



Aqueous extraction to high yield and quality of macauba (*Acrocomia aculeata*) pulp oil

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ABSTRACT

Aqueous extraction of oil from fresh pulp of macauba (*Acrocomia aculeata*) assisted with the commercial enzyme pool Cellic® CTec3 was evaluated at the laboratory and larger reactor scale. Effects of temperature and time of malaxation on the efficiency of extraction were studied by response surface methodology. Oil characteristics were determined by measurements of acidity, molar absorptivities K_{232} and K_{270} , carotene content and Deterioration of Bleachability Index. The aqueous extraction proved to be efficient for obtaining high quality oil. The best oil recovery efficiency was reached with the assistance of the buffered enzyme at mild conditions. Mass balance indicates an oil yield around three times higher than currently found in the traditional industry based on expelling of the dried pulp.

1. Introduction

Currently, oil palm is the main vegetable oil source in the global trade. Palm oil plus kernel oil account for 40% of the world supply, followed by soybean oil that supplies a further 27% (USDA, 2020). Their production issues are well known, allowing room for new sources of environmentally friendly oleaginous. In this way, there is an overgrowing tendency to insert the commercial exploitation of the macauba palm tree (*Acrocomia ssp.*), also known as “coco mbokaja”, native to Brazil, Paraguay and other tropical Latin American countries, in the world bioeconomy, due to its potential as an oleaginous source and the provision of environmental services (Castro et al., 2017; Evaristo et al., 2016; Coppel et al., 2018). Evaluation of spontaneously occurring plants indicate the potential to achieve oil yields close to or even higher than that of oil palm (*Elaeis guineenses*) (César et al., 2015; Colombo et al., 2018; Lanes et al., 2016; Mota et al., 2011; Motoike & Kuki, 2009; Pires et al., 2013). In addition, it is an outstanding alternative as the forest component in crop, livestock and forest production system (ICLF) (Castro et al., 2017; da Conceição et al., 2015; Montoya, 2016). Reports highlight its high carbon sequestration and nutrient cycling capacities (Ferreira et al., 2013; Evaristo et al., 2018), besides its positive effects on mitigation of climate changes (Moreira et al., 2018).

Macauba pulp and kernel oils can be used for several industrial purposes because of their fatty acid composition, with a predominance of, oleic/palmitic and lauric/oleic, respectively (Coimbra & Jorge, 2012;

Del Río et al., 2016). Pulp oil accounts for 85% of the total oil content of macauba fruit and kernel oil for another 15%, on average.

Pulp oil can contain up to 81% oleic acid contents (Ciconini, 2012), placing it the “high oleic” vegetable oil class. Furthermore, it has got high oxidative stability due to its large proportion of monounsaturated fatty acids and diglycerides, along with significant levels of carotenes (Oliveira et al., 2017; Del Río et al., 2016; Nunes et al., 2015; Ramos et al., 2008).

One of the main bottlenecks to the success of the value chain is the pulp oil extraction process. Usually, the fruit is naturally dried and pulped, and the oil is extracted by pressing. Pressing is demanding in energy and has a low efficiency. As a result, the oil recovery is low and its quality is below standards. Another alternative would be the use of organic solvents, such as hexane, used in the majority of oilseed processing (Cheng et al., 2019). However, its usage rises concerns regarding health and environmental safety (Tiwari, 2015; Telaka et al., 2020), plus the need for facilities that demand higher fixed capital investment and maintenance (Cheng et al., 2019). A better quality oil can be obtained if good agricultural and post-harvest practices are adopted (Da Conceição et al., 2015; Favaro et al., 2018), but they are not yet a widespread practice.

Considering these issues, aqueous extraction of fresh fruits, as used for olive oil (Boskou, 2006) and palm oil (Baryeh, 2001), could be a promising process. In such a process, oil is recovered by a sequence of mechanical steps, starting with the disruption of fruit tissues, fol-

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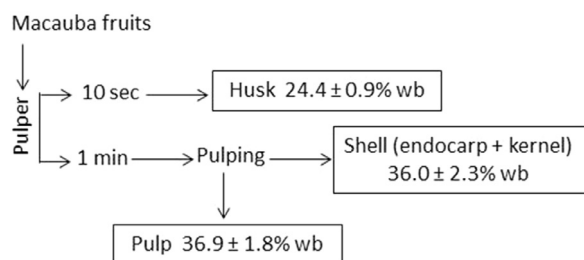


Fig. 1. Process steps for pulping macauba fruits and their respective yields.

lowed by coalescence of body oils, known as malaxation, and final separation of the aqueous, solid and lipid phases by centrifugation (Casas & Domínguez, 2017). There is no need of moisture or organic solvents. Aqueous oil extraction assisted with hydrolytic enzymes, added before the oil separation step, results in a greater oil recovery (Munder et al., 2020; Rosenthal et al., 1996, 2001; Sharma et al., 2002).

In this work it is reported the first evaluation of an aqueous extraction process applied to macauba pulp oil. Furthermore, the assistance of a commercial enzymatic pool added to the process was also tested. Based on this new approach the quality of the oil obtained was evaluated and the mass balance was performed to show the expected oil yield.

