil extraction methods that minimize time, solvent volume and oil degradation have been studied in order to optimize traditional methods, such as maceration, percolation and Soxhlet extraction (HELENO et al., 2016; HERNÁNDEZ-SANTOS et al., 2016; MARAN et al., 2017). Ultrasonic assisted extraction uses the energy of ultrasonic waves, which cause cavitation processes and lead to high shear forces that cause flaking,

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erosion, and breakage of the particles. Furthermore, they promote the hydration and swelling of the particles, which increase pore size, the diffusion process of the solute and, consequently, mass transfer (TIWARI, 2015; CHEMAT et al., 2017). This method is highly efficient and consumes little energy, making it a viable alternative to traditional methods. It can be successfully adopted in the processing of vegetable samples, besides its sustainable aspects (HERNÁNDEZ-SANTOS et al., 2016). The method also presents other advantages such as low cost and simple technique and equipment requirements (PERRIER et al., 2017).

Therefore, this paper aims to evaluate the quality and the fatty acid profile of the oil obtained from the munguba seeds, extracted by the ultrasound-assisted method.

MATERIALS AND METHODS

Harvesting spot

The munguba fruits were collected at the Parque Florestal in Sinop, Mato Grosso (S11 50'06,20'';W55 30'02,30''), and taken to the quality control laboratory at Federal University of Mato Grosso, Campus of Sinop. The botanical identification was performed at Mato Grosso North-central herbarium (CNMT) located at the Sinop Campus of the Federal University of Mato Grosso. Its exsiccate was stored under the registration number 4506.

Fruits preparation for extraction

The munguba fruits were dried in a forced convection oven at a temperature of 40 ± 2 °C for 48 – 72 hours. This caused the fruits to open, exposing their seeds, and reduced their moisture (<10%). The seeds were then ground using a ball mill.

Oil extraction

The ultrasound-assisted extraction was performed according to RAISER et al., (2018b) using hexane as the extraction solvent. The milled seeds were put in a 1000 milliliters bottle along with the extraction solvent in the ratio of 1:5 (weight/volume [w/v]). This mixture was submitted to an ultrasonic bath (Cristófoli®) for 2 hours at a frequency of 45 kHz and a temperature of 35 ± 2 °C. After that, the extraction liquid was vacuum filtered then evaporated in a rotary evaporator at a temperature of 50 ± 2 ºC, providing the oil which was stored at a temperature of 5 ± 2 ºC.

The sample was also extracted using a Soxhlet apparatus according to RODRIGUES et al., (2019), with some modifications. The sample was packed into the extraction chamber of the Soxhlet extractor; while the solvent hexane was poured into the round bottom flask of the extractor. The whole set-up was assembled on a heating mantle at 60 °C and allowed to reflux for 2 hours. The extract was evaporated using a rotary evaporator at a temperature of 50 ± 2 ºC.

Oil physicochemical characterization

The following tests were performed according to AOCS (2004): %FFA (% of free fatty acids) as oleic acid (Ca 5a - 40), iodine value (Cd 1d – 92), refractive index (Cc7-25), peroxide value (Cd 8b – 90), saponification value (Cd – 25), density (Cc 10a - 25) and melting point (capillary method) (Cc 3-25).

Viscosity was determined on a rotary viscometer (Myr - VR 3000). The sample was kept in a water bath and the viscosity values were measured according to the temperature variation, from 30 °C to 60 °C, using the L2 spindle and rotation speed of 200 rpm.

Thermogravimetric analysis (TG-DTG curves)

A thermogravimetric analyzer (Shimadzu TGA-50) was used to evaluate the oil's thermal stability, moisture, and ash content, providing TG-DTG curves. The samples were assessed in a nitrogen atmosphere, from 30 to 600 °C, at a heating rate of 10 °C min^{-1}, using an α-alumina pan (PARDAUIL et al., 2017).

Differential scanning calorimetry (DSC)

The DSC curve was assessed with a differential scanning calorimeter (DSC 60 Plus/ TAC (Shimadzu)), by weighing approximately 1 mg of the sample into a sealed aluminum pan and then performing the analysis in a nitrogen rate flow of 50 mL/min and a heating rate of 10 °C/min, until reaching 600 °C (PARDAUIL et al., 2017).

Fourier-transform infrared spectroscopy (FTIR)

The FTIR was used as an auxiliary method to characterize the munguba oil. The infrared spectra were obtained with a Fourier-transform infrared spectrophotometer (Shimadzu Iraffinity-1 (Shimadzu)) using an attenuated total reflectance (ATR-IFTR) accessory, in a wavelength range of 4000 to 500 cm^{-1}, 4 cm^{-1} resolution and 32 scans. A zinc selenide crystal was used to perform this analysis (ROHMAN & MAN, 2010).

