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Ultrasound-assisted extraction of red mombin seed oil (*Spondias purpurea* L.): phenolic profile, fatty acid profile and chemical characterization of the cake, residue from the oil extraction

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SUMMARY: The ultrasound-assisted method was used to extract oil from the red mombin seed, mainly aiming to analyze yield. A multivariate analysis served to define optimized parameters (6.46 minutes and S/S ratio of 1:23.10 mass:volume) for ultrasound-assisted extraction (UAE) with the objective of maximizing yield, using the response surface methodology (RSM) and desirability graph with central variables and axial points determined by the central composite rotatable design (CCRD). In addition to the optimization of oil extraction, oil was chemically characterized in terms of antioxidant capacity and nutritional aspects to test the quality and chemical characteristics of red mombin seed oil extraction residue (cake). Analyses showed 32% unsaturated fatty acids, such as palmitoleic acid, linolelaidic acid, and α-linolenic acid, and the presence of phenolic compounds, especially catechin. High dietary fiber content and the presence of phenolic compounds, such as chlorogenic acid, vanillin, and gallic acid, were found in the cake, which allows the possibility of incorporating this material into food products.

KEYWORDS: *Bioactive compounds; Emerging technologies; Fruit processing waste; Nutraceuticals; PUFAs; Red mombin cake.*

RESUMEN: Extracción asistida por ultrasonidos del aceite de semillas de ciruelas rojas (*Spondias purpurea *L.): perfil fenólico, ácidos grasos y caracterización química de la torta, residuo de la extracción del aceite. Se utilizó el método asistido por ultrasonido para extraer el aceite de semillas de ciruelas rojas, principalmente con el objetivo de analizar el rendimiento. Un análisis multivariante permitió la elección de los parámetros óptimos (6.46 minutos y una relación S/S de 1:23.10 masa:volumen) para extracción asistida por ultrasonido (EAU) con el objetivo de maximizar el rendimiento, utilizando la metodología de superficie de respuesta (RSM) y un gráfico de optimización, con variables centrales y puntos axiales determinados por el diseño giratorio de compuesto central (CCRD). Además de la optimización de la extracción del aceite, éste se caracterizó químicamente con respecto a la capacidad antioxidante y los aspectos nutricionales, para probar la calidad y las características químicas la torta, residuo de la extracción del aceite de semilla de ciruela roja. Los análisis mostraron un 32% de ácidos grasos insaturados, como el palmitoleico, linolelaídico y ácido α-linolénico, y la presencia de compuestos fenólicos, especialmente catequina. En la torta se encontró un alto contenido de fibra dietética y la presencia de compuestos fenólicos, como ácido clorogénico, vainillina y ácido gálico, lo que permite la posibilidad de incorporar este material en los productos alimenticios.

PALABRAS CLAVE: *Compuestos bioactivos; Nutracéuticos; PUFA; Residuos del procesamiento de frutas; Tecnologías emergentes; Torta de ciruela roja*

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1. INTRODUCTION

Fruit production has increased in Brazil since the 1990s, with cultivation conditions and novel technologies being applied in the market. Moreover, industry expansion regarding the processing of plant material is also developing, such that fruit processing may originate agricultural and industrial waste, adding up to 0.5 billion tons of waste worldwide, thus generating environmental, social and economic impacts (Banerjee *et al.,* 2017; Papargyropoulou *et al.,* 2014; Girotto *et al.,* 2015; Parfitt *et al.,* 2010).

The red mombin (*Spondias purpurea* L.) is a tropical fruit belonging to the Anacardiaceae family and is native to Central America, and is distributed on other continents, such as Asia and Africa, due to its ease of adaptation to varying climates (Engels *et al.,* 2012; Omena *et al.,* 2012; Maldonado-Astudillo *et al.,* 2017). The management, processing, and commercial activity of the seriguela are linked to regional development because its cultivation is still based on informal agricultural practices. Despite this, it is consumed and marketed in different ways, such as juices, nectars, sweets, and sorbets (Pereira and Santos 2016). The processing of the fruit is based on its composition so that in its ripe stage the seriguela, from northeastern Brazil, for example, has an average of 70% pulp, 14% peel and 16% seed (Maldonado-Astudillo *et al.,* 2014; Alia-Tejacal *et al.,* 2012). Therefore, the processing of seriguela generates on average 540 tons of seed residue (Pereira and Santos, 2016).

Red mombin seeds are a type of waste originated from juice and nectar industries and have been the subject of studies assessing nutritional quality of interest to industries, especially for foods and pharmaceuticals, due to compounds such as vitamin C, bioactive compounds, carotenoids, and oil (Omena *et al.,* 2012; Maldonado-Astudillo *et al.,* 2017). Within this context, red mombin seeds have been shown to present compounds with interesting biological activities, such as anticholinesterasic activity, due to high concentrations of chlorogenic acid, and antioxidant capacity, which has been attributed to compounds such as rutin, quercetin, rhamnetin, and kaempferol, which also present anti-inflammatory and antitumor activities (Engels *et al.,* 2012; Omena *et al.,* 2012; da Silva and Jorge 2014a; Lesjak *et al.,* 2018; Maldonado-Astudillo *et al.,* 2017; Silva e Lima and Meleiro 2012).

Conventionally, oil extraction from seeds is carried out by pressing followed by the use of solvents, such as n-hexane. However, this type of extraction is applied to oilseeds that present oil contents above 30%. Novel extraction methods are necessary in order to increase the yield and quality of seeds with lower oil concentrations. Ultrasound-assisted extraction (UAE) has been used as an alternative to reduce problems related to conventional extraction, such as high temperatures, long periods of extraction, and to reduce environmental concern regarding the use of high concentrations of solvents (Zhang *et al.,* 2008; Sicaire *et al.,* 2016).

