

ORIGINAL ARTICLE

Obtaining oil from macauba kernels by ultrasound-assisted extraction using ethyl acetate as the solvent

Extração assistida por ultrassom do óleo das sementes de macaúba utilizando acetato de etila como solvente

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Abstract

The objective of this work was to evaluate the efficacy of ethyl acetate as a solvent in the extraction of macauba kernel oil (MKO) using ultrasonic-assisted extraction (UAE). It was shown that more MKO oil could be extracted with the use of larger amounts of solvent, higher temperatures and longer extraction times. Thus the maximum oil yield (40.61%) was obtained by UAE at 60 °C for 45 min, using a solvent to kernel ratio of 12 (mL g⁻¹), obtaining a higher yield than that obtained with *n*-hexane under the same experimental conditions. UAE was favorable for this oil extraction ($p < 0.05$), presenting a yield close to that reported for classical extraction but with a shorter extraction time and smaller solvent volume. Lauric acid corresponded to ~44% of the MKO composition. The oils presented low free fatty acid contents (<0.80% wt), and the phytosterols, campesterol and β -sitosterol, were identified in the MKO with higher levels in the oil obtained by UAE.

Keywords: Ethyl acetate; Ultrasound; Macauba kernel; Solvent; Fatty acids; Classic extraction.

Resumo

O objetivo deste trabalho foi avaliar a eficácia do acetato de etila como solvente na extração de óleo de semente de macaúba (OSM), utilizando extração assistida por ultrassom (EAU). Verificou-se que o aumento na remoção de OSM pode ser obtido com o uso de maiores quantidades de solventes, maior temperatura e tempo de extração. Com isso, obteve-se o rendimento máximo de óleo (40,61%) proporcionado pela EAU a 60 °C, 45 min, e utilizando a razão solvente para grão de 12 (mL g⁻¹), o qual foi superior ao obtido por *n*-hexano, nas mesmas condições experimentais. A EAU foi favorável na extração de óleo ($p < 0,05$) e apresentou rendimento próximo ao relatado pela extração clássica; no entanto, na EAU, utiliza-se menor tempo de extração e volume de solvente. O ácido láurico



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corresponde a ~44% da composição do OSM. Os óleos apresentaram baixos teores de ácidos graxos livres (<0,80%) e os fitoesteróis, campesterol e β -sitosterol, foram identificados no OSM com maiores teores no óleo obtido pela EAU.

Palavras-chave: Acetato de etila; Ultrassom; Sementes de macaúba; Solvente; Ácidos graxos; Extração clássica.

1 Introduction

The Brazilian Macauba palm tree (*Acrocomia aculeata*) produces fruits of which the kernel represents approximately 6.3% (Evaristo et al., 2016), with a productivity of between 1500 and 5000 kg of oil per hectare per year. The Macauba palm reaches high productivity after 4 years of growth and keeps producing for over 100 years (Navarro-Díaz et al., 2014). The kernel contains from 35% to 52.9% of oil (Hiane et al., 2005; Coimbra & Jorge, 2011a; Lescano et al., 2015) which has a high concentration of saturated fatty acids, of which lauric acid represents 31.6% to 50% of the fatty acid composition (Coimbra & Jorge, 2012; Lescano et al., 2015; Alves et al., 2016; Río et al., 2016; Oliveira et al., 2017).

Ultrasonic assisted extraction (UAE), an emergency technique which, according to the literature, has not yet been applied to obtain oil from this matrix, could be used to obtain macauba kernel oil. UAE presents advantages such as increased mass transfer, better solvent penetration in the solute, higher yield, selectivity and improvement of the quality of the components present in the oil, faster extraction rates, lower temperature, decreased extraction time, and the natural compounds of interest are less degraded as compared to other extraction techniques (Chemat et al., 2011; Mason et al., 2011; Easmin et al., 2015; Grassino et al., 2016).

