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Comparação da estabilidade termo-oxidativa do óleo de murici (*Byrsonima crassifolia* L. Kunt) obtido por hidrólise enzimática assistida por ultrassom e método clássico

Comparison of the thermo-oxidative stability of murici oil *(Byrsonima crassifolia* L. Kunt*)* obtained by enzymatic hydrolysis assisted by ultrasound and classical method

Comparación de la estabilidad termooxidativa del aceite de murici (*Byrsonima crassifolia* L. Kunt) obtenida por hidrólisis enzimática asistida por ultrasonido y método clásico

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Resumo

A extração de óleo de várias matérias-primas por processo enzimático tem recebido grande importância por ser considerada tecnologia limpa. Além disso, o produto obtido por ação enzimática apresenta melhores rendimentos, bem como melhores parâmetros de qualidade química, além de melhores condições de estabilidade. Assim, o objetivo do presente trabalho foi otimizar o processo de extração de óleo de polpa de murici por hidrólise enzimática e

comparar a estabilidade termo-oxidativa do óleo de murici extraído por hidrólise enzimática assistida por ultrassom e o óleo obtido pelo método clássico (Soxhlet). Os resultados obtidos mostraram dependência da variável dependente - rendimento (%) - em função das variáveis independentes - concentração enzimática (%) e tempo de exposição ao ultrassom (min). A partir da análise estatística aplicada, observou-se que o óleo extraído por hidrólise enzimática apresentou maior estabilidade térmica (400 °C) em comparação ao obtido pelo método clássico (384 °C). A análise da estabilidade oxidativa mostrou maior propensão à oxidação do óleo obtido pelo método clássico comparado ao obtido pela hidrólise enzimática em 6 horas de indução a 110 °C. Assim, o óleo obtido por hidrólise enzimática possui propriedades superiores às obtidas pelo método clássico e, portanto, com melhor estabilidade termo-oxidativa.

Palavras chaves: Óleo de murici; Hidrólise enzimática; Estabilidade térmica; Estabilidade oxidative.

Abstract

The extraction of oil from various raw materials by enzymatic process has received great importance for being considered clean technology. In addition, the product obtained by enzymatic action has better yields as well as better chemical quality parameters, also better stability conditions. Thus, the objective of the present work was to optimize the murici pulp oil extraction process by enzymatic hydrolysis and to compare the thermo-oxidative stability of murici oil extracted by ultrasound-assisted enzymatic hydrolysis and the oil obtained by the classic method (Soxhlet). The results obtained showed a dependence on the dependent variable - yield (%) - as a function of the independent variables - enzyme concentration (%) and exposure time on ultrasound (min). From the applied statistical analysis, it was observed that the oil extracted by enzymatic hydrolysis showed greater thermal stability (400 °C) compared to that obtained by the classical method (384 °C). The analysis of oxidative stability showed greater propensity to oxidation the oil obtained by classical method compared to that obtained by enzymatic hydrolysis in 6 hours of induction at 110 °C. Thus, the oil obtained by enzymatic hydrolysis has properties superior to that obtained by the classical method and, thus, with better thermo-oxidative stability.

Keywords: Murici oil; Enzymatic hydrolysis; Thermal stability; Oxidative stability.

Resumen

La extracción de aceite de diversas materias primas mediante procesos enzimáticos ha recibido gran importancia por ser considerada tecnología limpia. Además, el producto obtenido por acción enzimática tiene mejores rendimientos, mejores parámetros de calidad química y mejores condiciones de estabilidad. Por lo tanto, el objetivo del presente trabajo fue optimizar el proceso de extracción de aceite de pulpa de murici por hidrólisis enzimática y comparar la estabilidad termooxidativa del aceite de murici extraído por hidrólisis enzimática asistida por ultrasonido y el aceite obtenido por el método clásico (Soxhlet). Los resultados obtenidos mostraron una dependencia de la variable dependiente - rendimiento (%) - en función de las variables independientes - concentración de enzima (%) y tiempo de exposición a ultrasonido (min). Del análisis estadístico aplicado, se observó que el aceite extraído por hidrólisis enzimática mostró una mayor estabilidad térmica (400 °C) en comparación con el obtenido por el método clásico (384 °C). El análisis de la estabilidad oxidativa mostró una mayor propensión a la oxidación del aceite obtenido por el método clásico en comparación con el obtenido por hidrólisis enzimática en 6 horas de inducción a 110 °C. Así, el aceite obtenido por hidrólisis enzimática tiene propiedades superiores a las obtenidas por el método clásico y, por lo tanto, con una mejor estabilidad termooxidativa.

Palabras clave: Aceite de murici; Hidrólisis encimática; Estabilidad térmica; Estabilidad oxidative.