Thus, UAE presents advantages compared to conventional extractions, since this method increases the transfer from mass into liquid using cavitation forces because the bubbles formed during this process disturb cell walls, therefore increasing the release of components of interest into the medium (Chielle *et al.,* 2016). This prevents damages to the structures and properties of compounds in matrices because the procedure can be conducted at low or even room temperatures. Therefore, the amount of n-hexane used in oil extraction decreases, less time is needed, and maintaining performance becomes desirable, which is an essential issue for industries due to economic and environmental reasons (Zhang *et al.,* 2008; Sicaire *et al.,* 2016).

Thus, the objective of the present study was to optimize the ultrasound-assisted extraction of red mombin seed oil, evaluating yield as the main variable, using the response surface methodology (RSM) under various extraction conditions, such as time and solid:solvent ratio. In addition, the study also aimed to characterize the oil obtained regarding its antioxidant capacity and nutritional aspects, to test its quality and evaluate the characteristics of red mombin seed oil extraction residue, herein called "cake", for possible applications of this material.

2. MATERIAL AND METHODS

2.1. Standards and chemical reagents

The chemical reagents ABTS ((2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), β-carotene, TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), DPPH (2,2-di-

phenyl-1-picrylhydrazyl), Tween 40, and phenolic compound standards (gallic acid, catechin, chlorogenic acid, caffeic acid, ferulic acid, *trans*-cinnamic acid, vanillin, quercetin, *m*-coumaric acid, *p*-coumaric acid, *o*-coumaric acid) were obtained from Sigma-Aldrich (Saint Louis, Missouri, USA). Methanol (HPLC grade) was obtained from J-TBacker (Alfragide, Lisbon, Portugal). In turn, the Folin-Ciocalteu reagent was obtained from Dinâmica (Indaiatuba, São Paulo, Brazil). Acetone, acetic acid, gallic acid, linoleic acid, methanol, and chloroform were obtained from Vetec (Saint Louis, Missouri, USA), while sulfuric acid was obtained from Merck (Darmstadt, Hessen, Germany). Ethanol, was obtained from Synth (Diadema, São Paulo, Brazil), while n-hexane and potassium persulfate were obtained from Neon (São Paulo, São Paulo, Brazil). All reagents were analytical grade, and stock and buffer solutions were prepared using distilled water.

2.2. Red mombin seed preparation

Red mombin seeds were donated by the company Frutos do Brasil, located in the municipality of Goiânia, state of Goiás, Brazil. Fruits were originally collected from the municipality of Santa Maria da Vitória, state of Bahia (13° 23' 41" S, 44° 11' 19" W, 436 m), Brazil, during the 2016/2017 harvest. Seeds were washed and sanitized using a 200 ppm sodium hypochlorite (NaOCl) solution for 30 minutes to eliminate all vegetative forms of microorganisms, thus avoiding seed deterioration during the storage period. The drying process was carried out by placing a portion of red mombin seeds in a forced-air convection oven at 50 °C (TE 394/4, Tecnal, Piracicaba, Brazil) until they reached constant weight, stored in low-density polyethylene bags, and kept in a freezer at -18 °C until analyses. Red mombin seeds were defrosted at room temperature and dried using a forced-air convection oven (TE 394/4, Tecnal, Piracicaba, Brazil) at 50 °C until they reached constant weight. Seeds were pulverized using a knife mill for subsequent oil extraction.

2.3. Ultrasound-assisted extraction (UAE) and experiment design

Ultrasound-assisted extraction (UAE) was used to extract red mombin seed oil. An ultrasonic bath (USC2800A, Logen Scientific, São Paulo, Brazil; frequency 40 Khz; internal dimension: 293 x 235 x 150 mm) was used for this procedure. Extraction was carried out under various experimental conditions which were defined in pre-tests and presented two independent factors: time (x1, 5 - 15 min), and solid:solvent ratio (x2, 1:5 - 1:20) (mass:volume).

Table 1. Ultrasonic-assisted red mombin seed oil extraction matrix for the construction of the central rotational compound design (DCCR) and the experimental result of the yield

Variables	Levels				
	Axial (-)	-1	0	+1	Axial (+)
Time (x1,min)	3	5	10	15	17
S/S ratio (x2,m:v)	1.90	5	12.5	20	23.1
No	Blockes	Time (x1, min)	S/S ratio (x2, m:v)	Oil Yield (%)	
				Experimental	Predicted
1	1	-1 (5)	-1(1:5)	4.99	8.73
2	1	-1(5)	1(1:20)	58.93	58.99
3	1	0(15)	-1(1:5)	7.99	15.19
4	1	0(15)	1(1:20)	54.90	58.41
5[c]	1	0(10)	0(1:12.5)	44.97	45.71
6 [c]	1	0(10)	0(1:12.5)	47.98	45.71
7[*]	2	-1.41(3)	0(1:12.5)	42.96	45.92
8[*]	2	+1.41(17)	0(1:12.5)	52.00	45.92
9[*]	2	0(10)	-1.41(1:1.90)	0.00	-6.23
10[*]	2	0(10)	+1.41(1:23.10)	60.89	59.86
11[c]	2	0(10)	0(1:12.5)	44.92	45.71
12[c]	2	0(10)	0(1:12.5)	44.97	45.71

[C] Central point; [*] Axial point.

N-hexane was used as the solvent and the temperature was held at 30 °C during extraction. The extract obtained was filtered using a qualitative filter paper into previously dried and zeroed 50 mL beakers to determine lipid content (P_0). Next, the material was placed in an oven at 105 °C and dried until reaching constant weight. Lipid weight was then determined. Relationships between response and variables were established according to a central composite rotatable design (CCRD) (Table 1).