In the ultrasound-assisted process, the phenomenon called cavitation occurs, due to the formation of low frequency ultrasonic waves (above 20 kHz), which propagate in a liquid medium, creating something called microbubble shock. These shocks increase or decrease their size, generating compression and expansion until the bubbles implode near the solid, promoting the rupture of the vegetable cell wall and aiding in the extraction of the intracellular material (Zheng & Sun, 2005; Tao & Sun, 2013; Ferreira et al., 2014; Chemat et al., 2017).

In the application of new extraction methods, the challenge is to establish solvents that can be an alternative to the use of *n*-hexane, which is the solvent most commonly applied, without significantly compromising the oil extraction efficiency. This concern is due to the numerous disadvantages of using *n*-hexane, such as damage to human health, high toxicity, high flammability, air pollution, and the fact that it is obtained from a non-renewable source (Hammond et al., 2005).

Since macauba kernel oil can be used for human consumption, the use of a food grade solvent would be of interest. Of these, ethyl acetate has presented high yields in the extraction of vegetable oils (Randhava et al., 2011; Lohani et al., 2015; Purohit & Gogate 2015), such as those obtained from macauba pulp (Trentini et al., 2016), chia (Mello et al., 2017), *Hevea Brasiliensis* (Reshad et al., 2015), radish (Silva & Silva, 2016) and *Croton gratissimus* seeds (Jiyane et al., 2018).

The present study aimed to evaluate the effect of the process variables on the ultrasonic-assisted extraction of macauba kernel oil, and to establish conditions that maximize oil extraction by this technique using ethyl acetate as the solvent. In parallel, classical extraction (Soxhlet) with *n*-hexane was carried out to compare the yield obtained and the quality of the extracted oil.

2 Material and methods

2.1 Sample preparation

Acrocomia aculeata was obtained from Parana State - Brazil (Global Positioning System coordinates 23°15' and 34°08' S; 52°46' and 37°46' W). The macauba fruits were pulped with a stainless steel knife, separating the kernel, which was dried at 60 °C for 6 hours to a moisture content of 2.91% ± 0.12%. After drying, the kernels were comminuted in a blender (Britannia) and separated by granulometry using Tyler-type sieves on a mechanical shaker (Marconi, MA750).

2.2 Classical extraction

The classical extraction was carried out using Soxhlet equipment as described by Silva et al. (2017), with *n*-hexane (Anidrol, 98.5%) as the solvent. For each extraction, 5 g of kernel and 150 mL of solvent were used, refluxing at a temperature of 69.1 °C for 480 min. After the extraction time, the solvent was removed and the oil yield calculated according to Equation 1.

$$\text{Oil yield (\%)} = \frac{w_o}{w_a} \times 100 \quad (1)$$

where w_o (g) is the mass of extract obtained and w_a (g) is the initial mass of kernel fed into the extractor.

2.3 Ultrasound-assisted extraction

For each experiment, 3 g of sample were added to a flask together with the solvent ethyl acetate (F Maia). The flask was positioned in the centre of an ultrasound bath (Ultronique, Q 5.9/40A, frequency 40 KHz, Eco-Sonics) and coupled to a condenser connected to a thermostabilized bath at 10 °C (Marconi/MA184). The kernels were separated by filtration, and the solvent removed to calculate the oil yield from Equation 1.

The experiments were carried out using the maximum ultrasound bath power (165 W), kernels with a mean diameter of 0.841 mm, and a Box-Behnken design with 17 runs, to determine the influence of the process variables on the oil yield and to determine the levels needed to maximize the oil yield. **Table 1** shows the variables considered, as well as the levels adopted.

Table 1. Variables and levels of the Box-Behnken design used in the UAE.

Variable	Level		
	-1	0	1
Temperature (°C), X ₁	30	45	60
Solvent to kernel ratio (g mL ⁻¹), X ₂	4	8	12
Time (min), X ₃	15	30	45

The Statistica 8.0 software (STATSOFT™, Inc.) was employed to evaluate the effect of the process variables on the oil yield ($p = 0.05$). The experimental data was fitted to a second-order polynomial model, as presented in the papers of Mello et al. (2017), Rodrigues et al. (2017) and Silva et al. (2017). Additional confirmation experiments were subsequently carried out to verify the validity of the statistical model.