1. Introduction

World technological development has been shown to be progressive in the direction of biotechnological processes, mainly focused on the interests of replacing conventional chemical processes with enzymatic processes, due to the irreversible trend of prevalence of environmental policies (Sheldon & Woodley, 2018).

Given this perspective, the use of biotechnological processes in the most diverse sectors of the food industry have stood out more and more in improving their production methods. Depending on this, the use of enzymes in industrial processes is of great interest, especially regarding the advantages presented in relation to catalysts of chemical nature, when these are more advantageous in terms of specificity, energy consumption and in the conditions reaction velocity (Prasad & Roy, 2018). Thus, the application of enzymes in the extraction processes of vegetable oils has emerged as an alternative in solving problems with the use of solvents potentially offensive to the environment (Liu et al., 2019).

Vegetable oils are often subjected to heat treatments and also exposed to oxygen, factors compromising the condition of its stability. Thus, the use of vegetable oils for various purposes has led to the need to better assess its quality and degree of resistance, mainly its storage stability and the thermal processes that can cause loss of nutritional and functional quality (Reda & Carneiro, 2007).

The oil contained in the pulp of the fruits of the muricizeiro (*Byrsonima crassifolia*) represents approximately 23% of wet lipids (Monteiro et., 2015). It also has a lipid profile rich in mono and polyunsaturated fatty acids, consisting of more than 80% of its composition, may be equivalent to the values found for olive oil (Rezende & Braga, 2003). Also, due to its potential constitution by compounds of an antioxidant nature, among which murici oil is a product with high added value in terms of maintaining its stability.

Several methods have been used in order to assess the extensibility of thermal and oxidative deterioration of vegetable oils, and which are based on thermo analytical and accelerated oxidation induction assay. These methods are more advantageous than conventional methods, because they are more precise, sensitive, require less sample and results are obtained more quickly. They are also widely used in the quality control of vegetable oils, because they provide, quickly, data on oil stability in terms of its thermal behavior (Santos et al., 2002; Freire et al., 2012; Oliveira et al., 2019).

Thermal analysis allows a wide range of application for measuring physical properties, study of chemical reactions, thermal stability assessment, determination of material composition and development of analytical methodology. Dynamic thermogravimetric techniques, in turn, can be used to stimulate the level of stability of these oils and fats (Faria, et al., 2002).

Oxidative stability tests express the ability to maintain fatty acid oxidation stability in terms of an induction period (PI) in the formation of volatile organic acids resulting from accelerated oxidation conditions, and its determination is based on the increase in electrical conductivity (Knothe et al., 2006).

In order to provide contributions on alternative methods for extracting vegetable oils, and mainly in the evaluation of its stability conditions in the face of processing situations, the present research consists of evaluating the thermo-oxidative stability of murici oil extracted by enzymatic hydrolysis assisted by ultrasound.

2. Material and Methods

This article is a quantitative explanatory research (Pereira et al., 2018) developed by the first author in the course co-conclusion work under the guidance of the fourth author. In this study, different enzymatic concentration and time of murici oil extraction were optimized to finally compare the thermo-oxidative stability of the oil extracted from the best condition of ezyme extraction with the oil obtained by the conventional method using a chemical solvent.

Several researchers, such as Ribeiro et al. (2016) and Polmann et al. (2019) deal with the use of enzymes for oil extraction, where it can be observed that enzymatic hydolysis improves in the oil recovery process as well as the quality of this product being better when compared to that obtained by the conventional extraction method. Next, the experimental methodologies used in the development of the work will be presented.

2.1 Sample

The present study was carried out in the cereals, chemistry and vegetable laboratories of the food engineering department of the Federal University of Maranhão (UFMA). The murici fruits were collected in the rural area of Amarante - MA (Latitude: 5°34'8" South and Longitude: 46°44'16" West). The collection occurred at random, from the ground, on the same day of the fall, with the fruits in an ideal stage of commercial maturation (yellowish pulp) and suitable for fresh consumption. The collected fruits were washed in running water and packed in clean and dry plastic bags, and stored in a freezer with a controlled temperature of - 22 °C, until the moment of use for extracting the pulp. After selection, sanitization, rinsing and weighing steps, the fruits were pulped in a mechanical pulper, packed in polyethylene bags and stored under freezing at -22 °C until further analysis.

2.2 Centesimal composition of murici pulp

The analysis of the proximate composition of the murici pulp was carried out according to the methodology of the Instituto Adolf Lutz - IAL (2008) regarding moisture content (012/IV), protein (037/IV), lipids (032/IV), ashes (018/IV), β-carotene (123/IV) and carbohydrates by 100% difference - {moisture + protein + lipids + ashes}.