2. Material and methods

2.1. Materials

Macauba fruits, produced in an experimental area of Embrapa Cerrados (Planaltina, DF, Brazil; 15°36'09.6"S 47°43'34.5"W), were collected after abscission. A canvas mat was used to avoid fruit contact with the soil. Fruits were taken from the field 5 days maximum after falling to prevent microbial decay. Spoiled and broken fruits were discarded, and the selected fruits were washed and directed to the oil aqueous extraction.

All chemicals were of analytical grade and used as received without any further purification. The enzyme pool was Cellic® CTec3 (Novozymes - Araucária-PR, Brazil) with 220 FPU/mL and 240 mg de protein/mL. One FPU unit is the amount of enzyme required to release a fixed amount of glucose equivalent to that obtained from a 50 mg piece of Whatman n°1 filter paper, in a 1 min time.

2.3. Separation of macauba fruit fractions

Fractions of macauba fruits were separated using a pulper device especially designed for this fruit. It is based on a hybrid hammer-squirrel-cage mill (RM Ltda, Brazil). It takes 10 seconds to remove the exocarp (husks) and an extra minute to finalize the pulping. The time of pulping is important to avoid the rupture of the endocarp. The pulp, husk and shell (endocarp and kernel) fractions were freshly weighed to estimate the mass balance. Fig. 1 shows the sequence of procedures and the weight of each fraction. The pulp was stored in polyethylene bags and kept at -8 ± 2 °C until further processing.

2.4. Aqueous oil extraction

The extraction efficiency of macauba pulp oil was evaluated firstly using 5 grams per experimental unit, taken from 200 grams of homogenized pulp. Malaxation was carried out in an orbital shaker, which allowed the determination the most adequate time and temperature for the process. With these settings, a larger amount of pulp, 200 grams per batch, was tested in a closed reactor with malaxation performed in a similar way as the one used at large scale. Experimental details are presented in Sections 2.4.1 to 2.4.4.

2.4.1. Preparation of pulp suspension

Aqueous suspension batches of macauba pulp were prepared using 200 g of wet pulp (110 g dry weight) suspended in distilled water or 0.1 M citrate buffer (pH 5.0), in a ratio of pulp-to-liquid of 1:2.5 (w/w) and homogenized using a food blender (Mixer Phillips 1336, USA) for 10 seconds at 13,500 rpm. This suspension was made to provide sufficient homogenous samples for the experiments.

2.4.2. Aqueous oil extraction

Aliquots of 17.5 g of pulp suspension prepared as mentioned in Section 2.4.1, equivalent to 5 grams of wet pulp (2.75 g dry matter), were transferred to a 125 mL Erlenmeyer and another 12.5 g of distilled water was added to make a final pulp-to-water mass ratio of 1:5, which was the best one determined previously. Malaxation was carried out using an orbital shaker (Orbital Shaker-Incubator New Lab NL/343/01, Brazil) at 50 rpm, also determined previously. The effects of temperature and time on the oil extraction efficiency were evaluated during the malaxation step using a 2² full-factorial design (Table 1). The mixture was centrifuged for 15 minutes centrifugation at 8000 rpm/7084 x g (Hettich Refrigerated Centrifuge ROTINA 380R, Germany), to separate the four phases: the oil, oily cream, aqueous and solid fractions. Each of these fractions was cautiously removed and set aside. The solid fraction was washed twice with 20 mL distilled water and centrifuged as described above to further removal of the entrapped oil. All three fractions in the sequential washings were added to their respective first round fractions. Solid fraction was dried at 60 ± 2 °C for 12 h and the remaining oil content determined. All the combined oil and oily cream fractions were centrifuged at 8000 rpm (7084 x g) to obtain sufficient oil to perform the acidity analysis.

2.4.3. Enzyme assisted aqueous oil extraction

The aqueous oil extraction was evaluated with the assistance of the commercial cellulolytic and hemicellulolytic enzymes pool Cellic® CTec3 HS (15 FPU per gram of pulp dry matter). The same sample mass and settings as described in Section 2.4.2 were used, with an addition of 0.27 mL of enzyme pool suspension. However, instead of water, the pulp was homogenized with 12.5 g of 0.1 M pH 5.0 citrate buffer. The enzymatic reaction was stopped by heating the flask in a boiling water bath for 10 min, followed by cooling in a cold water-bath at about 8 °C. Separation of the fractions followed as described in Section 2.4.2.

Effects of temperature and time of reaction on the enzyme assisted oil extraction were evaluated in a 2² central composite experimental design, as presented in Table 3.