Gas chromatography-mass spectrometry (CG-MS)

Prior to the analysis, the munguba oil was esterified according to MILINSK et al. (2011) with

modifications, as follows: 50 µL of the oil was mixed with 0.5 ml of a 0.5 mol/L potassium hydroxide methanolic solution and the mix was heated to 70 °C for 10 min. After that, 400 µL of a hydrochloric acid and methanol mix (1:4 v/v [volume/volume]) was added to the previous mixture, and the final solution was heated to 70 °C for 20 minutes. Finally, 1 mL of hexane was added to it and the mixture was vigorously stirred for 5 minutes. After the esterification, 100 µL of the supernatant was collected and diluted with 900 µL of hexane.

The oil's fatty acid composition was determined in a gas chromatograph (Agilent Technologies 7890A) coupled to a mass spectrometer (Agilent Technologies 5975C), using helium as the carrier gas (1.0 mL/min) in the following conditions: 1 µL of sample injection volume; 3:1 split; column: HP5-MS; MS Source: 230 °C; MS Quad: 150 °C; gradient: 140 °C (2 minutes), 4 °C/min until reaching 180 °C, 0.5 °C/min until reaching 200 °C, 5 °C/min until reaching 250 °C (3 minutes); Full time: 70 minutes. The fatty acids were identified based on the standard retention time (Lipid Standards Sigma-Aldrich: FAMEs mixtures C14:0 – C22:0) injected under the same conditions.

Total phenolic content determination

The extraction of the phenolic compounds from the munguba oil was performed according to SCHONS et al. (2017), using 2 g of oil mixed with 5 mL of methanol 60% (v/v) and 2 mL of hexane. This mix was stirred for 10 minutes, then left resting for 10 minutes, followed by 10 minutes of centrifugation at 3000 rpm, aiming to separate the oily phase from the hydroalcoholic phase, the latter was the portion containing the phenolic content of the sample.

The total phenolic content determination was performed following the Folin-Ciocalteu method with modifications (SCHONS et al., 2017). To build the calibration curve, gallic acid was used in concentrations ranging from 1 to 10 µg/mL and total phenolic content values were presented as gallic acid equivalent (microgram of gallic acid equivalents per gram of oil - µgEAG/g).

RESULTS AND DISCUSSION

Oil physicochemical characterization

The ultrasound-assisted extraction presented a 47.70 % yield of a light yellow colored oil. JORGE & LUZIA (2012), OLIVEIRA et al. (2019) and RODRIGUES et al. (2019) obtained yields of 38.39%, 40.4% and 43.42%, respectively, using the Soxhlet extraction method. These yields are considered high, and variations found in the literature can be attributed to several different reasons, such as soil characteristics, weather changes, plant injury, and fruit ripening time, as well as method and solvent used in the oil extraction. Aiming to compare the yield of the ultrasound-assisted method, a Soxhlet extraction was performed using the same dried fruit sample for 2 hours, same time gap used in the ultrasound-assisted method, which provided a yield of 27.20 %, confirming the ultrasound method is more efficient.

The analyzed physicochemical parameters are shown in table 1.

The % of free fatty acids (%FFA) can provide some information about the oil and indicate possible oxidation and deterioration, which can be used as preliminary data about its quality. The munguba oil presented an acid value of 2.06 ± 0.04 %, a value inferior to those found by CAMARGO (2008) and SILVA (2011) using munguba oil extracted by the Soxhlet method (4.31% and 2.97%, respectively). The acidity of an oil can be influenced by its moisture, therefore, the absence of moisture in the oil may be one of the reasons for its low acid value. Furthermore, the mentioned acid values suggest that the munguba oil has naturally higher acid values.

The iodine value and the refractive index evaluation contribute to the determination of the oil's fatty acid unsaturation. Several authors who studied oils in general, observed that the higher the iodine value, higher is the refractive index and, usually, higher is the unsaturation level of the oil's fatty acids (LI et al., 2015; PAUCAR-MENACHO, 2015; GODSWILL et al., 2018).

The iodine value found for the munguba oil was 43.77 ± 0.47 g I_2/100g and the refractive index was 1.465 (40 °C). The presented values suggest a smaller amount of unsaturated fatty acids, showing that most of the munguba oil's fatty acids are saturated. These results were confirmed by the fatty acid profile performed by GC-MS (Table 2). These results are consistent with those found by JORGE & LUZIA (2012), who obtained iodine and refraction index values of 27.4 g I_2/100g and 1.4569 (40 °C), respectively.