2.3 Obtaining the enzyme complex

The enzyme complex was obtained according to the methodology developed by Souza et al. (2019) therefore, using a solid-state fermentation process and canola cake as a substrate. The fermentation agent was the Aspergillus oryzae NRRL 1911.

2.4 Experimental design for extracting murici pulp oil

To evaluate the effect of the enzyme complex and ultrasound on the yield of murici pulp oil, a central composite rotational design (CCRD) was used. Two variables (enzyme concentration and exposure time on ultrasound) were chosen for the surface response methodology (RSM) to verify their effects on oil yield. The two significant variables studied at five coded levels (-1.4142, -1, 0, +1, +1.4142) are shown in Table 1. CCRD resulted in a total of 11 experimental tests, including 4 factorial design, 4 axial points and 3 central point repetitions (Table 2).

Table 1 - Levels of factors chosen for CCRD as a function of enzyme concentration (%) and exposure time on ultrasound.

Factors	**Unity**	**Levels of coded variables**				
		-1.4142	**-1**	**0**	**+1**	**+1.4142**
Enzyme concentration	%	0.05	0.08	0.15	0.22	0.25
Time	min	20	27	45	63	70

Source: Authors.

Table 2 – Central composite rotational design (CCRD) for oil yield obtained by enzymatic hydrolysis assisted by ultrasound.

Test		**Independent variables**		**Dependent variable**
Rand	**Test**	**Enzyme concentration (°C)**	**Extraction time (min)**	**Yield (%)**
11	1	0.08(-1)	27(-1)	0.9167
4	2	0.08(-1)	63(+1)	1.3955
10	3	0.22(+1)	27(-1)	1.3105
6	4	0.22(+1)	63(+1)	2.2134
1	5	0.05(-1.41)	45(0)	1.1169
5	6	0.25(+1.41)	45(0)	3.5561
7	7	0.15(0)	20(-1.41)	1.1135
8	8	0.15(0)	70(+1.41)	2.4398
2	9	0.15(0)	45(0)	2.1009
9	10	0.15(0)	45(0)	2.0919
3	11	0.15(0)	45(0)	2.1099

Source: Authors.

The CCRD results were expressed by the following second order polynomial, using a multiple regression technique, according to the following equation 1:

$$Y = \beta 0 + \sum \beta iXi + \sum \beta iiX2i + \sum \beta ijXiXj \quad (1)$$

where: Y is the predicted answer, β_0 is the intercept term, β_i is the linear coefficients, βii is the quadratic coefficients, β_{ij} is the interaction coefficient and X_i and X_j are the coded independent variables.

2.5 Extraction of murici pulp oil

2.5.1 Extraction by ultrasound-assisted enzymatic hydrolysis

For the enzymatic extraction in aqueous medium, assisted by ultrasound of murici oil, proceeded according to the pre-arranged methodology proposed by Freitas et al. (1993), with adaptations in the sense of inserting the assistance of the ultrasonic bath to the extraction procedures, making use of this, the conditions proposed in the methodology described by Watanabe et al. (2005).

Ultrasound-assisted reactions were conducted in a beaker with a capacity of 600 mL. In each experiment, the amount of murici pulp used was 50 g, subsequently diluted in distilled water (300 mL). A thermal pretreatment to inactivate the enzymes of natural composition of the fruit was applied to the pulp samples diluted in distilled water, in a water bath at a temperature of 85 °C for 15 min. Then the medium was cooled until it reached the reaction temperature, around 40 ± 2 °C.

Then, the enzyme extract was added, in the proportions determined in experimental design, all calculated in relation to the pulp mass used. The extractions followed with the immersion of the beaker containing the diluted pulp and enzymatic extract in an ultrasonic bath Limp Sonic, model LS-3D-2 with capacity of 2,8 L, frequency of 40 kHz, maximum power 90 W and dimensions of the 13.7x24.0x10.0 cm. A mechanical stirrer of the brand was added to the set Fisatom and model 713D, used over complementary extraction time (absence of cavitation activity of ultrasound) and a skewer-type digital thermometer Tramontina, model IM-910.0150E for temperature control over the reaction time.

At the end of each reaction time, 20 ml of n-hexane was added to the reactor in order to interrupt the reaction and decrease the viscosity of the medium. The sample was then centrifuged at 3,000 rpm for 30 minutes. The phase containing the oil was collected using a Pasteur pipette and the aqueous phase resulting from the centrifugation was then discarded.

The collected oil was kept in an oven at 50 °C, for evaporation of the solvent and elimination of residual moisture, until constant weight was obtained. The final product was quantified by gravimetric method, as well as its yield and, then, packed in glass bottles wrapped in aluminum foil to avoid exposure to light, and stored under refrigeration.