2.4.4. Aqueous oil extraction at a larger scale in closed reactor

A stainless-steel jacketed reactor with total capacity of 5 L (Kiloclave Type 4, Buchi GmbH, Switzerland) equipped with an anchor type stirrer and a temperature sensor was used to evaluate the efficiency of the aqueous oil extraction on a larger scale using 206.5 g of wet pulp (113.6 g dry matter), homogenized as described in Section 2.4.2. A circulating bath (Julabo, Germany) containing mineral oil controlled the temperature of malaxation, 45 ± 2 °C during 107 minutes; the best operational settings determined according Section 2.4.3. The reactor worked at 20 rpm. Three different aqueous extraction treatments were compared under the same conditions mentioned above (Fig. 2): 1) distilled water; 2) 0.1 M citrate buffer solution (pH 5.0) containing Cellic® CTec3; 3) 0.1 M citrate buffer solution (pH 5.0) containing Cellic® CTec3 as described in Section 2.4.3. The final slurry was transferred to a beaker, heated directly with an electrical resistance (90 ± 2 °C for 10 min), cooled down naturally (around 30 minutes) to room temperature and centrifuged (4700 rpm/3709 x g, 30 min, Sorvall RC 6 plus, Thermo Scientific, USA). Separation of the oily cream, solid fraction, pulp oil, and the washings of the solid fraction followed the procedure described in the section 2.4.2.

Table 1

Experimental design matrix and recovery efficiency of macauba pulp oil obtained in aqueous extraction at lab scale.

Run	Coded level of variables		Actual level of variables		Response	
	A	B	Time (minutes)	Temperature (°C)	Oil recovery efficiency (%), Y	
					Observed	Predicted
1	-1	-1	30	30	68.3	67.3
2	1	-1	30	50	72.3	71.3
3	-1	1	90	30	73.5	72.5
4	1	1	90	50	67.4	66.4
5	0	0	60	40	68.1	69.4
6	0	0	60	40	67.0	69.4
7	0	0	60	40	69.2	69.4

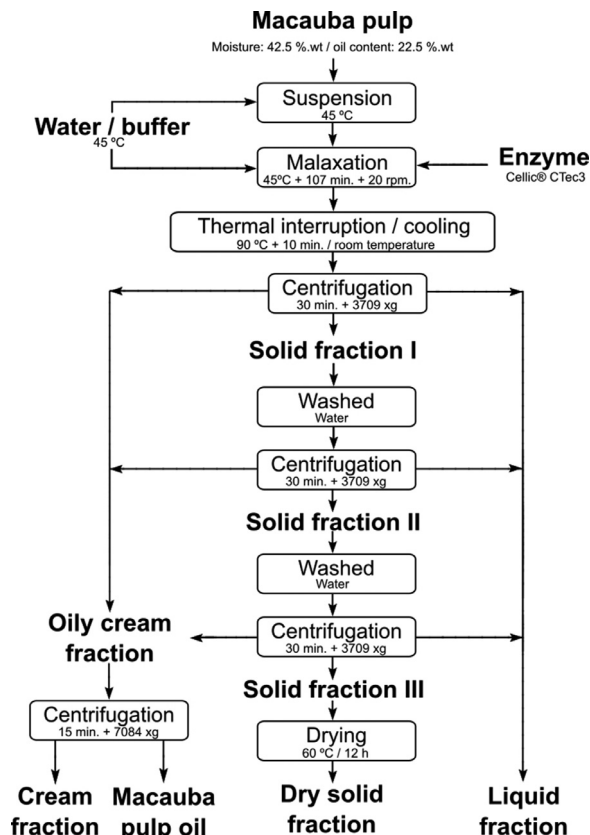


Fig. 2. Steps of the treatments to obtain macauba pulp oil by aqueous extraction in closed reactor.

2.5. Moisture and oil content

Pulp moisture content was determined at 105 ± 2 °C (AOAC, 2005). Oil contents in the pulp and in the solid fractions were measured with an Ankom XT15 extractor (ANKOM Technologies, Macedon, NY AOCS), Am 5-04 method (AOCS, 2004).

2.6. Oil Recovery Efficiency

The ratio of the absolute initial oil mass in the pulp with the final oil mass in the solid fraction was used to obtain the extraction efficiency, as described by Munder et al (2020):

$$\eta(\%) = \frac{m_{OP} - m_{OS}}{m_{OP}} \times 100 \quad (1)$$

Where: η is the oil recovery efficiency (%); m_{OP} is the mass of oil in the pulp (g); m_{OS} is the mass of oil in the solid fraction (g).

2.7. Solvent extraction

In order to have a standard for comparison, macauba pulp oil was extracted by solvent using refluxing hexane in a Soxhlet apparatus for 8 hours. The pulp was previously dehydrated by freeze drying to avoid any possible bias effect on acidification if a high temperature was used in an ordinary oven.

2.8. Chemical analysis of the pulp oil

The acidity index of the pulp oil was determined with a potentiometric titrator Metrohm (Model Titrando 809, Metrohm, Switzerland) according method Ca 5a-40 (AOCS, 2004), expressed as free fatty acid in oleic acid (%). The specific extinction coefficients, also known as molar absorptivities, (k_{232} -value and k_{270} -value) were measured using method Ca 6a-40 (AOCS, 2004). Total carotenes content was measured according to Rodriguez-Amaya & Kimura (2004), solubilizing the oil in petroleum ether and reading at 450 nm using a Shimadzu UV/VIS spectrophotometer (Shimadzu, Japan). The Deterioration of Bleachability Index (DOBI) was determined after solubilizing 0.05 g of pulp oil in 10 mL de iso-octane, and reading the absorbance at 446 nm and 269 nm (Shimadzu UV/VIS, Japan). DOBI is expressed as:

$$DOBI = A_C / A_{SO} \quad (2)$$

Where A_C is the absorbance at 446 nm (corresponding to carotenes); A_{SO} is the absorbance at 269 nm (corresponding to secondary oxidation compounds).