The oil yield obtained by UAE (under the conditions of maximum yield) with ethyl acetate was compared to the extraction yield obtained using *n*-hexane under the same experimental conditions. For comparative purposes, the extraction was also carried out without ultrasound (orbital shaker Marconi, MA 839/A) shaking ~3 g of kernels in glass flasks together with the solvent at 40 rpm. Finally, the effect of particle size on the oil yield obtained by UAE (under the conditions of maximum yield with ethyl acetate) was evaluated using 0.640 mm particles.

2.4 Oil characterization

The crude oil obtained from the macauba kernels was characterized in relation to its fatty acid composition and phytosterol contents. The analyses were carried out using a gas chromatograph (GC-MS Shimadzu, QP2010 SE) with an automatic injector, equipped with a mass spectrophotometer and flame ionization detector. To determine the fatty acid composition, the samples were derivatized following the methodology adapted from Santos Júnior et al. (2014) and analysed using the chromatographic conditions described in detail by Rodrigues et al. (2017). The components present in the samples were identified by comparing with a fatty acid methyl ester mixture (Supelco) and quantified using methyl heptadecanoate (Sigma-Aldrich, >99% purity) as the internal standard.

To determine the phytosterols and free fatty acid contents, the samples were derivatized with N,O-bis(trimethylsilyl) trifluoroacetamide (Sigma-Aldrich) and analysed according to Trentini et al. (2016). The phytosterols and free fatty acids were quantified using 5 α -cholestane (Sigma-Aldrich, > 99% purity) and methyl heptadecanoate (Sigma-Aldrich, > 99% purity) as the internal standards, and identified by a comparison of their spectra with those presented in the NIST14.lb and NIST14.lbs spectral libraries.

The data collected were subjected to ANOVA using Excel® 2010 software and Tukey tests ($p = 0.05$) to evaluate differences between the results. The experiments were carried out in duplicate.

3 Results and discussion

3.1 Classical extraction

Using the classical extraction (Soxhlet) 51.17% \pm 1.21% of oil was obtained. According to reports in the literature, the macauba kernel contains 35% to 52.9% of oil by weight (Hiane et al., 2005; Coimbra & Jorge, 2011b; Lescano et al., 2015). The differences in oil contents from macauba kernels can be justified according to the region where the fruit was cultivated, as well as the drying time and temperature to which the kernels were exposed before oil extraction (Coimbra & Jorge, 2011a).

3.2 Ultrasound-assisted extraction

Table 2 shows the experimental conditions used to obtain MKO by UAE using ethyl acetate as the solvent, and the oil yield obtained under these conditions. It can be seen that the oil yields varied from 28.25% to 39.24%. **Table 3** presents the effects of the operational variables analysed on these values.

Table 2. Experimental conditions applied and oil yields obtained in the experiment to assess the effects of the process variables using a Box-Behnken design.

Run	Variable			Oil Yield ² (%)
	X ₁ ¹	X ₂ ¹	X ₃ ¹	
1	-1	-1	0	29.16
2	1	-1	0	33.13
3	-1	1	0	33.06
4	1	1	0	37.99
5	-1	0	-1	30.02
6	1	0	-1	33.08
7	-1	0	1	33.45
8	1	0	1	39.24
9	0	-1	-1	28.25
10	0	1	-1	33.87
11	0	-1	1	31.14
12	0	1	1	35.36
13	0	0	0	32.54
14	0	0	0	32.67
15	0	0	0	32.18
16	0	0	0	32.39
17	0	0	0	31.78

¹as in Table 1. ²obtained by Equation 1.

Table 3. Model coefficients and *p*-values of the model used in the UAE extraction of macauba kernel oil.