The experiment design consisted of 12 trials, including two central points, determined to assure data repeatability (runs 5, 6, 11, and 12), and two axial points per block. Extraction yield was selected to combine independent variables. Thus, maximum yield shown by the design was chosen for multiple extractions, from which the solvent was removed using a rotary evaporator at 50 °C and traces of the solvent were removed using a vacuum oven (MA 120/TH, Marconi Equipamentos Para Laboratórios Ltda, Piracicaba, São Paulo, Brazil) for 24 hours at 30 °C. The oil extracted was stored in amber bottles and covered with aluminum foil until the characterization and oxidative profile analyses.

A full description of the experimental design requires a cubic model. Lipid yield (Y) is related to independent variables coded as x_i and x_j, according to the second-degree polynomial, such as in equation 1, where β_0 is the interaction coefficient, β_i is the linear term, β_{ii} is the quadratic term, and β_{ij} is the interaction term.

$$Y = \beta_0 + \sum \beta_i x_i - \sum \beta_i x_i^2 + \sum \beta_{ii} xj - \sum \beta_{ii} x_j^2 - \sum \beta_{ij} xixj \quad (1)$$

2.4. Determination of extraction yield

After extraction, the determination of red mombin seed oil yield followed the methodology described by Chanioti and Tzia (2017):

$$Yield\ (\%) = \frac{P_0}{P_a} * 100 \quad (2)$$

Where P_0 and P_a refer to lipid content in the sample and sample weight (g), respectively.

2.5. Characterization of red mombin seed cake and oil

The objective of characterizing the red mombin seed extraction residue, or cake, was to test its possible technological applications. Analyses were conducted in triplicate and presented proximal composition, such as moisture, ashes, proteins, and total carbohydrates (TC) by difference (TC = 100 - (moisture + ashes + proteins + lipids)), as well as hydrogenionic potential, total titratable acidity, expressed in g·100 g^{-1} of organic acids, soluble solids (AOAC 2016), and lipids using the Bligh-Dyer method (Bligh and Dyer, 1959). Results were expressed in g·100 g^{-1}. Caloric value was calculated according to Atwater and Woods (Atwater and Woods, 1896).

With the aim of characterizing red mombin seed oil, the following tests were conducted: hydrogenionic potential; acidity index; refraction (AOCS 2013); and peroxides, using a semi-quantitative Quantofix® Peroxide kit (Macherey-Nagel GmbH & Co.KG, Düren, Germany).

2.6. Cake extracts

The preparation of extracts to evaluate the antioxidant capacity of the cake followed a modified version (Zieliński and Kozłowska 2000). In sum, five different extracts were obtained: ether extract (ETE); alcoholic extract (ALE); aqueous extract (AQE); methanol:acetone:water extract (50% methanol:70% acetone:distilled water) (MAWE), at a proportion of 2:2:1; and hydroalcoholic extract (70% ethanol (v/v)) (HAE). In order to obtain ETE, 2.5 g of the sample were homogenized under agitation and sheltered from light using a 1:20 (m/v) ethyl extracting solution for one hour at room temperature. The extract was then filtered, transferred to a volumetric flask, and the final volume was adjusted according to the extracting solution volume initially used. The residue recovered was used to obtain other extracts following the same procedure, although with ethanol to obtain ALE and distilled water to obtain AQE.

Regarding the preparation of MAWE, 20 mL of 50% methanol were added to the sample and left to rest for 1 hour. The extract was then filtered and transferred to a 100-mL volumetric flask. Soon after, 70% acetone was added and the same procedure was again conducted, such that the filtrate was added to the same flask that had been used previously. At the

end, the volume was completed using distilled water. In turn, HAE was prepared by adding 50 mL of 70% ethanol and macerated for one hour, followed by filtration. At the end of the extractions, all extracts were placed in amber bottles and stored in a freezer at -18 °C until the determination of antioxidant capacity (Rufino *et al.,* 2010).

2.7. Extracts of the red mombin seed oil

A methanolic extract was used to evaluate reducing capacity (Wang *et al.,* 2017). In sum, 1 g of oil was weighed and placed into a centrifuge tube with 2.5 mL of n-hexane and 3 mL of a methanol:water solution (60:40, v/v). The mixture was agitated for 3 minutes in a vortex equipment, and the tube was then placed in a refrigerated centrifuge (5403, Eppendorf AG, São Paulo, Brazil) at 3,500 g for 10 minutes at 4 °C. Thus, the lower methanolic layer was separated and this operation was repeated three times. The methanolic extract was evaporated at 35 °C using a forced-air convection oven until completely dry. Next, the extract was resuspended in 1 mL of the methanol:water solution (60:40, v/v). The extract was stored in an amber bottle and refrigerated at 4 °C until analyses.

In order to determine antioxidant capacity using the DPPH and ABTS methods, the red mombin seed oil and isopropyl alcohol (1:10 p/v) extract was prepared based on its solubilization (Martins *et al.,* 2013).

2.8. Antioxidant capacity

Red mombin seed cake extracts – ETE, ALE, AQE, MAWE, HAE – and oil methanolic extract were used to determine reducing capacity using the Folin-Ciocalteu method, expressed in mg of gallic acid equivalents per 100 g of sample. The cake extracts and the isopropyl alcohol extract were used to determinate DPPH (2,2-diphenyl-1-picrylhydrazyl), expressed in IC_{50} ($mg \cdot mL^{-1}$) and ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), expressed in µmol of Trolox equivalents per gram of sample. In addition, the determination of antioxidant capacity using the ferric reducing antioxidant power (FRAP),expressed in µmol of $FeSO_4$ per gram of sample, and β-carotene/linoleic acid method, expressed in percentage of protection, was only made for the cake extracts (Rufino *et al.,* 2010; Zieliński and Kozłowska 2000).