2.9. Chemical analysis of the aqueous fraction from the oil extraction

The soluble solids and total sugars in the aqueous fraction (waste water) were determined to evaluate the enzyme role on cell depolymerization and characterize their potential for further use. Total soluble solids (°Brix), were evaluated using an Abbé refractometer (Electron Machine, model DSA E-Scan, USA) at 25 °C, and total sugar content was evaluated according to the method of Dubois et al., 1956.

2.10. Experimental design and statistical analysis

The effects of the temperature and time of malaxation on the efficiency of oil extraction were verified using experimental design. The experiments were conducted following a 2^2 full-factorial design for the extraction with water (Section 2.4.2), and a central composite rotational design (CCRD) for the enzyme-assisted pulp oil extraction (2.4.3), with five replications for the central point. The results were analyzed using the software Statistica version 10 (StatSoft, 2011). The Response Surface Methodology (RSM) was applied in the CCRD for modeling and analyzing the extraction efficiency under the influence of variables to optimize this response (Montgomery, 2012). The significance of the model was determined by the analysis of variance (ANOVA) (Box et al., 1978).

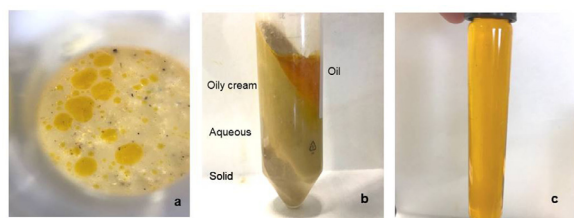


Fig. 3. Macauba pulp oil aqueous extraction. Coalescence of the bodies oil after malaxation (a); Phases separation after centrifugation (b); Macauba pulp oil recovered (C).

2.11. Mass balance

Mass balance was performed for the enzyme-assisted aqueous oil extraction in buffered medium considering the mass of pulp (1000 g wet pulp) and water at the entrance of the reactor and the expected oil, solid fraction and waste water contents in the outlet of the process.

3. Results and discussion

The overall pulping recovery in this pilot scale was 97%, encompassing 36.4% pulp, 24.4% husk and 36.2% shell (endocarp + kernel) (Fig. 1). The 3% loss is due to the fresh pulp that sticks to the pulper or remains with the endocarp. Scaling up this operation is the first step to an important technological advance to reduce costs on a successful aqueous extraction process.

3.1. Aqueous oil extraction

The usual process for extracting oil from macauba pulp usually begins with the drying of the whole fruit, followed by pulping and extraction by continuous pressing. In the first assay of this study, the oil was obtained directly from the fresh fruit by an aqueous process using water as diluent (Section 2.4.2). It was observed that pulp oil coalesced on the liquid surface, forming large oil drops with malaxation at low speed rotation (50 rpm) (Fig. 3a). Four phases formed after centrifugation, a clear oil fraction on the top, an oil-water emulsion fraction (oily cream), an aqueous phase and a solid fraction consisting of the insoluble compounds and the remaining oil (Fig. 3b). The separated pure oil is shown in Fig. 3c. The oil-water de-emulsification was not studied in this work, but it is an essential process that deserves further attention.

The overall efficiency of oil recovery ranged from 67.0% to 73.5% of the 41.2% dry basis total oil content measured in the fresh fruit (Table 1). The highest efficiency was achieved with 30 °C and 90 minutes of malaxation (run 4).

As isolated factors, both temperature and time of malaxation showed no direct influence on the efficiency of pulp oil extraction with water, according to the analysis of variance (ANOVA) (Table 2). However, their interaction was significant, with an inverse effect, as can be noted from the Pareto chart (Fig. 4). A best understanding of this interaction is reached with the help of the contour plot (Fig. 5). Higher efficiencies are obtained with lower temperature and longer times, or their opposites. As for large-scale facilities, such a pattern helps to set up good operational conditions that consider logistics and costs of equipment, energy, and workforce, for example.

The efficiency of oil recovery obtained in this work is greater than those previously reported for macauba pulp oil extraction either by pressing or using solvents. Costa (2016), for instance, achieved 32.6% efficiency when pressing dried pulp under 330 kgf/cm², and 58% under 578 kgf/cm², in both cases the yield was lower than that obtained with the aqueous extraction. Figueiredo et al. (2016) reported an efficiency of only 17.8% by pressing dried fruits. Using solvents, the authors got better results, 55.3% with hexane and 70.2% with isopropanol after 8 hours of extraction, which is still lower than that obtained in the aqueous

Table 2

Analysis of variance (ANOVA) of the full 2² factorial design to evaluate temperature and time effects on aqueous extraction of macauba pulp oil.

Factor	QS	DF	MS	F	p
A - Temperature (°C)	1.8421	1	1.8421	1.5615	0.3378
B - Time (min)	0.0175	1	0.0175	0.0148	0.9140
A x B	24.9863	1	24.9863	21.1812	0.0441
Lack of fit	9.6851	1	9.6851	7.6917	0.1091
Pure error	2.3592	2	0.9379		
Total QS	38.2787	6			
R ²	0.7013				
R ² -Adjusted	0.4026				

QS: quadratic sum; DF: degrees of freedom; MS: mean square. A linear term of the temperature; B linear term of the time; AB interaction term between the factors A and B

F Fisher-test; p significance at the 95% confidence ($p < 0.05$).

