

Otimização do processo de extração de antioxidantes presentes na bacaba (*Oenocarpus distichus* Mart.) utilizando metodologia de superfície de resposta

Optimization of the antioxidants extraction process from the bacaba (*Oenocarpus distichus* Mart.) using response surface methodology

Optimización del proceso de extracción antioxidante presente en bacaba (*Oenocarpus distichus* Mart.) utilizando la metodología de la superficie de respuesta

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Paula Beatriz Rocha Sampaio

ORCID: <https://orcid.org/0000-0003-1321-9960>

Universidade Federal do Tocantins, Brasil

E-mail: p.beatrizsampaio@gmail.com

Andressa Sousa Pereira

ORCID: <https://orcid.org/0000-0002-9631-5079>

Universidade Federal do Tocantins, Brasil

E-mail: dressousa@gmail.com

Caroline Roberta Freitas Pires

ORCID: <https://orcid.org/0000-0002-1427-7276>

Universidade Federal do Tocantins, Brasil

E-mail: carolinerfpires@mail.uft.edu.br

Rodolfo Castilho Clemente

ORCID: <https://orcid.org/0000-0002-0766-9968>

Universidade Federal do Tocantins, Brasil

E-mail: castilho@mail.uft.edu.br

Guilherme Nobre Lima do Nascimento

ORCID: <https://orcid.org/0000-0003-4185-0872>

Universidade Federal do Tocantins, Brasil

E-mail: guilherme.nobre@uft.edu.br

Resumo

A bacaba (*Oenocarpus distichus* Mart.) é um fruto que provém de palmeiras nativas da família Arecaceae e apresenta importante valor nutritivo e socioeconômico para comunidades

rurais. Apesar de muito popular, este fruto possui poucas características químicas catalogadas. O presente estudo teve como objetivo otimizar o processo de extração de antioxidantes da bacaba averiguando o intervalo de extração mais eficiente e o melhor tempo de rotação, utilizando os solventes etanol e a mistura metanol/acetona, tendo-se como resposta o conteúdo de fenóis totais e a atividade antioxidante. A mistura dos solventes Metanol/Acetona foi o solvente mais eficiente para a extração de antioxidantes da bacaba, com tempo de extração de 90 minutos e 20 minutos de intervalo de rotação a 5.000 rpm.

Palavras-chave: Bacaba; Antioxidantes; Planejamento experimental.

Abstract

Bacaba (*Oenocarpus distichus* Mart) is a fruit that comes from palm trees native from the Arecaceae family and presents important nutritional and socioeconomic value for rural communities. Although being very popular, this fruit has very few cataloged chemical characteristics. The present study aimed to optimize the bacaba antioxidant extraction process by investigating the most efficient extraction interval and the best rotation time, using ethanol and methanol/acetone solvents, with the content of total phenolics and antioxidant activity. Methanol acetone solvent mixture was the most efficient solvent for extracting antioxidants from bacaba, with an extraction time of 90 minutes and 20 minutes of rotation interval at 5,000 rpm.

Keywords: Bacaba; Antioxidants; Experimental planning.

Resumen

La bacaba (*Oenocarpus distichus* Mart.) Es una fruta que proviene de palmeras nativas de la familia Arecaceae y tiene un importante valor nutricional y socioeconómico para las comunidades rurales. Aunque es muy popular, esta fruta tiene pocas características químicas catalogadas. El presente estudio tuvo como objetivo optimizar el proceso de extracción antioxidante de bacaba mediante la determinación del intervalo de extracción más eficiente y el mejor tiempo de rotación, utilizando los disolventes etanol y la mezcla de metanol / acetona, teniendo el contenido como respuesta. fenoles totales y actividad antioxidante. La mezcla de solventes de metanol / acetona fue el solvente más eficiente para la extracción de antioxidantes de bacaba, con un tiempo de extracción de 90 minutos y 20 minutos de intervalo de rotación a 5.000 rpm.

Palabras clave: Bacaba; Antioxidantes; Planificación experimental.

1. Introduction

The Amazon region possesses a large diversity of plant and vegetable species yet underexploited for their phenolic compound contents (Carvalho et al, 2016). Among these, *Oenocarpus* palm trees, from the *Arecaceae* family, are widely distributed across the Amazon and have great economical, ecological, and food potential (Carvalho et, 2016).

Currently, exploitation of *Oenocarpus* fruits has been mainly based on extractive harvest, since neither domestication nor rational cultivation has been reported for this species. (Keaujalytè et al, 2015). Bacaba locally consumed is made into drinks, jelly, ice cream etc (Finco, 2016).

Bacaba's fruits present in average, 16,04 mm and 13,92 mm of longitudinal and transversal diameter respectively, weighing 2,23g, its peel presents violet color, with mesocarp (pulp) of predominantly orange color (Sousa, Oliveira & Mortorano, 2015).

The mesocarpo of bacaba is rich in fat, carbohydrates, and total dietary fibers (39.3 g 100g⁻¹ fresh weight). It is also a good source of unsaturated fatty acids, with a profile similar to that of olive oil. The total soluble solid of bacaba fruits is 7.89°B and pH 5.3 – 4.8, with a gross energy values of 606.3 ± 12.8 kcal 100g⁻¹ fresh weight. In addition, bacaba is a promising source of phenolic compounds; it has a high anthocyanin content and significant antioxidant capacity (Puerari et al, 2015).