2.9. Phenolic compound profiles in red mombin seed cake and oil

The extract was used to identify phenolic compounds in the cake through the chromatographic method (Ramaiya *et al.,* 2013a). For this extraction, 2.5 g of red mombin seed cake was homogenized in 20 mL of 70% HPLC grade methanol (v/v) for one hour in an ultrasonic bath at room temperature. The extract obtained was centrifuged at 14,000 rpm for 15 min at 4 °C and filtered using a qualitative filter paper. In order to inject samples, extracts were filtered again using 0.45-µm porous membrane filters.

In turn, the extract used to identify phenolic compounds in red mombin seed oil using the chromatographic method was prepared following Wang *et al.* (2017). The extract was refrigerated at 4 °C until analysis.

2.10. Phenolic compound profiles

The quantification and identification of phenolic compounds in red mombin seed oil and cake were carried out using high-performance liquid chromatography (HPLC-DAD/UV-Vis) (Gonçalves *et al.,* 2017), with a Shimadzu model (Shimadzu Corporation, Kyoto, Japan) equipped with four high-pressure pumps (model LC-20AT), photodiode array detector (model SPD-M20A), degassing unit (model DGU-20A5), CBM-20A interface, CTO-20AC column oven, and an autosampler (model SIL-20A). Separation was done by means of a Shimadzu Shimpack ODS GVP-C18 column (4.6 x 250 mm, 5 mm) connected to a pre-column (Shimadzu-pack ODS GVP-C18, 4.6 x 10 mm, 5 µm). The mobile phase consisted of 2% (v/v) acetic acid in deionized water (mobile phase A) and a 70:28:2 (v/v) ratio of methanol:water:acetic acid (mobile phase B) at a flow rate of 1.0 $mL \cdot min^{-1}$ with a gradient elution program: 20% B (0-5 min), 45% B (25-43 min), 80% (50 min), 0% B (55-65 min), and execution time of 65 minutes. Injection volume was 20 µL and analyses were conducted at 15 °C. Phenolic compounds were detected at 280 nm. Standard solutions were diluted in methanol (HLPC grade) and calibration curves were obtained by injecting ten different concentrations in duplicate. Phenolic compounds were identified by comparing retention times and standards (gallic acid, catechin, chlorogenic acid, caffeic acid, ferulic acid, *trans*-cinnamic acid, vanillin, quercetin, *m*-coumaric

acid, *p*-coumaric acid, *o*-coumaric acid). Results were expressed in mg of phenolic compound·100 g^{-1} of the sample.

2.11. Fatty acids profile

Methylation of the oil extracted (AOCS, 2013) was carried out with the objective of producing fatty acid methyl esters (FAME). The esters which resulted from this esterification step underwent a gas chromatography analysis using GC 2010 SHIMADZU equipment with flame ionization detector (FID) and capillary column (100 m x 0.25 mm x 0.2 μm). The chromatographic conditions were: a) Injector: 1-μL of the sample was injected with execution time of 60 min operating in the split mode, using helium drag gas at a flow rate of 1.0 mL·min^{-1}; b) Column: initial temperature of 140 °C, held for 5 minutes, and raised at a rate of 4 °C·min^{-1} to 240 °C. The stationary-phase column consisted of biscyanopropyl polysiloxane. Fatty acids were identified and quantified by comparing the retention time of esters in the Supelco 37 standard of the components in the FAME mixture (CRM47885 - CAS 75-09-2) with the samples.

2.12. Statistical analysis

The design and coefficient of the predictive models were obtained using Statistic 12 software (Statsoft, Tulsa, USA). The analysis of variance (ANOVA) was used to analyze data from red mombin seed oil extraction tests and indicate significant differences ($p < 0.05$). The variable presenting the lowest p value (or highest F value) indicated the most significant effect ($p < 0.05$) of responses. The response surface methodology (RSM), combined with the desirability function, was used to evaluate process optimization. The results from the red mombin seed cake and oil characterization analyses were expressed in mean ± standard deviation and were conducted in triplicate. The analyses of residue antioxidant capacity were submitted to a 5%-probability Tukey's test for extract variables.

3. RESULTS AND DISCUSSIONS

3.1. Effects of ultrasound-assisted extraction on red mombin seed oil yield

The experimental value and the predicted yield of red mombin seed oil extraction carried out through various experiments, under various conditions, are shown in Table 1.

The highest oil extraction yield was obtained in experiment 10, in which extraction conditions were 10 min and solvent amount was 23.10 mL of solvent per gram of solid. In light of the yield obtained, Table 2 demonstrates the effect of independent variables on the dependent variable. The independent variable solid:solvent (mass:volume ratio) presented a positive effect ($p < 0.05$) on the linear and quadratic term, while "time" and "interaction among factors" did not have any effect on red mombin seed oil extraction ($p > 0.05$).

3.2. RSM analysis

Model adequacy was evaluated using the analysis of variance (ANOVA), shown in Table 3. The regression model adjusted for the experiment results presented a coefficient of determination (R^2) of 0.9682, which indicates that 96.82% of total variability of responses was attributed to the experimental variables studied. In turn, the adjusted R value of 0.9417 showed a direct relationship between experiment values and predicted yield values. The F value of the model was 36.5808 and was lower than the p value (0.05), which along with the p value for lack of ad-

TABLE 2. **Analysis of the effect of the conditions (Time and S/S ratio) on the yield of red mombin seed oil extraction.**

Factors	Efects	SD	t (3)	p
Mean	45.7115	0.7565	60.4212	0.0000
S/S ratio (g·mL^{-1}) (L)	46.7398	1.0699	43.6854	0.0000
S/S ratio (g·mL^{-1}) (Q)	-18.8956	1.1962	-15.7962	0.0000
Time (min) (L)	2,9404	1.0699	2.7482	0.0708
Time (min) (Q)	-1.8622	1.1962	-1.5568	0.2173
S/S ratio x Time (L)	-3.5146	1.5130	-2.3228	0.1028

L: linear; Q: quadratic. ($p < 0.05$).