The peroxide value analysis aims to investigate the oxidative degradation of the oil, considering the maximum value established by the CODEX ALIMENTARIUS (2019), which is 10 meq/Kg. The value found for the munguba oil was 3.75 ± 0.02 meq/Kg, which shows that the oil was in good state of conservation.

Table 1 - Physicochemical parameters of munguba oil extracted by the ultrasound-assisted method.

Physicochemical Parameters	Value
%FFA (oleic acid)	2.06 ± 0.04
Iodine value (g I_2/100g)	43.77 ± 0.47
Refractive index (40 °C)	1.465
Peroxide value (meq/Kg)	3.75 ± 0.02
Saponification value (mg KOH/g)	181.70 ± 2.78
Density (g/mL)	0.9290
Ash content (% m/m)	1.84 ± 0.00
Moisture (%)	ND*

* ND = Not detected.

The saponification value analyzes the presence of high and low molecular weight fatty acids in the oil, hence, the higher the saponification value, the lower is the fatty acid's molecular weight (MORETTO & FETT, 1998). The value for the munguba oil was 181.7 ± 2.78 KOH/g, which indicates the presence of low molecular weight fatty acids. SILVA (2011) and JORGE & LUZIA (2012) found similar results, 172.0 mg KOH/g and 208.0 mg KOH/g respectively, analyzing an oil extracted by the Soxhlet method. The saponification value presented by the munguba oil highlights its potential for use in the manufacture of edible products, since this result falls within the range established by the CODEX ALIMENTARIUS for crude vegetable oils (168 – 265 mg KOH g^{-1}), comparable to mustard seed oil, palm oil, babassu oil and soybean oil.

Density and viscosity values reflect the degradation processes the oil goes through, since their increase is a result of polymerization reactions that cause the formation of higher molecular weight products within the oil. Such modifications in oil structure may, for instance, compromise the characteristics of cosmetic and pharmaceutical formulations containing this oil. Thus, density and viscosity values work as additional parameters in the evaluation of the oil's quality, as well as for the optimization of operational processes that involve, mainly, the material flowability.

Oil density is influenced by the amount of unsaturation of its fatty acids, hence polyunsaturated fatty acids have higher density values than saturated fatty acids (DENG et al., 2018). Munguba oil presented a relative density value of 0.9290 g/mL at 25 °C, a characteristic value for oils with high amounts of saturated fatty acids, information confirmed by the GC-MS analysis.

Viscosity measures the internal resistance of a fluid's flow. This property varies in vegetable oils, depending on the amount of unsaturated fatty acids, fatty acid chain length and its ramifications. Figure 1 shows the decline of munguba oil's dynamic viscosity

Table 2 - Fatty acid composition of the munguba oil extracted by the ultrasound-assisted method.

Fatty acid	Percentage in weight*
Palmitic C16:0	75.27
Oleic C18:1	8.19
Linoleic C18:2	6.91
Stearic C18:0	4.37
Elaidic C18:1	1.13
Arachidic C20:0	0.27
Myristic C14:0	0.26
Behenic C22:0	0.01

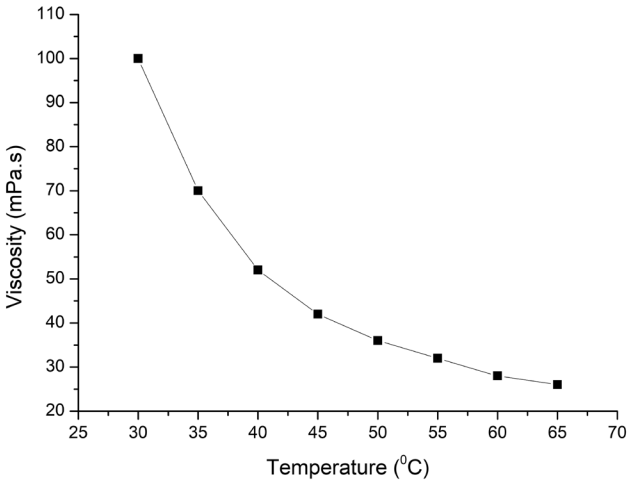

Figure 1 - Viscosity of the munguba oil extracted by the ultrasound-assisted method versus temperature increase.

as temperature increases. The variation in viscosity values is higher under lower temperatures and it goes down as temperature goes up. This happens due to a decrease in the intermolecular forces, which leads to a separation of the fatty acid molecules, consequently causing a downturn in the viscosity values (DAVIES, 2016; SAJJADI et al., 2016).