2.5.2 Classical Soxhlet extraction

Oil was extracted with the hexane solvent in a soxhlet extractor. A 50 g sample of murici pulp was weighed on filter paper and disposed of as a cartridge, later being introduced into the extractor, in turn coupled to a flat-bottomed balloon.

Volume of hexane used was that necessary to cover the entire cartridge containing the sample. The extraction was carried out continuously for 8 hours, using constant temperature and the extracted oil was dried in an oven at 50 °C, for two hours, to eliminate the remaining solvent. The extract was then quantified by gravimetry and placed in glass flasks covered with aluminum foil.

2.6 Chemical analysis for murici oil quality control

The analyzes were performed according to the methodology of IAL (2008), regarding the acidity index (325/IV), refractive index (327/IV) and saponification index (328/IV).

2.7 Evaluation of thermal stability of the oil by thermogravimetry (TG/DTG)

The thermogravimetric analysis of the extracted murici oil was carried out in a thermobalance with simultaneous thermal analysis TG/DTG, Shimadzu, model DTG-60, in oxygen atmosphere with flow of 100 mL min^{-1}, and heating rate of 10°C min^{-1}.

The oil mass used for the analysis was approximately 8 mg for both oil samples (by enzymatic extraction and solvent extraction) arranged in an aluminum crucible. The variation of the oil mass as a function of temperature was verified in the range of 25 to 900 °C.

2.8 Evaluation of oxidative stability of oil by Rancimat

For the oxidative stability tests of extracted murici oil, the samples were subjected to aging in equipment Rancimat of Methorm, model 873, making for such applicability of the method governed by the norm EN 14112.

Therefore, 2 g of oil sample was extracted enzymatically and by classic solvent extraction. Both analyzes were performed in duplicate.

The samples were subjected to aging at a temperature of 110 °C, under constant flow of dry atmospheric air, at a flow rate of 10 L h^{-1}. The analysis lasted 6 hours.

2.9 Statistical analysis

The t test was used to verify the difference between oil extracted by enzymatic hydrolysis and conventional as for ORD (Oil decomposition range), MMLT (Maximum mass loss temperature) and TOx (Oxidation temperature). The significant differences at $p < 0.1$ and statistical analysis was performed by analysis of variance (ANOVA), using SAS software (SAS Institute, Cary, NC) Version 9.1.3.

3. Results and Discussion

3.1 Centesimal composition of murici pulp

The values for the centesimal composition and β-carotene data of murici pulp are shown in Table 3.

Table 3 - Centesimal composition and β-carotene for murici pulp.

Component	**Average±SD**
Moisture (%)	74.78±0.00
Lipids (%)	7.58±0.15
Protein (%)	0.52±0.00
Ashes (%)	1.89±0.74
Carbohydrates (%)	15.23

Source: Authors.

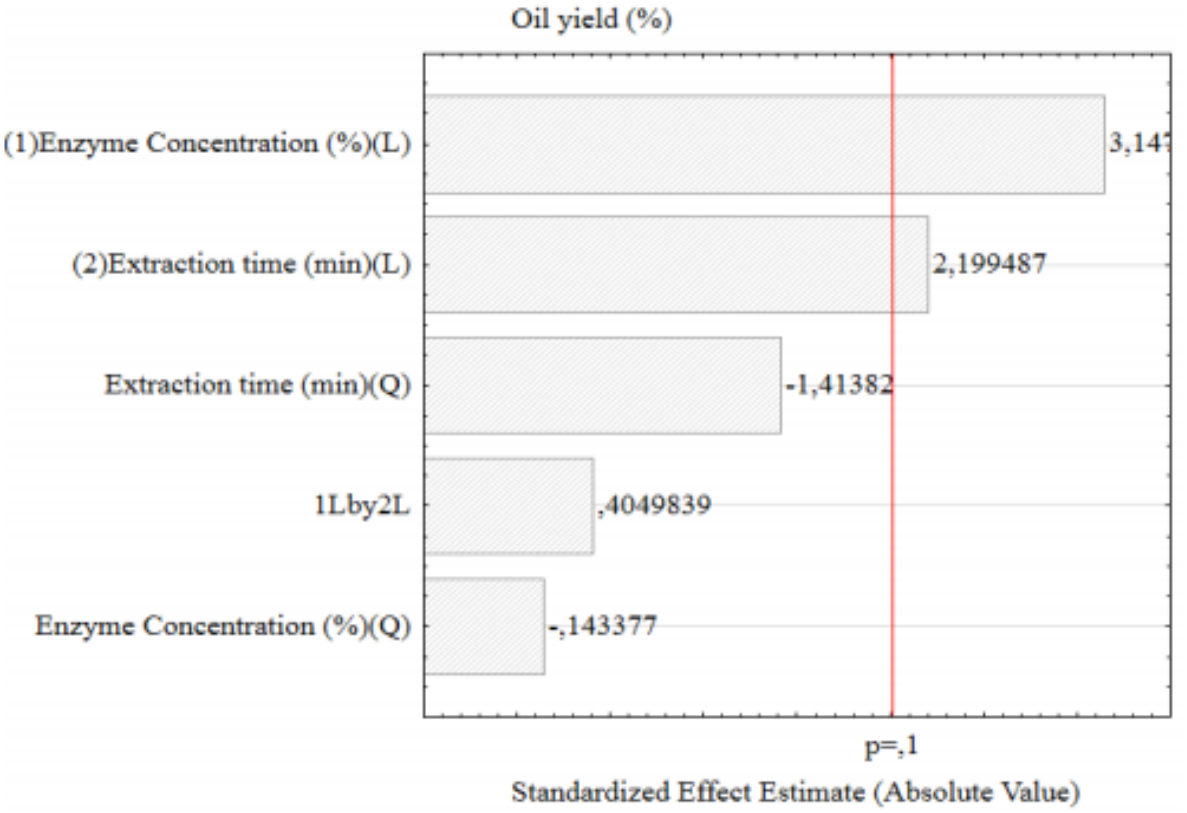
The pulp presented moisture (74.78%) as the major majority constituent, followed by carbohydrates (15.23%) and lipids (7.58%).