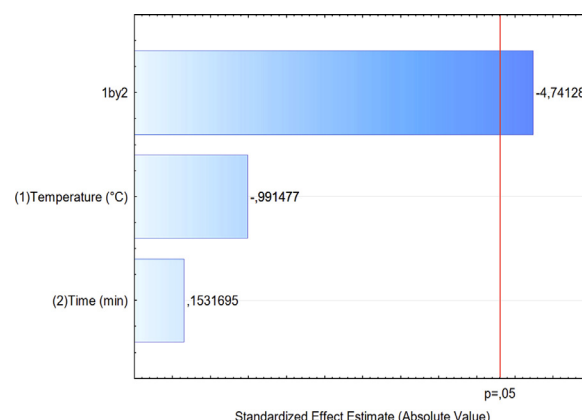


Fig. 4. Pareto chart of effects of time and temperature upon extraction efficiency of macauba pulp oil with water using a 2² full experimental design.

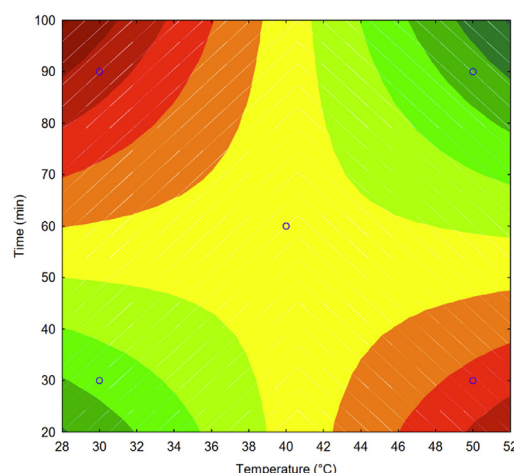


Fig. 5. Contour plot of the aqueous extraction efficiency of the macauba pulp oil considering temperature and time of malaxation.

extraction. Furthermore, processing fruits just after harvesting may prevent changes in oil quality characteristics that could occur with drying of the fruits, either at room temperature or in faster energy-demanding processes. Indeed, the straightforward fruit processing, with short storage, quick pulping and mild extraction conditions assured a low fatty acid release despite the high moisture content all along the process. The free fatty acid content was 0.43% oleic acid, which is far below the 5% maximum concentration for crude vegetable oils (Anvisa (2005); Codex Alimentarius, 1999). Macauba pulp oil can contain acidity levels

Table 3

Experimental design matrix and recovery efficiency of pulp oil aqueous extraction assisted by the enzyme pool Cellic® CTec3.

Run	Coded level of variables		Actual level of variables		Response	
	A	B	Time (min)	Temperature (°C)	Oil recovery efficiency (%), Y	
					Observed	Predicted
1	-1	-1	30	30	58.9	58.2
2	1	-1	90	30	84.3	81.6
3	-1	1	30	50	59.6	64.0
4	1	1	90	50	83.3	87.4
5	-1.41	0	18	40	58.0	56.0
6	1.41	0	102	40	89.1	88.7
7	0	-1.41	60	26	66.5	69.4
8	0	1.41	60	54	83.1	77.6
9	0	0	60	40	76.1	79.6
10	0	0	60	40	80.2	79.6
11	0	0	60	40	77.6	79.6
12	0	0	60	40	80.9	79.6
13	0	0	60	40	83.4	79.6

Table 4

Analysis of variance (ANOVA) of the main effects and interactions of factors on the oil recovery efficiency from macauba pulp.

Factor	QS	DF	MS	F	p
Model					
A – Time (min)	1081.26	1	1081.26	130.6901	0.0003
B – Temperature (°C)	66.471	1	66.47	8.0342	0.0471
A x B	0.7228	1	0.7228	0.0874	0.7822
A ²	94.27	1	94.27	11.3939	0.0279
B ²	66.34	1	66.34	8.0189	0.0472
Residual	120.02	7	17.15		
Lack of fit	86.93	3	28.98	3.5023	0.1286
Pure error	33.09	4	8.27		
Total QS	1412.14	12			
R ²	0.9150				
R ² -Adjusted	0.8543				

QS: quadratic sum; DF: degrees of freedom; MS: mean square. A linear term of the temperature; B linear term of the time; AB interaction term between the factors A and B.

F Fisher-test; p significance at the 95% confidence (p<0.05).

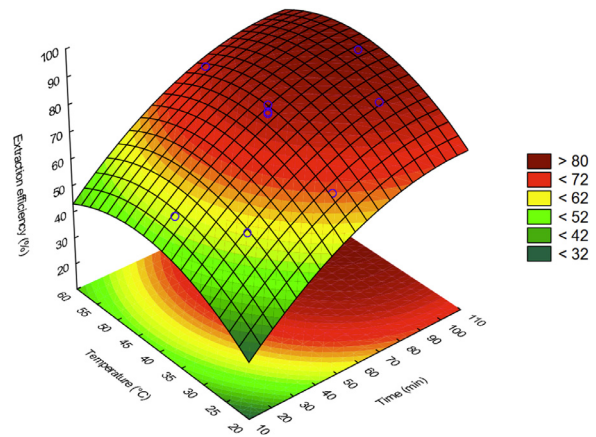


Fig. 6. Surface response plot of the efficiency of enzymatic assisted extraction of macauba pulp oil.

up to 30% oleic acid (Favaro et al., 2017; Tilahun et al., 2019) because of prevalent unsuitable storage or processing conditions.