Variables	Effect ^a	<i>p</i> -value ^b	Coefficient ^c
Mean/Interaction	33.15	<0.0001	33.14
X ₁ (L)	4.43	<0.0001	2.21
X ₁ (Q)	-1.40	0.0001	-0.70
X ₂ (L)	4.64	<0.0001	2.32
X ₂ (Q)	0.38	0.0858	0.19
X ₃ (L)	3.49	0.0001	1.74
X ₃ (Q)	-0.22	0.2510	-0.11
X ₁ x X ₂	0.48	0.2407	0.24
X ₁ x X ₃	1.36	0.0175	0.68
X ₂ x X ₃	-0.70	0.1145	-0.35

^aEffects of the independent variables on the dependent variables. ^bstatistical significance $p < 0.05$. ^ccoefficients of the second-order polynomial model (Equation 2). L = linear effect; and Q = quadratic effect.

From **Table 3** it can be seen that an increase in the magnitude of the variables tested can result in a notable increase in the removal of oil from the kernel ($p < 0.005$). The quadratic effect of the temperature and effect of the interaction between temperature and time were also significant ($p < 0.05$).

An analysis of the results indicated that the largest amount of oil was extracted using a higher solvent-to-sample ratio. This was due to the increased diffusion efficiency with a greater availability of solvent in the extraction medium (Gayas et al., 2017; Perrier et al., 2017; Samaram et al., 2015). In addition, Reshad et al. (2015) commented that the lower yields obtained using low solvent to sample ratios may be related to greater agglomeration of the particles at lower ratios.

Chanioti & Tzia (2017) evaluated the effect of the solvent/sample ratio on the UAE of oil from olive pomace and observed higher oil yields with a ratio of 4 to 12. Similar effects were reported by Mello et al. (2017) and Rodrigues et al. (2017) for UAE of oil from chia seeds and macauba pulp, respectively.

An increase in temperature contributed to the miscibility of the oil in the ethyl acetate (Eikani et al., 2012) and facilitated disruption of the plant tissue (Samaram et al., 2015), thus improving oil extraction due to better elution of the oil from the solvent (Gayas et al., 2017). Other authors who evaluated the application of UAE to obtain oil from different plant matrices also obtained higher yields with temperature increases of 40 °C to 60 °C (Chanioti & Tzia, 2017).

The viscosity and density of the solvent decreased with increasing temperature (Hemwimol et al., 2006; Ramandi et al., 2012), resulting in an increase in the mass diffusion coefficient (Shalmashi, 2009), causing the easier occurrence of cavitation bubbles, thus reducing the tensile strength of the liquid (Hemwimol et al., 2006). Furthermore, an increase in the vapor pressure of the solutes was observed with the use of higher extraction temperatures, favouring the collapse of the cavitation bubbles in ultrasound-assisted processes (Gutte et al., 2015). From the thermodynamic parameters of the extraction of hempseed oil presented by Kostić et al. (2013), it can be seen that an increase in temperature favoured process spontaneity due to the lower Gibbs free energy values.

The increase in time aided in greater solvent penetration of the matrix, consequently obtaining a higher oil yield. For example, in the comparison of runs 6 and 8, under the same conditions of temperature and solvent-to-sample ratio, the yield was ~15% higher when the time was increased from 15 to 45 min.

Hashemi et al. (2018) carried out the extraction of *Aloysia citriodora* oil for 15, 30, and 45 min and observed no differences in the yield of oil extracted between 30 and 45 min. As cited by Assami et al. (2012), ultrasound releases up to 80% of the oil in just 30 min, since, according to Khoei & Chekin (2016), long extraction periods may cause a decrease in the oil yield due to weakening of the cavitation effect. In addition, Coelho et al. (2017) reported that at the beginning of extraction the mass transfer rate is higher because the solvent is in contact with the surface region of the sample, and after a certain period the extraction yield is reduced due to low diffusion of the oil.