3.2 Optimization of oil extraction by enzymatic action

The experimental tests were followed according to the CCRD conditions and the oil yield of murici pulp was recorded. The yield results are shown in Table 2, showing that the yield ranged from 0.92% by weight (Test 1) to 3.56% by weight of oil (Test 6), corresponding to 12.9% and 46.91% efficiency of oil extraction from murici pulp, respectively. Thus showing that the use of enzyme for oil extraction has a positive effect on oil recovery.

From the Pareto Chart (**Figure 1**), it can be observed that the linear factors of the independent variables: enzyme concentration (%) and extraction time (min), were significant ($p < 0.1$) in the extraction of murici pulp oil.

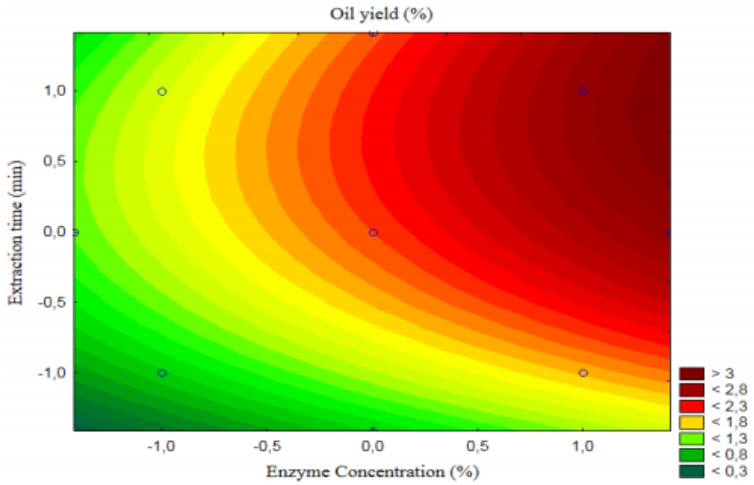
Figure 1 - Pareto graph of murici pulp oil yield obtained by enzymatic hydrolysis of CCRD.



Source: Authors.

As seen in Figure 2, increasing the enzyme concentration and the time of extraction in ultrasound increases the oil yield. However, when the independent variables rise too much, it can cause a negative effect (Figure 1).

Figure 2 - Yield contour region as a function of enzyme concentration (%) and extraction time (min) on ultrasound.