3.2. Aqueous enzyme assisted extraction

The use of hydrolytic enzymes to assist the aqueous extraction of the oil under certain conditions resulted in higher efficiency of oil extraction, varying from 58.0% to 89.1% (Table 3). The ANOVA indicates that temperature (p<0.05) and duration (p<0.001) of malaxation have significant effects, but their interaction is not significant (p>0.05) (Table 4). The main significant effect (p<0.001) corresponds to the first order term of the time of malaxation, followed by its quadratic term (p<0.05). The mathematical model for the significant effects of temperature and time is as follows:

$$\eta = -23.7926 + 0.9441A - 0.00415A^2 + 2.8812B - 0.00142B^2 \quad (3)$$

Where η is the oil efficiency recovery (%); A is temperature (°C) and B is the time (minutes) of enzymatic malaxation.

ANOVA model shows no lack of fit (p>0.05). R² value (multiple correlation coefficient) is 0.91; meaning that the regression and the model can explain 91% of the variables' response and can be used for predictive purposes.

The response surface methodology (RSM) plot (Fig. 6) showed that maximum efficiency of pulp oil extraction (89%), within the range of the

experimental conditions evaluated, would be achieved at 45 °C and 107 min of malaxation. On the other hand, it should be stressed that time of malaxation has a major effect on the oil recovery efficiency, whereas temperature has a slight effect. Therefore, it could be possible to have gains in oil recovery with enzyme assistance even at mild temperatures, with energy savings and maintenance of oil quality.

This is the first report on the use of enzyme-assisted malaxation to extract macauba pulp oil without pressing. Most studies on the subject are focused on olives (Aliakbarian et al., 2008), oil palm (Teixeira et al., 2013), and oilseeds (De Faveri et al., 2008). It is a proof of a technological concept towards the development of an efficient aqueous extraction process, although based on an indirect method, as made by Munder et al. (2020) to determine the oil extraction efficiency from sunflower seeds. Further studies are needed to set a reliable procedure to measure directly the amount of oil released from the original tissue and from the demulsified fraction.

3.3. Aqueous oil extraction in closed reactor and mass balance

The recovery efficiency of the aqueous extraction of macauba pulp oil in the scale up experiment was 73.5% (Table 5), matching that obtained with the best condition measured in the laboratory-scale experiment for the same treatment (Table 1). The assistance of the enzyme pool Cellic® CTec3 increased that efficiency to 85.7%, which was further improved to 88.7% when buffer solution was used instead of water

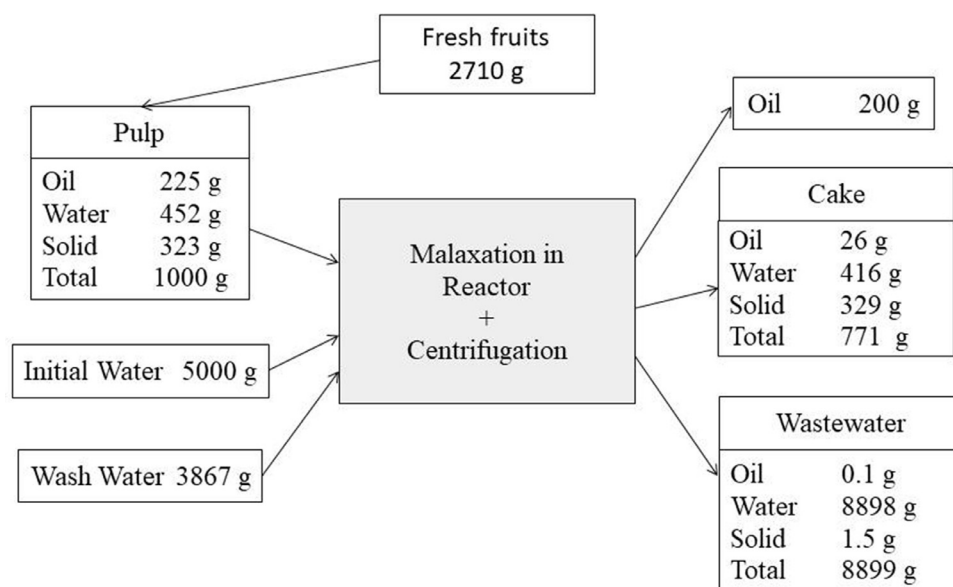


Fig. 7. Mass balance of enzyme-assisted aqueous oil extraction from macauba pulp in buffered medium.

Table 5

Oil recovery efficiency and expected oil yield of macauba pulp oil by aqueous extraction in closed reactor.