TABLE 3. **Analysis of variance (ANOVA) of the model adjusted for yield.**

Source	Sum of squares	DF	Mean square	Fvalue	Ftab	R^2	Radj
Model	4976.259	5	995.2518	36.5808	4.39		
Residual	163.241	6	27.2069	-	-		
Lack of fit	156.373	3	52.124	22.7670	9.28		
Pure error	6.868	3	2.289	-	-		
Correlation Total	5139.500	11	-	-	-	0.9682	0.9417

justment indicated that the model precisely adjusted to the experimental data.

The mathematical model codified for red mombin seed oil extraction yield is a second-order polynomial and is represented by equation 3.

$$Y = 45.71 + 46.74x_1 - 18.90x_1^2 \quad (3)$$

A tridimensional (3D) response surface graph was constructed and is shown in Figure 1. These types of graphs are useful to determine maximum and minimum points. In turn, contour graphs are useful to determine how variables influence the desired responses (Li *et al.,* 2012). Thus, the effects of solid:solvent ratio, time, and response analyzed were assessed and the yield value increased until it reached a level > 60%, with a solid:solvent ratio of 1:23.10. In addition, taking into account the industrial extraction process, in order to reduce energy and time expenditure, experiment 2 showed a yield close to the maximum, at around 59%, but with half the extraction time applied in experiment ten. In order to reach maximum yield value, the minimum time required is approximately 2 min.

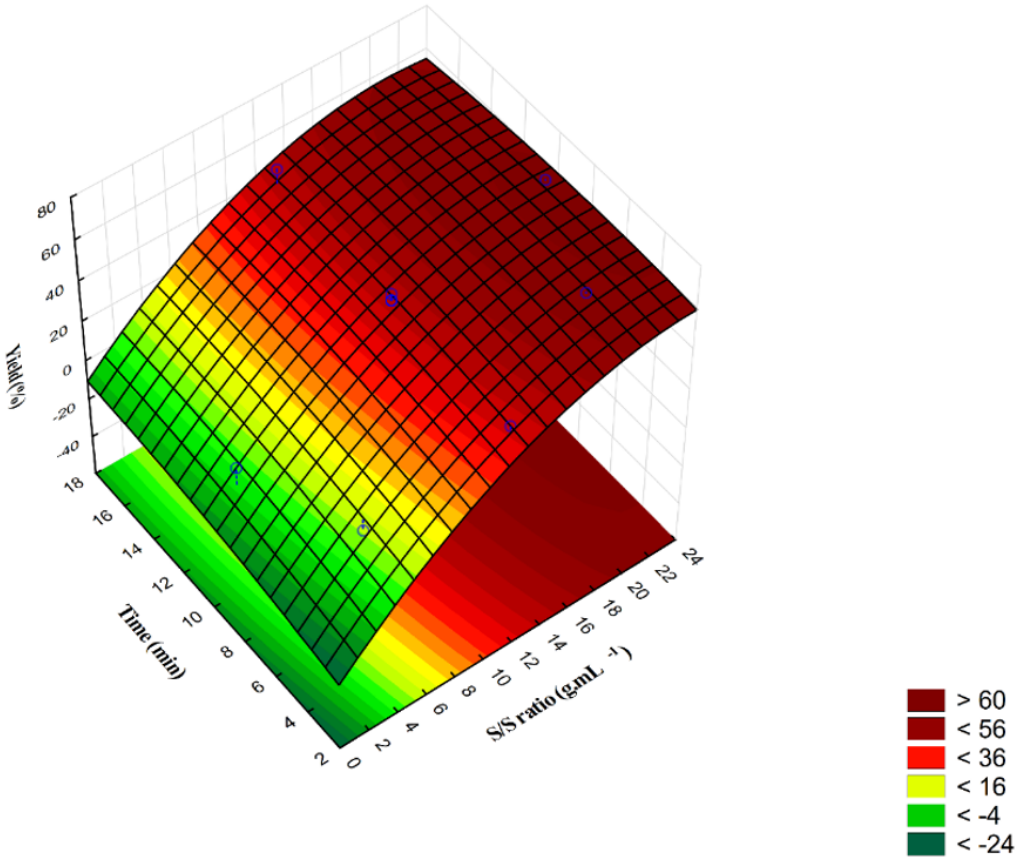

FIGURE 1. Response surface of the yield from red mombin seed oil extraction.

3.3. Optimization of the conditions for red mombin seed oil extraction

The desirability function was applied to optimize the ultrasound-assisted extraction of red mombin seed oil. The optimized values are shown in Figure 2.

As observed in Figure 2, the optimized point to obtain a yield value above 60% would be after 6.46 min, with a solid:solvent ratio of 1:23.10. Under these optimized conditions, the predicted value for extraction yield was 60.89%, while the experimental value observed was 62.85 % (n = 3), thus validating the model. The strong correlation observed between real and predicted values confirms that the model was adequate to reflect the optimization expected. Only the optimized values of extracted red mombin seed oil and its cake were characterized.

A satisfactory adjustment of the quadratic model was observed for the ANOVA and parametric analyses, such as R^2. The results showed that predicted and experimental values were significantly different. However, the present study showed that the combination of emerging ultrasound extraction technology and natural raw material, such as the residue from red mombin seeds, is an economic alternative for traditional extraction methods, considering industry demands and sustainable development. However, more advanced studies should be conducted, using less harmful solvents and other extraction conditions.