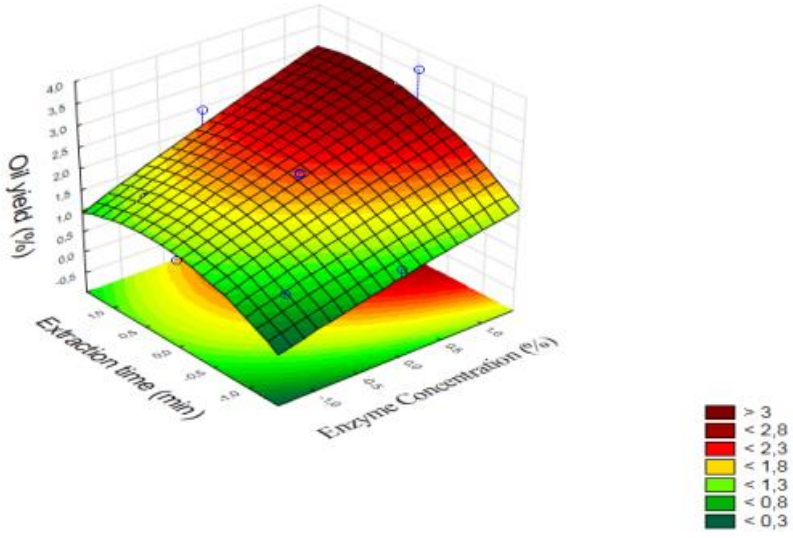


Source: Authors.

The results reveal dependence on income (Y) and independent variables – enzyme concentration (%; X_1) and extraction time (min; X_2) - and the linear model generated by the RSM (Figure 3) expressed in Equation 1 was positively significant (p = 0.0117).

$$Y = 2.10 + 0.58X_1 + 0.41X_2 \quad (1)$$

Figure 3 - Effect of the interaction of the murici pulp oil extraction variables by enzymatic hydrolysis on the yield (Y).



Source: Authors.

The quality of fit of this predictive model was assessed by the determination coefficient (R^2 = 0.7726) and p-value of the model (p <0.1). Although the determination coefficient was low, the model can still predict 77.26% of the yield as a function of the independent variables.

The analysis of variances (ANOVA) for yield (Table 4) was performed to adjust the response model with the 11 tests.

Table 4 - Analysis of variance (ANOVA) and model adjustment for response surface of murici pulp oil yield obtained by enzymatic hydrolysis.

	Yield	
Factors	**F-value**	**p-value**
Model	8.1413	0.0117^{**}
Linear		
$\mathbf{X_1}$	3.1473	0.0254^{**}
$\mathbf{X_2}$	2.1995	0.0791^{*}
Quadratic		
$\mathbf{X_1}$	-0.1434	0.8916^{ns}
$\mathbf{X_2}$	-1.4138	0.2165^{ns}
Interaction		
$\mathbf{X_1X_2}$	0.4049	0.7022^{ns}
Lack of fit	2.3352	0.1733^{ns}
$\mathbf{R^2}$	0.7726	

X_1 = Enzyme concentration (%), X_2 = Extraction time (min), R_2 = Determination coefficient, Significance level = $^{**}p\leq0.05$, $^{*}p\leq0.10$ e $^{ns}p>0.10$

Source: Authors.

Table 4 presents the analysis of variance (ANOVA) in which it can be observed which factors were significant, at a level of 10% of significance, for the optimization of oil extraction from murici pulp.

In the present study, the highest yield obtained by extracting murici pulp oil by CCRD was 3.56%, corresponding to an efficiency of 46.91% of the total oil, under conditions of 0.25% of the enzyme concentration and 45 min of the extraction time. Ribeiro et al., (2016) when applying experimental planning for the extraction of sesame oil by enzyme action, he reported that the efficiency of the extraction ranged from 20.33 to 36.65% of oil. Polmann et al., (2019) used commercial protease in their study to verify the extraction efficiency of pecan oil aided by CCRD taking into account as independent variables – pH, temperature, substrate concentration, agitation and enzymatic load - and observed that the yield varied from 14.9% to 55.9% of pecan oil.

Many studies have used an enzyme in oil extraction because it is considered green technology and also in obtaining better quality oil in several aspects. The use of protease in oil extraction is closely related to a lipid molecule that is in the middle of a protein region, this way the protease acts in the hydrolysis of the cell wall protein, making the oil molecule available for better recovery (Jiang et al., 2010).

Thus, in the present work, the efficiency of oil extraction through the use of enzymatic hydrolysis was observed, as was also observed by other mentioned authors.

3.3 Thermal stability of murici oil obtained by enzymatic hydrolysis and classic method

It was observed that the process of extracting oil from murici pulp affects the decomposition ranges of the oil as well as the oxidation temperature (Table 5). Data from the t-test suggest that the extraction method had a significant effect ($p = 0.0030$) on the decomposition range of murici pulp oil, so the use of chemical caused a greater decomposition range (214.5 °C), while extraction by enzymatic hydrolysis resulted in a lower decomposition range (194 °C). Mothé & Azevedo (2002) state that the oil decomposition range, measured by the difference between the two decomposition temperatures (Tf - Ti), is called the reaction interval, and the shorter this interval, the more stable the material at decomposition. The extraction method also had a significant effect ($p = 0.0088$) on the oxidation temperature of murici pulp oil. These thermal properties the oil extracted by enzymatic hydrolysis is more thermally stable, being able to maintain its properties during thermal processing.