Treatments	Oil recovery efficiency %	Whole fruit pulp oil yield ¹ %
Water	73.5	6.1
Cellic CTec3 + water	85.7	7.1
Cellic CTec3 + buffer	88.7	7.4

¹ Based on 36.9% fresh pulp in the whole fruit, with 22.5% oil content (wet basis).

(Table 5). Again, these results indicate that it is feasible to achieve high efficiency of oil extraction at a larger scale with the aqueous process.

The extraction of the oil from seeds and other biomass using aqueous conditions is based on the insolubility of the oil in water. The water-soluble components of the biomass are diffused in water, releasing oil from the fiber structure. The increase in the efficiency of extraction is made possible by partial degradation of the structural polymer of the cell wall by the enzyme, promoting easy and efficient oil extraction (Kalia et al., 2001).

The fresh fruits evaluated in this study had an average pulp mass of 36.9%, with 22.5% oil content, which is near the average seen in macauba fruits. Thus, the total pulp oil content was 8.3%, considering the whole fruit. In the aqueous treatment, the oil yield was 6.1%, increasing to 7.1% and 7.4% in the water with enzyme and buffer plus enzyme treatments, respectively. These values are about three times larger than those currently obtained by mechanical pressing at large scale in the industry, around 1.9%, based in the whole fruit mass (C. Daher, personal communication, May 8, 2020, Paraguay). Therefore, it can be concluded that the aqueous extraction is advantageous in comparison to the traditional pressing of dried pulp.

The mass balance of the overall process of the enzyme-assisted aqueous oil extraction in buffered medium is presented in Fig. 7. Starting with 1.000 g of wet pulp and 88.7% oil extraction efficiency, 200 g of oil was obtained after the de-emulsification process. The wet cake mass (771 g) that was separated after centrifugation was composed mostly wet fiber material, containing around 54% moisture and 11.3% oil entrapped into the tissue cells. This remaining oil could be removed by an additional solvent process, as it is usually made in the olive oil industry to produce the bagasse grade olive oil.

The whole process consumes a total of 8,867 g of water (Fig. 7), 8,899 g being recovered as wastewater after the malaxation. It contained soluble proteins, sugars and other minor compounds. Evaluations of this wastewater confirmed that the enzyme pool Cellic® CTec3 effectively acted upon the structural carbohydrates, releasing larger contents of sugars (Table 6). There is at least twice as much soluble solids (°Brix) and total sugars (g/L) in both water and buffer enzyme-assisted treatments than in the water extraction treatment. The recovery of these products could be important not only to the environment but as a source of added value to the production chain, such as the production of bioproducts by fermentation processes (Hodaifa et al., 2008).

3.4. Physicochemical characterization of macauba pulp oil extracted in closed reactor conditions

Overall, the aqueous extraction preserved the quality of the macauba pulp oil during the processing in the closed reactor. The most efficient treatment, enzyme dissolved in buffer, resulted in a more favorable oil quality, as measured by acidity, extinction coefficients and DOBI (Table 7). The acidity of the oil extracted only with water was the lowest, less than 0.5% oleic acid. The assistance of the enzyme pool resulted in a slightly greater release of free fatty acids. However, it is within the acceptable range of low-grade acidity, as compared to the maximum for palm oil, 5% (CODEX Codex Alimentarius, 1999), since there is no such standard for macauba yet. Such a slighter higher acidity is probably caused by greater disruption of cells and their organelles, allowing larger interaction of the released oil with the water and endogenous lipases, with hydrolysis of the triacylglycerols.

The oil obtained by solvent extraction (Section 2.7) showed a higher acidity (4.16%) than that obtained by aqueous extraction (Table 7). The working temperature of the solvent, around 72 °C, during 8 h of reflux, might have favored the release of fatty acids. The mild process conditions and the relatively short time for aqueous extraction contributed to the maintenance of low acidity, which is clearly an advantageous effect. Even the abundant presence of water, that from the pulp itself and that added for the malaxation, seemed not to be an issue.

The extinction coefficients K_{232} nm and K_{270} nm are indicative of the oxidation status of the oil (Shahidi & Wanasundara, 2002). K_{232} is shown to be highly correlated to the development of peroxides in macauba pulp oil in a first oxidative degradation (Tilahun et al., 2019). The extraction with water and solvent showed similar K_{232} figures, higher than those measured in both enzyme-assisted treatments

Table 6

Soluble solid and total sugars in the liquid fraction (wastewater) of enzyme pool Cellic® CTec3 assisted aqueous extraction of macauba pulp oil with buffered solution.

Aqueous extraction	Centrifugation	Soluble solids (Brix)	Total Sugars (g/L)
Water	1st	2.0±0.1	10.0±0.4
	2 nd + 3 rd	1.6±0.4	8.7±0.0
Cellic® CTec3 in water	1st	4.3±0.1	23.4±0.4
	2 nd + 3 rd	1.0±0.1	6.6±0.3
Cellic® CTec3 in buffer	1st	4.3±0.1	20.8±0.0
	2 nd + 3 rd	1.2±0.1	4.3±0.0

Table 7

Quality parameters of macauba pulp oil obtained in closed reactor by aqueous extraction and by solvent.