3.4. Phenolic compound and fatty acid profiles

The identification of phenolic compounds in red mombin seed cake and oil is shown in Table 4. A few classes of phenolic compounds could be identified in red mombin seed cake, such as simple phenols, cinnamic acids, and flavonoids. This shows that after extracting the oil, the residue still presented phenolic compounds with biological activities of interest,

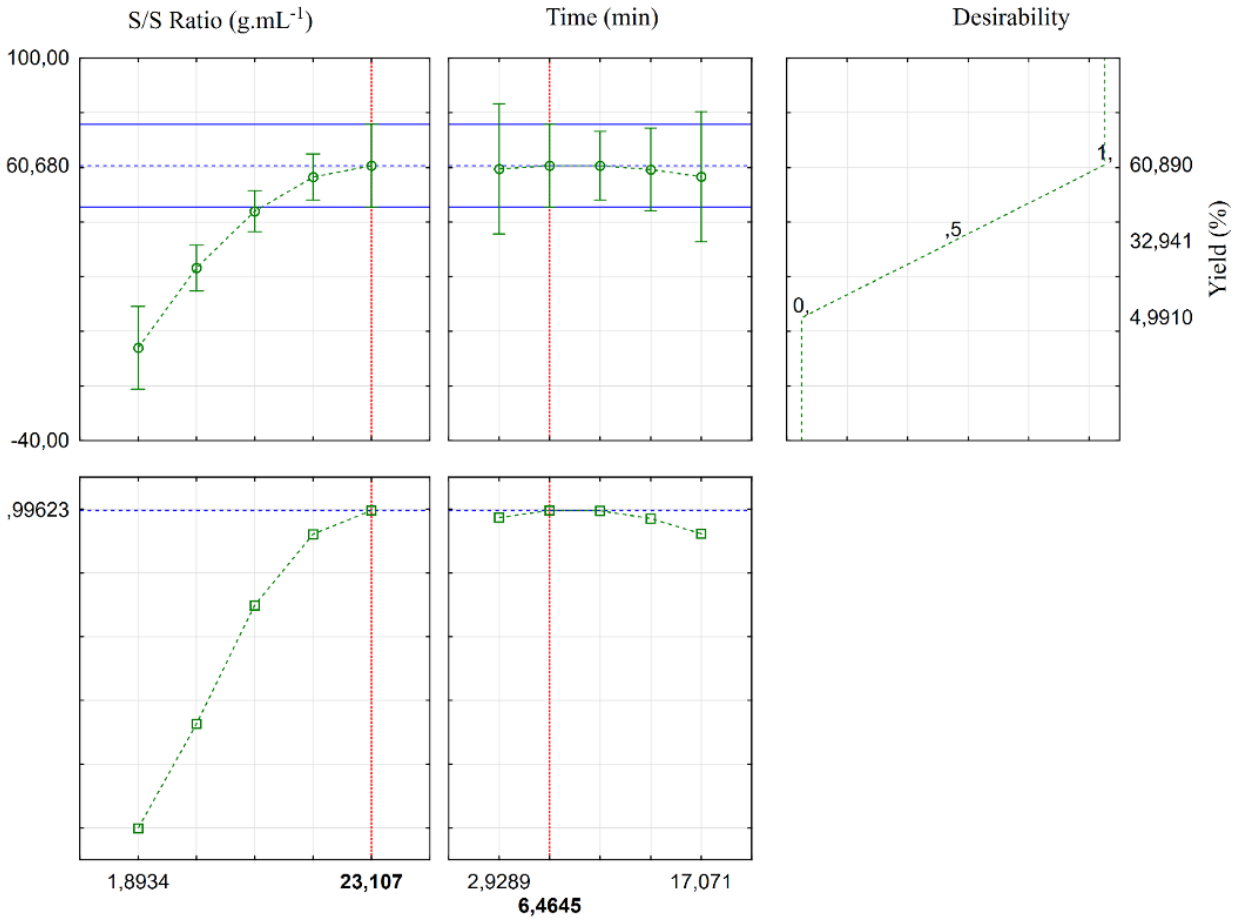

FIGURE 2. Desirability graph of the yield from the red mombin seed oil extraction.

TABLE 4. **Profile of phenolic compounds of the cake and oil and fatty acids of red mombin seed oil obtained by USA.**

Phenolic compounds	**Retention time (min)**	**Phenolic profile (mg·100g^{-1})**	
		Red mombin seed cake	**Red mombin seed oil**
Acid galic	6.82	2.71±0.24	0.56±0.02
catechin	10.80	30.70±4.79	N.D.
Chlorogenic acid	12.56	N.D.	1.08±0.04
Caffeic acid	15.05	N.D.	N.D.
Vanillin	17.65	N.D.	0.52±0.06
M-coumaric acid	24.42	N.D.	N.D.
Ferulic acid	27.79	N.D.	N.D.
Quercetin	37.59	N.D.	N.D.
Trans-cinnamic acid	51.66	0.36±0.02	N.D.
Fatty Acid		**Red mombin seed oil (g·100g^{-1})**	
Saturated			
C8:0		0.50±0.00	
C12:0		0.57±0.24	
C13:0		1.04± 0.57	
C14:0		1.30±0.42	
C15:0		0.48±0.17	
C16:0		16.32±0.87	
C18:0		8.69±0.15	
C20:0		40.77±2.89	
C22:0		0.22±0.02	
C24:0		0.29±0.02	
Monounsaturated			
C16:1		0.47±0.05	
Polyunsaturated			
C18:2 *trans* n-6		30.89±2.14	
γ C18:3 n-6		0.43±0.03	
C18:3 n-3		1.56± 0.19	
SFA		70.18	
MUFA		0.47	
PUFA		32.68	
PUFA/SFA		0.47	
n-6/n-3		20.07	

Mean±SD (n=3). N.D.: Not detected; SFA: Saturated Fatty Acid; MUFA: Monounsaturated Fatty Acid PUFA: Polyunsaturated Fatty Acid.

such as antioxidant, antimicrobial, and anti-inflammatory activities attributed to gallic acid, catechin, and *trans*-cinnamic acid (Chen *et al.,* 2011; Kang *et al.,* 2018; Samavat *et al.,* 2016).