The thermal stability of the oils obtained by conventional method and enzymatic hydrolysis presented three important events. In the first and second events, they are associated with the decomposition range that occurred between 200 - 415 °C and 205 - 400 °C for murici

pulp oil obtained by conventional method and enzymatic hydrolysis, respectively. These events are associated with loss of materials more susceptible to volatilization of unsaturated and saturated short chain fatty acids (Mothé & Azevedo, 2002). Maximum mass loss temperature at 360 °C for both samples. In which this temperature is equivalent to the greater resistance of the oil to the effects of degradation (Mothé & Azevedo, 2002). The third event is associated with the oxidation temperature which for murici pulp oil obtained by conventional method was started at 384 °C, while for oil obtained by enzymatic hydrolysis of the pulp it was started only at 400 °C. Suggesting that the extraction method has an effect on the thermal stability of the oil.

Table 5 - Average temperatures obtained during the thermal stability analysis - ODR (Oil decomposition range; °C), MMLT (Maximum mass loss temperature; °C) and TOx (Oxidation temperature; ° C) - of oil obtained by enzymatic hydrolysis and classic method.

Extraction method	**ORD (°C)**	**MMLT (°C)**	**TOx (°C)**
Classic	215	360	384
Enzymatic hydrolysis	195	360	400

Source: Authors.

From the onset temperature onset (T_{onset}), verified in the thermogravimetric curve (360 °C), murici oil extracted by enzymatic hydrolysis and by classical method can be considered more thermally stable than several edible oils, such as sunflower, soy, canola, corn and olive (Dweck & Sampaio, 2004).

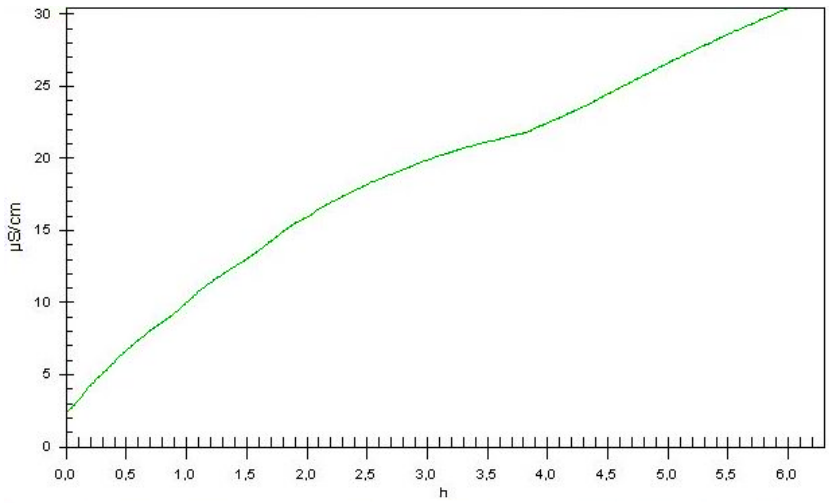
The extraction of oil by enzymatic action becomes a good alternative, because according to the results of the present research and results of the literature previously mentioned, the action of enzymes results in a product with better thermal stability.

3.4 Oxidative stability of murici oil obtained by conventional method and enzymatic hydrolysis in Rancimat

The murici oils extracted by classic method with solvent (Soxhlet) and by enzymatic hydrolysis had their oxidative stability evaluated by the accelerated method in Rancimat.

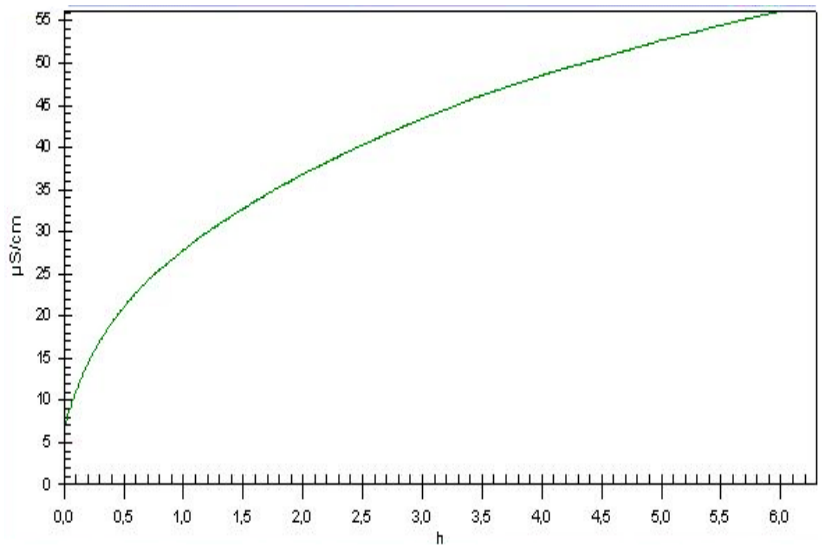
Figures 4(a) and **4(b)** show the characteristic curves for the Induction Period (PI) for oxidation for the respective samples.