Treatment	Free Fatty Acid (oleic acid %)	Extinction coefficients		Total carotenes (µg/g)	DOBI ¹
		K ₂₃₂	K ₂₇₀		
Water	0.4±0.1c	3.9±0.1a	0.28±0.003b	107.0±5.2b	0.74±0.00c
Enzyme A ²	1.5±0.0b	2.9±0.2b	0.33±0.020a	109.0±0.0b	1.00±0.00b
Enzyme B ³	1.2±0, bc	2.4±0.1c	0.27±0.013b	101.6±1.2b	1.20±0.04a
Solvent	4.2±0.4a	3.7±0.0a	0.30±0.005ab	131.8±4.6a	0.81±0.00c

¹ Deterioration of Bleachability Index. ² Cellic® CTec3 + water. ³ Cellic® CTec3 + buffer.

(Table 7). Secondary degradation, indicated by K₂₇₀, meaning the formation of trienes, shows a more random pattern, with oils extracted either by solvent or by aqueous extraction presenting similar trends (Table 7). Since there is few reports on these parameters for macauba oil, it is difficult to assert that these values should be considered as indicative of a significant oil degradation. For comparison, K₂₃₂ figures are close to those found in a study with oil extracted by expelling the pulp from whole dried fruits at 80-90 °C for 7 to 8 hours, while K₂₇₀ is about half (Nunes et al., 2015). Authors claim that the obtained oil was of good quality. Therefore we could assume that the aqueous extraction resulted in oil with suitable oxidative status.

The total carotene content on the evaluated aqueous extractions varied from 101.6 µg/g to 109.0 µg/g, about 20% lower than extracted with hexane (Table 7). Probably the hexane gets a better drag because of its high hydrophobicity. Even though, these values are situated in the low range reported for macauba pulp oil, within 103 to 568 µg/g (Ciconini, 2012; Oliveira et al., 2017; Del Río et al., 2016; Nunes et al., 2015; Valerio et al., 2019; Tilahun et al., 2019). Such a broad range of carotene content is related not only to the processing conditions, but also to the genetic diversity within this species.

The DOBI value reflects the freshness and the ripeness of the oil palm fruits, besides the presence of contaminants, being related to the degree of oxidation and to the capability of bleaching of the palm oil (Lin, 2001; Zschau, 2001). As a quality control, stresses the ratio between total carotenes content and the development of degraded compounds from oxidative reactions. It helps setting up the oil refining, including the type and amount of activated earth to bleach the oil (Lin, 2001).

DOBI grade for palm oil is determined as: <1.68 (sludge palm oil); 1.78 – 2.30 (poor); 2.31-2.92 (fair); 2.93-3.24 (good); >3.24 (excellent) (Zschau, 2001). DOBI figures in the aqueous extractions of macauba pulp oil are in the range 0.74 to 1.20, being 0.81 in the solvent extraction (Table 7). If compared with palm oil, the current figures would rank the evaluated macauba pulp oil as sludge grade. However, oil palm and macauba pulp oil have different carotenes contents and composition. Crude palm oil has around 500 to 1224.5 µg carotenes/g oil (Benade, 2003; Tan et al., 2017), while the maximum reported so far for macauba is 568 µg/g, as mentioned above. Adding to that, crude palm oil has a β-carotene content up to 57% (Yap et al., 1991), while in macauba oil it goes up to 70 to 80% (Ramos et al., 2008). Also, the difference in the fatty acid composition of both oils, such as predominance of palmitic acid in palm oil and oleic acid in macauba oil, as well other

minor components (Oliveira et al., 2017; Tan et al., 2017) may result in a specific spectral response at 269 nm. All together, these intrinsic characteristics of each palms species may result in different ratios by which DOBI is calculated.

Currently, there is no DOBI standards established to macauba pulp oil, and, to our best knowledge, this is the first report of its evaluation. It is important such register to contribute to a further set up of proper standards for macauba oils.

This first report on oil aqueous extraction shows the potential of this process to provide feasible yields and high-quality macauba pulp oil. The oil extraction efficiencies were higher, whether assisted or not by the enzyme pool Cellic® CTec3 than the current expeller process. Furthermore, the aqueous extraction uses fresh fruits with no need of previous drying, which makes the process less energy and time-consuming. The yields and intrinsic quality of oil are kept even when fruit pulp is processed at a larger scale.

Further studies are necessary to broad the knowledge on this process, including evaluations of other isolated or combined enzymes, and scale up of unitary operations. It includes mechanisms to demulsify the cream fraction and deeper data gathering on the composition of the residues of the process (liquid and solid fractions). This could lead to find out alternatives to mitigate or, even better, to add value by the exploitation of byproducts. Thus, it is worthy to take further steps to combine the positive aspects of macauba palm as a crop, with a clean and efficient processing to provide a novel sustainable vegetable oil source.

4. Conclusion

This is the first report on aqueous extraction of oil from macauba pulp. Results clearly demonstrated that the aqueous extraction, that uses mild processing settings, has the potential to surpass the oil recovery efficiency of traditional system with expelling of the dried pulp. High quality oil was obtained. The assistance of the commercial enzymatic pool Cellic® CTec3 improved the oil recovery efficiency up to 88.7%, meaning 7.4% oil yield of the initial fruit mass.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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