Regarding the oil extracted from red mombin seeds, there were also only a few classes of phenolic compounds present, such as benzoic acids, gallic acid, vanillin, and cinnamic acids, such as chlorogenic acid. These compounds may contribute to antioxidant capacity (Omena *et al.,* 2012). In comparison with the oil extracted from other seeds, all compounds identified in the present study were also found in camellia seed oil (*Camellia* L.) (Wang *et al.,* 2017; Fang *et al.,* 2015), while only vanillin

was reported in oils extracted from soybean, grape seed, pumpkin seed, and rice bran (Siger *et al.,* 2008). Moreover, gallic acid has also been identified in olive oil (Rosenblat *et al.,* 2008). Despite the presence of these compounds in oils extracted from other seeds, quantitative differences were observed among them.

The composition of fatty acids in red mombin seed oil, identified using gas chromatography with a flame ionization detector (GC-FID), is described in Table 4. This analysis yielded a total of 14 fatty acids. The most abundant were eicosanoic acid, followed by linolelaidic acid (a *trans* isomer of linoleic acid), palmitic acid, and finally, stearic acid. In total, the oil comprised 67.92% saturated fatty acids (SFA), 31.63% polyunsaturated fatty acids (PUFA), and only 0.45% monounsaturated fatty acids (MUFA).

Considering this percentage, data from the literature indicate that PUFA reduces total cholesterol and regulates levels of HDL and LDL cholesterol in the blood (Berto *et al.,* 2015). Other authors stated that n-3 fatty acids have immunosuppressor effects, and that n-6 fatty acids help regulate immune responses when activated (Perini *et al.,* 2010). Therefore, high n-6:n-3 ratio values may interfere in mechanisms related to inflammation, especially when there is an increase in n-6 PUFA due to their pro-inflammatory effect, and because enzymes involved in PUFA metabolism are mapped in a region that is often associated with cancer (Azrad *et al.,* 2013). The n-6:n-3 ratio values were close to those found in a study on *umbu* seed oil (a fruit from the Anacardiaceae family), for which values for this ratio were between 13.4 and 18.0 (Dias *et al.,* 2019).

In addition to the n-6:n-3 ratio, the PUFA:SFA ratio also provides important information. According to the Department of Social Health and Security (Department of Health and Social Security 1994), diets should present PUFA:SFA ratios ≥ 0.45 to be considered beneficial to human health. However, when this ratio is below the stipulated value it can lead to an increase in cholesterol levels in the blood (Berto *et al.,* 2015). Thus, despite the ratio between n-6 and n-3 PUFA, this relationship can be considered beneficial in case of consumption of red mombin seed oil.

3.5. Physicochemical characterization of red mombin seed cake and oil

The physicochemical characterization of red mombin seed oil is presented in Table 5. The refraction index represents the content of unsaturated fatty acids and indicates if this seed's oil presents edible oil qualities, given that the digestion of edible plant oils is determined by PUFA composition (da Silva and Jorge 2014; Rezig *et al.,* 2018). The refraction index value obtained (1.475) indicated high levels of unsaturated fatty acids, as in soybean oil (1.477), grape seed oil (1.472), and *cajá-manga* seed oil (1.4598) (Nehdi *et al.,* 2012; FAO/WHO 1995). The acidity index was similar to the *cajá-manga* seed oil (*S. mombin* L.), which belongs to the same family (Anacardiaceae) as the red mombin. However, *cajá-manga* seeds present lower values than those recommended by FAO/WHO, indicating possible oxidative degradation of the material evaluated. The presence of peroxides was not observed, indicating that lipid oxidation did not occur in this oil (Böger *et al.,* 2018). Therefore, the process used to obtain the oil preserved its final quality (Eromosele and Paschal, 2003).

The reducing capacity of red mombin seed oil was partially attributed to the phenolic compounds pres-

Table 5. **Characterization of red mombin oil and antioxidant profile.**

Parameters	**Red mombin seed oil**	**CODEX ALIMENTARIUS (2003)**	**BHT**
Refractive index	1.475±0.00	-	-
Peroxide index	N.D.	15 meq·kg^{-1}	-
Acidity level (%oleic acid)	1.33±0.05	4.0 mg KOH·g^{-1}	-
CR (mg AGE·100g^{-1})	38.43±1.80	-	-
DPPH (IC_{50}) (mg·L^{-1})	186.64±1.17	-	85.2±0.7
ABTS (μmol de Trolox·g^{-1})	206.20±1.72	-	-

N.D.: Not detected

ent in the methanolic fraction extracted, since three phenolic compounds were identified (gallic acid, chlorogenic acid, and vanillin). However, based on the total reducing capacity (38.43 mg GAE·100 g^{-1}) found in the present study, and given the interfering substances in this analysis, it is known that this assessment is not specific to phenolic compounds but also for compounds from the -OH functional group. Therefore, since the oil studied was a raw extract, other compounds such as vitamin C, sugars, amino acids, amines, and sulfur-containing compounds may interfere with the final results (Chen *et al.,* 2015). In addition, only 6.84% (2.63 mg·100 g^{-1}) of the total reducing capacity was related to phenolic compounds. The same behavior was observed in the methanolic fraction of grape seed oil (165.5 mg GAE·100 g^{-1}/6.14 mg·100 g^{-1}), passion fruit seed oil (262.3 mg GAE·100 g^{-1}/9.28 mg·100 g^{-1}), and pumpkin seed oil (268.6 mg GAE·100 g^{-1}/1.21 mg·100 g^{-1}) (da Silva and Jorge, 2014).

In turn, the antioxidant capacity of red mombin seed oil was completely determined using an extract prepared with isopropyl alcohol because other compounds with different polarities were considered. Thus, the DPPH method showed that 186 mg·mL^{-1} of antioxidants would be necessary to reduce the initial concentration of oxidation reactive species by 50%. Antioxidant capacity through ABTS showed a higher value than what was observed by Da Silva and Jorge (2014a) for grape seed oil (138.8 μmol of Trolox.g^{-1}).