Figure 4(a) - Oxidative stability of murici oil obtained by enzymatic hydrolysis of the pulp.



Source: Authors.

Figure 4(b) - Oxidative stability of murici oil obtained by the classic pulp method



Source: Authors.

The induction periods for both samples were not observable at the proposed analysis time (6 hours). Therefore, it was only possible to determine that this induction period is over 6

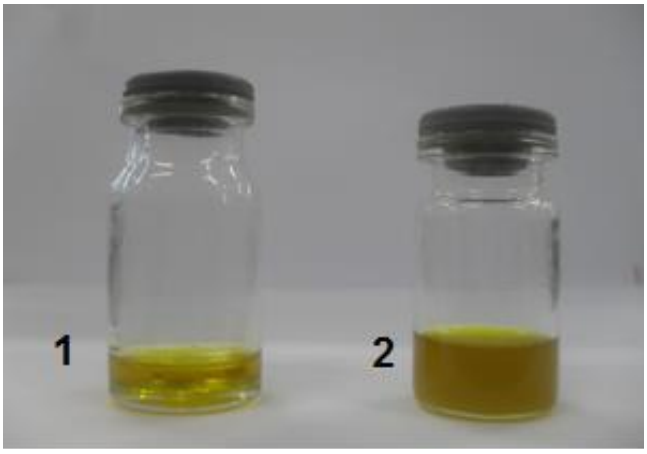
hours, considering that the region corresponding to the sudden increase in conductivity was not identifiable, when this would occur.

From the comparative evaluation between the extraction methods under study, it was observed that although the induction periods were not detectable in the analysis time used, the oil obtained by enzymatic route was less prone to the oxidative process, when it showed an electrical conductivity value (30 μS/cm) lower than that for the oil obtained by the classical method (55 μS/cm) in the 6 hours of submission of the samples to the conditions of analysis.

3.5 Physico-chemical characterization of murici oil obtained by enzymatic hydrolysis of the pulp

The oils extracted by enzymatic hydrolysis and by classical method are shown in **Figure 5**. The physicochemical characterization was performed only with the oil obtained by enzymatic hydrolysis of the murici pulp, as it presents the lowest decomposition range of the oil and requires a higher temperature for its oxidation, demonstrating better thermal stability.

Figure 5 - Murici oil obtained: 1 - enzymatic hydrolysis assisted by ultrasound; 2 - classic solvent method.



Source: Authors.

The physical-chemical parameters (Table 6) evaluated for murici oil extracted by ultrasound-assisted enzymatic hydrolysis were shown to be within the specifications of limits established for other edible oils, according to RDC n° 270 of ANVISA (BRASIL, 2005).

Table 6 – Physical-chemical parameters of murici oil extracted enzymatic hydrolysis.

Parameters	Unit	Murici oil
Acid index (AI)	mg KOH/g	2.27±0.07
Saponification index (SI)	mg KOH/g	123.47±0.00
Refractive index	-	1.463

Source: Authors.

This characteristic allows to classify the oil extracted by enzymatic route with better properties for use in cosmetic preparation. However, when used for biodiesel production, it needs previous treatments to correct both the saponification index and the acidity index. Since high levels of saponification and acidity can hinder the product washing process and decrease the performance of transesterification reactions.

4. Conclusions

In this research, the effect of enzymatic concentration and time of oil extraction from murici pulp was evaluated, which resulted in better yield when using higher concentration of enzymes. So the present research contributes in the research area in which they involve the use of enzymes as green technology, since they contribute in the best yield of obtaining a product without generating by-products that could contaminate the environment.

The initial objective of comparing thermal and oxidative stability was achieved and showed evidence that the best stability is obtained when the oil obtained was enzymatically.

The experimental planning for the extraction by enzymatic hydrolysis, assisted by ultrasound, of the oil contained in the murici pulp proved to be significant in the oil yield, although the yield was low compared to the classic method using chemical solvent. Despite this disadvantage in yield, the oil obtained by enzymatic hydrolysis showed better thermo-oxidative stability.

It is expected that the oil obtained by enzymatic route can be evaluated in the future for the quality of bioactive compounds and antioxidant capacity, which could become a great potential for the production of cosmetics.

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