Thermogravimetric analysis (TG-DTG curves)

The TG-DTG curves (Figure 2) provide moisture and ash content values. The oil presented an absence of moisture, which reinforces the quality of the extraction process, favoring the stability of the oil since it brings down the possibility of hydroelectrolytic oxidation, enzyme action and microbial growth. The ash content provides preliminary information about nutritional value, due to the presence of minerals, as well as identifying possible adulteration and presence of impurities. Results for ash content (1.84 ± 0.00 %) in the munguba oil suggest small amounts of inorganic compounds and/or impurities. On that matter, RODRIGUES et al., (2019) found an ash content value of 4.160% in the munguba seed, showcasing high amounts of minerals, like potassium and zinc. TG-DTG curves also allowed investigation of the oil's thermal stability. The oil remained stable until 300 °C and from that point on, the mass decreased by 98.16%, due to decomposition processes and/or oil oxidation.

Differential scanning calorimetry (DSC)

The DSC curve (Figure 3) presented both endothermic and exothermic events. The first endothermic peak occurred at 32.2 °C and refers to the melting point of the oil, corroborating the value of 32.0 °C provided by the test performed with the capillary tube (Cc 3-25). The enthalpy involved in this process was -54.87 J/g. Endothermic and exothermic peaks presented over 300 °C are probably due to oil degradation, which matches the results observed on the TG-DTG curve. The DSC curve is an excellent tool used in the quality control of oils, since it is a fast analysis method, uses small amounts of sample, and can be used in its identification and purity evaluation.

Fourier-transform infrared spectroscopy (FTIR)

FTIR is commonly used in molecular structure identification, providing absorption bands directly related to functional groups, and allows monitoring of changes in the quality of a substance (BENDINI et al., 2007; ROHMAN & MAN, 2010).

The main absorption bands presented in the infrared spectrum (Figure 4) show symmetrical and asymmetrical bond stretching at 2924 and 2854 cm^{-1}, indicating a saturated carbon chain (C - H), and a prominent axial bond stretch band (C = O) at 1747 cm^{-1}. Some more stretching bands can be found in the fingerprint region, which occurs between 1650 and 500 cm^{-1}, however, some specific bands stand out, like

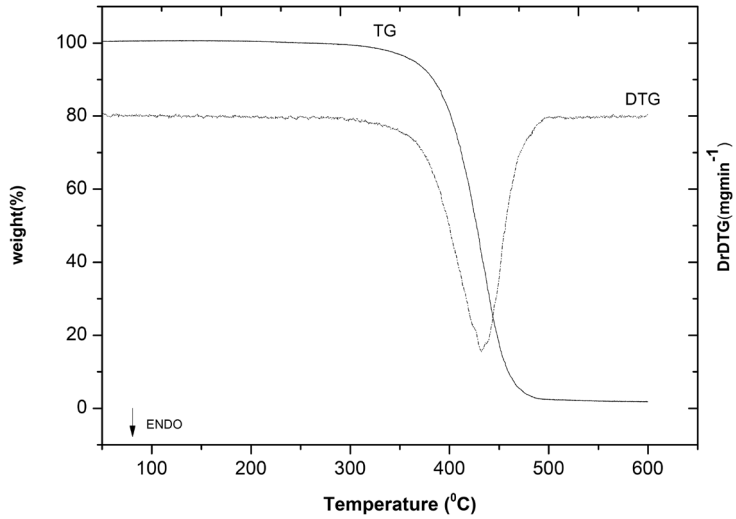

Figure 2 - Thermogravimetry and derivative thermogravimetry curves of munguba oil extracted by the ultrasound-assisted extraction method.

the one related to ester groups bond stretches (1149 and 1107 cm^{-1}) and the one related to the rocking vibration from the CH_2 bond (722 cm^{-1}). Furthermore, it's worth noting that the exact band position and the detection intensity are directly dependent on the fatty acids' composition (SILVERSTEIN et al., 2005; SHI et al., 2017).

Gas chromatography-mass spectrometry (CG-MS)

GC-MS analysis shows that the fatty acids present in the munguba oil are mostly saturated (Table 2), confirming the results provided by the iodine value, refractive index, density and viscosity analysis.

The oil presented 80.18% of saturated fatty acids and 19.82% of unsaturated fatty acids.