The chemical characterization of red mombin seed cake, after oil extraction, is shown in Table 6. Moisture values were shown to guarantee cake preservation and stability, since water content was below the value (15%) recommended in Codex alimentarius (FAO/WHO) (1999). With this moisture content it is possible that the cake would help with mixture fluidity and maintaining ingredient portions when used in a food product (Zago *et al.,* 2015). Considering that red mombin seeds before oil extraction presented approximately 1.87 g·100 g^{-1} of lipid content, 43% of the initial content was recovered.

Since the cake is considered a raw material with a high content in dietary fibers, especially insoluble fibers (ANVISA 2012), it can be considered a functional co-product. Fibers present biological functions of interest, such as assisting intestinal transit and influencing the modulation of intestinal microbiota, generating various metabolic products and functions depending on the microorganisms present (Danneskiold-Samsøe *et al.,* 2019). According to the Institute of Medicine of the National Academy of Sciences (FNB/IOM 2009), mean fiber intake should be 14 g for every 1,000 kcal. Thus, by consuming 100 g of red mombin seed cake, a total of 83.73 g of dietary fiber is absorbed, which represents six times the recommended intake value.

Table 6. Proximal and chemical composition of red mombin seed cake.

Parameters	Red mombin seed cake
Moisture (g·100g^{-1})	4.31 ± 0.21
Ash (g·100g^{-1})	2.42 ± 0.05
Protein (g·100g^{-1})	4.81 ± 0.21
Lipids (g·100g^{-1})	0.82 ± 0.02
Total carbohydrates (g·100g^{-1})	87.74 ± 0.20
Insoluble fiber (g·100g^{-1})	80.45 ± 0.50
Soluble fiber (g·100g^{-1})	3.28 ± 0.50
Dietary fiber (g·100g^{-1})	83.73 ± 0.50
Caloric value (Kcal·100g^{-1})	377.64 ± 0.57
pH	3.78 ± 0.03
TTA (g·100g^{-1})	0.36 ± 0.01
SS (°Brix)	3.00 ± 0.58

Some authors state that, in fact, waste produced by agricultural and industrial activities are potential sources of nutrients and bioactive compounds (Banerjee *et al.,* 2017; Girotto *et al.,* 2015), especially for pharmaceutical industries (Mirabella *et al.,* 2014). In this case, the presence of bioactive compounds was observed by determining antioxidant capacity using various methods (Table 7) and solvents. Part of the values obtained in these trials could be attributed to the phenolic compounds identified using HPLC-DAD/UV-Vis. Statistical differences ($p < 0.05$) were observed among the solvents evaluated in all antioxidant capacity methods investigated. MAWE was the solvent that extracted the most compounds present in the cake because the mixture of solvents involved (50% methanol:70% acetone:distilled water (2:2:1) (v:v:v)) can act directly on cell walls and membranes, favoring the process of compound extraction and consequently causing their lixiviation (Lapornik *et al.,* 2005).

Taking into account the existing literature on the nutritional compounds and antioxidant capacity of

Table 7. **Antioxidant profile of the red mombin seed cake.**

Extract	RC (mg AGE.100g^{-1})	DPPH (IC_{50})	ABTS (µmol de Trolox.g^{-1})	FRAP (µmol de FeSO4.g^{-1})	β-Carotene bleaching (%Protection)
ETE	6.94±0.13^{e}	85.16±5.97^{e}	305.67±2.85^{d}	80.35±0.69^{e}	41.34±5.45^{c}
ALE	45.05±1.00^{d}	13.62±1.26^{d}	166.01±3.72^{e}	70.07±0.70^{d}	34.32±3.97^{d}
AQE	106.33±4.16^{c}	6.96±0.87^{c}	356.83±3.50^{c}	256.74±5.90^{c}	62.83±1.32^{a}
MAWE	493.87±6.27^{a}	1.90±0.21^{a}	2734.73±2.16^{a}	984.30±2.81^{a}	54.39±1.62^{b}
HAE	454.55±2.87^{b}	2.33±0.03^{b}	1274.98±5.21^{b}	811.45±4.24^{b}	44.63±3.67^{c}

Results correspond to means ± standard deviation of three replicates. Lowercase letters on the same line and uppercase letters in the same column do not differ statistically from the Tukey test at 5% ($p < 0.05$). RC: reducing capacity; ETE: ether extract; ALE: alcoholic extract (95% alcohol); AQE: aqueous extract; MAWE: methanol + acetone + distilled water extract; HAE: hydroalcoholic extract (70% alcohol).

red mombin seed cake, the present study represents the first contribution to the subject. Red mombin seed cake presented interesting nutritional values from a preservation and fiber content point of view. Conveniently, the cake that originates from the extraction of red mombin seed oil may eventually be used as an ingredient for new types of food, with the objective of providing nutritional enrichment.

4. CONCLUSIONS

The red mombin seed is considered a waste in the processing of pulps by the food industry, causing numerous environmental problems, so this study collaborates to reduce this organic waste. Thus, it was possible to obtain a maximum yield in the extraction of the oil in experiment ten under the conditions of 23.10 mL of solvent per gram of seed and extraction time of 6.46 minutes, obtaining a 60% yield, contributing to the discovery of new sources of antioxidant compounds, mainly from agro-industrial waste, aiming for the development and application of new active products by the pharmaceutical medicinal industries and nutraceutical companies due to the presence the unsaturated fatty acids and phenolic compounds.

Futhermore, in this study it was also possible to obtain other conditions for the extraction of oil from the red mombin seeds that benefit the industry in terms of energy and process savings, maintaining a yield close to the maximum and with reduced time.

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DATA AVAILABILITY

The data referring to this article are part of the development of the master's thesis and are not disclosed in any repository.

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