For the longer extraction times used in the present study, an increase in temperature favoured the recovery of macauba kernel oil ($p < 0.05$). The solvent-to-sample ratio and its interaction with time and temperature had no effect on the oil yield ($p > 0.05$) since the solubility of the oil in the solvent is favoured by an increase in the amount of solvent, but after reaching equilibrium the excessive amount of solvent was not feasible.

An analysis of the interaction between the variables of temperature and time showed that an increase in both favoured oil extraction ($p < 0.05$). Since the use of higher temperatures alters the properties of the solvent, the solubility of the oil increased, aiding in its extraction since it took longer for the solvent to penetrate the intracellular matrix.

Equation 2 presents the correlation between the experimental variables ($p < 0.05$) and the response variable (oil yield). The F-test, $F_{\text{CALC}} (33.85) > F_{\text{TAB}} (3.74)$ indicated that the predictive equation (Equation 2) was capable of representing the experimental data for the range of factors investigated.

$$\text{Oil yield (\%)} = 33.17 + 2.22X_1 + 2.32X_2 + 1.75X_3 - 0.69X_1^2 + 0.68X_1X_3 \quad (2)$$

Using Equation 2, the maximum possible oil yield was determined to be 40.61% for extractions carried out at 60 °C for 45 min using a solvent-to-kernel ratio of 12 (mL g⁻¹). Thus the experiments were carried out in triplicate under the optimum conditions, resulting in an oil yield of 40.44 ± 0.58%, a result not different from that estimated using the predictive equation ($p > 0.05$). Under these experimental conditions the extraction with n-hexane gave an oil yield of 30.97 ± 0.98%, demonstrating the efficiency of oil removal using ethyl acetate.

The extraction was also carried out using the maximum yield conditions determined by Equation 2 in an orbital shaker without the use of ultrasound, resulting in the removal of 35.97% ± 0.03% of oil from the macauba kernels. A comparison of this result with the use of UAE indicated an increase of ~11.42% in the yield due to the use of ultrasound, which was due to the ultrasonic waves aiding in the rupture of the cell wall of the vegetable matrix, providing a higher oil yield (Vinatoru et al., 1999). The mass transfer rates are favoured by the use of UAE due to the implosion of the cavitation bubbles at the surface of the matrix, which favours exchange at the surface between the solvent and the matrix (Sicaire et al., 2016). Perrier et al. (2017) reported that the speed of oil diffusion from rapeseed flakes to solvent was favoured by the use of ultrasound. The percentage increase in yield was similar to that already reported in previous studies evaluating the effect of the application of ultrasound in the extraction process (Mello et al., 2017; Rodrigues et al., 2017).

The results showed that the use of UAE with ethyl acetate as the solvent obtained ~79% of the yield obtained with the classical n-hexane extraction; however, this yield was obtained in a shorter time (8 h to 45 min) and a lower sample-to-solvent ratio (30 to 12). To obtain greater removal of the oil from the macauba kernel using UAE, experiments were carried out under optimum conditions using a smaller particle size (0.640 mm), which increasing the yield to 51.24% ± 1.1%. The particle size had the greatest impact on the extraction yield since the use of smaller particles favours the interfacial area between the solid and the liquid (Suganya & Renganathan, 2012; Reshad et al., 2015).

Menezes et al. (2018) reported that using the classical method the extraction process is based on the diffusion of oil via contact between the sample and the solvent, and thus a large solvent volume is required. However, using UAE the cavitation phenomenon can locally change the temperature and pressure, promoting mass transfer, thus requiring a smaller volume of solvent to extract the same quantity of oil.

3.3 Oil characterization

Table 4 shows the fatty acid compositions, phytosterols and free fatty acid (FFA) contents of the macauba kernel oils obtained by UAE and using the classical extraction method. The data presented in this table show that the oils obtained with the two techniques had low but similar FFA contents (~0.80% wt).

Table 4. Fatty acid compositions, phytosterols and FFA contents of the macauba kernel oils obtained by the two extraction methods.