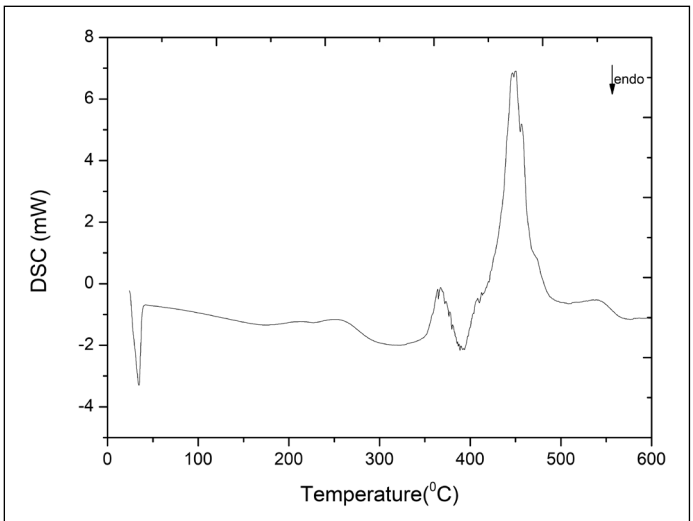

Figure 3 - Differential scanning calorimetry curve of the munguba oil extracted by the ultrasound-assisted extraction method.

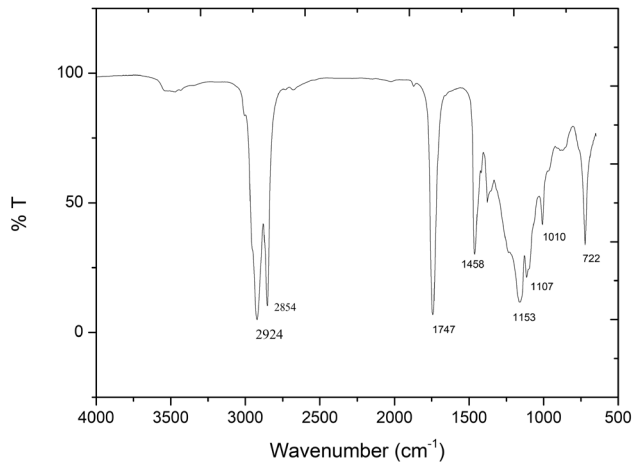

Figure 4 - Fourier-transform infrared spectrum of the munguba oil extracted by the ultrasound-assisted extraction method.

Palmitic acid was found at a higher percentage (75.27%) amongst the saturated fatty acids, followed by oleic acid (8.193%) and linoleic acid (6.906%). Other authors who studied the munguba oil also found palmitic acid as its main fatty acid (SILVA, 2011; JORGE & LUZIA, 2012; RODRIGUES et al., 2019; OLIVEIRA et al., 2019), and RODRIGUES et al. (2019), and OLIVEIRA et al. (2019) found fatty acid percentages similar to this study, using the Soxhlet oil extraction method. Variation in fatty acid amounts might be due to climate and soil specific characteristics, seasonal period and weather changes to which the plants were exposed, as well as the extraction method.

The presence of palmitic acid, the main fatty acid found in the oil, favors the use of the oil in pharmaceutical and cosmetic products. KIM et al. (2008) studied the stimulating effects of fatty acids in the diclofenac permeation on the skin and found that palmitic acid showed a major potential as an adjuvant. RAISER et al. (2018a), developed and evaluated the quality and stability of cosmetic formulations containing 3 and 5% of munguba oil and found that the spreadability of formulations was optimized with the increase of oil amounts in it.

Total phenolic content determination

The phenolic content determination was performed in order to check the natural antioxidant content of the oil, even if in small quantities, since phenolic compounds have a positive influence on human health. Plus, the presence of these compounds helps to avoid lipid oxidation, reducing chances of oil deterioration, as well as of the products developed from it.

The Folin Ciocalteu method performed with the munguba oil presented as results, a line equation ($y = 0.0098x + 0.0292$), with a coefficient of determination $r^2 = 0.9995$ and phenolic content of 55.02 ± 1.87 μgEAG/g. RODRIGUES et al. (2019), evaluated the total phenolic content in the munguba seed and found a value of 775.17 μgEAG/g of dried matter. The results from the oil (55.02 ± 1.87 μgEAG/g), when compared with the results from the munguba seed, suggest that the oil is responsible for about 7% of the total phenolic compounds in the seed, which can contribute to biological and nutritional use.

CONCLUSION

The results from this study show that the ultrasound-assisted extraction method provided efficiency to the extraction process and satisfactory yielding, making it a suitable alternative to the traditional methods, since it is an easy method to perform, which uses low amounts of solvent, is quick, efficient and low cost. Furthermore, the provided oil presented good quality characteristics, thermal stability and contained important fatty acids and an interesting amount of phenolic compounds, making it a suitable and sustainable option for use as an adjuvant in the manufacturing of food products, cosmetics, pharmaceuticals and biofuels.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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