Fatty Acids (g per 100 g of oil)	Method of Extraction	
	Classical	UAE
Caprylic (C8:0)	4.84 ± 0.00 ^a	4.39 ± 0.09 ^a
Capric (C10:0)	4.16 ± 0.00 ^a	4.07 ± 0.01 ^a
Lauric (C12:0)	44.25 ± 0.01 ^a	44.21 ± 0.01 ^a
Myristic (C14:0)	7.83 ± 0.00 ^a	7.87 ± 0.02 ^a
Palmitic (C16:0)	5.79 ± 0.00 ^a	5.83 ± 0.02 ^a
Stearic (C18:0)	2.47 ± 0.01 ^a	2.53 ± 0.01 ^a
Oleic (C18:1)	28.01 ± 0.01 ^a	28.43 ± 0.04 ^a
Linoleic (C18:2)	2.64 ± 0.01 ^a	2.67 ± 0.01 ^a
∑saturated	69.34	68.90
∑unsaturated	30.65	31.10
Phytosterols (mg per 100 g of oil)		
Campesterol	11.49 ± 0.09	13.92 ± 1.87
β-sitosterol	39.73 ± 2.67	52.77 ± 1.02
Total	51.23 ^a	65.96 ^b
Free fatty acids (wt%)		
	0.80 ^a ± 0.03	0.76 ^a ± 0.05

Means followed by different lowercase letters (comparative method) and uppercase letters (comparison of solvents) did not differ statistically ($p > 0.05$).

Lauric, oleic and myristic acids were the main fatty acids in the MKO, representing ~80% of the oil composition, giving similar values for the different extraction methods used ($p > 0.05$). Similar fatty acid profiles for macauba kernel oil were reported by other authors, as can be seen in the papers of Coimbra & Jorge (2011a), Lescano et al. (2015), Alves et al. (2016) and Oliveira et al. (2017).

The oil extracted from the macauba kernel was composed predominantly of saturated fatty acids (~69%), with lauric acid as the most abundant component. This highly saturated composition is an advantage in terms of oxidative stability (Hiane et al., 2005). In addition, lauric acid-rich oils have wide applications in the pharmaceutical, cosmetic and biodiesel industries (Coimbra & Jorge, 2012; César et al., 2015).

As shown in **Table 4**, the phytosterols, campesterol and β-sitosterol were identified in the macauba kernel oil. UAE promoted a greater extraction of these compounds ($p < 0.05$), obtaining 65.96 mg per 100 g of oil. Rotta et al. (2018) and Silva et al. (2017) extracted the oil from passion fruit and radish seeds, respectively, and reported that under the conditions evaluated the oil obtained by ultrasound showed a greater concentration of phytosterols when compared to conventional extraction. Possibly, ultrasound favoured the selectivity of these compounds, mainly due to the shorter contact time between the solvent and the sample, as well as the greater agitation (cavitation) of the system, promoting a more effective breakdown of the interactions and release of the compounds.

Río et al. (2016) evaluated the extraction of oil from macauba pulp and kernels using a hydraulic press, and identified the presence of campesterol, stigmasterol and sitosterol in the oil obtained. Trentini et al. (2017) extracted macauba kernel oil using supercritical carbon dioxide and obtained campesterol contents of 1.71 mg per 100 g of oil and β-sitosterol contents of 22.99 mg per 100 g of oil.

4 Conclusions

This study showed that UAE allowed for high oil yields when compared with the commonly used techniques and solvent (Soxhlet and *n*-hexane, respectively). The combination of ethyl acetate and ultrasound for the extraction of MKO proved to be an interesting alternative, besides guaranteeing a similar performance to that of the Soxhlet method, with a reduced extraction time and lower solvent consumption. Within the experimental range tested, the application of the highest temperatures, solvent-to-kernel ratios and times provided higher oil removal rates from the kernel, and thus the maximum oil recovery was obtained at 60 °C for 45 min with a solvent-to-kernel ratio of 12 (mL g⁻¹). The oils obtained presented high lauric acid contents (~44%) and UAE provided oil with a higher phytosterol content.

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