Phenolic compounds, a specific group of secondary metabolites, play an important role in combating oxidative stress in the human body by maintaining a balance between oxidant and antioxidants (Van Hung, 2016). Phenolic compounds possess one or more hydroxyl groups, and generally are categorized as phenolic acids, flavonoids, coumarins, and tannins (Van Hung, 2016).

Phenolic acids in plants have been connected with diverse functions, including fruits, vegetables, and grains and are physically dispersed throughout the plant in seeds, leaves, roots, and stems. In addition to their roles in plants, phenolic compounds in our diet may provide health benefits associated with reduce risk of chronic diseases such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective, and vasodilatory effects (Van Hung, 2016).

Thus, evaluations, extraction, separation and purification of natural antioxidants are very important. Furthermore, effective extractions of antioxidants from plant material is very helpful for full utilization of natural resources (Xu & Dong Ping et al, 2016).

Response surface methodology (RSM) is an efficacious mathematical and statistical technique for simultaneously evaluating of several experimental parameters (Xu, Dong-Ping et al, 2016).

Considering the potential that bacaba fruit has as a functional food source of nutrients, the potential oleaginous and byproduct generator, the focus of this work is to improve bacaba's antioxidant extraction process by exploring the efficacy of the solvents: ethanol, and methanol/acetone, determining the influence of the contact time and the efficiency of the rotation time in the centrifuge across the response surface.

2. Experimental Part

Samples

Bacaba's fruits (*Oenocarpus distichus* Mart.) were obtained in a popular market located in the city of Palmas - TO, between November and December of 2015. The fruits were at the maturation point to be commercialized.

The weight of the fruits collected totaled 3 kg. After the harvest, the fruits were directed to the Food Technology Laboratory of the Federal University of Tocantins (UFT), where they were selected considering the quality criteria related to the color of the fruit peel (violet) and absence of perceptible rottenness.

Then, the fruits were sanitized and placed in a chlorine solution of 100ppm for 10 minutes. Soon after, the fruits were pulped and stored in a freezer at -18 °C. At the time of the analysis, the samples were removed from the freezer and left in the refrigerator to be defrosted. Then 1.0 gram of the bacaba pulp was weighed in analytical scale, and finally the samples were packed in test tubes.

Then, the fruits were washed and placed in a chlorinated solution of 100ppm for 10 minutes for sanitization. Soon after, the fruits were pulped manually and the pulp packed in a freezer at -18°C.

At the time of the analysis, the samples were removed from the freezer and left in the refrigerator to be defrosted.

Conducting the Experiment

We weighed 1.0 gram of the bacaba pulp on analytical balance and we sampled the portions in test tubes. We conducted the experimental part with two solvents: ethanol at 85% concentration and methanol/acetone in concentrations of 50% and 70%, respectively.

For each solvent, we conducted a complete planning, with 12 assimilated samples, including four factorial (levels -1 and +1), four axial (levels $\pm \alpha$) and four replicates at the central point.

We assessed two factors at five levels of variation: extraction interval (18, 30, 60, 90, and 102 minutes), and rotation time (8,0, 10, 15, 20, and 22,0 minutes) with 4 central points for each solvent tested, taking into account the total phenol content and the total antioxidant activity.

Both experiments were performed with a rotation of 5,000 rpm. In the experimental planning matrix, the values of the variables are represented in the coded form, the factors and respective levels being presented in Table 1.

Table 1. Factors and levels tested (coded values are in parentheses) for the Central Composite Design with axial points.

Factors: Extraction time/rotation time						
	Lower axial point ($-\alpha$)	Lower Level (-1)	Intermediate level (0)	Upper level (+1)	Upper axial point ($+\alpha$)	
Extraction	18,0	30	60	90	102,0	
Rotation	8,0	10	15	20	22,00	

Source: Authors.

Table 1 shows the extraction interval and the rotation time to which the samples were submitted. We weighed 1 g of the samples to obtain the extract, which were extracted according to the design outlined in Table 1.

Total Antioxidant Activity

The methodology employed to determine the antioxidant activity was based on the extinction of the absorption of the 2,2-diphenyl-1-picryl hydrazyl radical (DPPH; 60 μM), adapted from the methodology proposed by Rufino et al. (2009), by calculating the percentage of radical sequestration of the DPPH from the standard.

We added 0.1 mL of each sample extract to 3.9 mL of DPPH solution. For the control, we added 0.1 mL of methanol along with the DPPH in place of the extract. We conducted the readings after 120 minutes in a spectrophotometer at 515 nm and the results are expressed as percentage of free radical sequestration (% FRS) according to the following equation:

$$\% \text{FRS} = (\text{Ca} - \text{Sa}) \times 100 / \text{Ca}$$

Ca = Control Absorbance

Sa = Sample absorbance

Total Phenolic Compounds

We made the determination of the total phenolic content in the extract by the method proposed by Waterhouse (2002), employing the Folin-Ciocalteu reagent. We added 0.5 ml of extract from each sample to the tubes containing 2.5 ml of 10% Folin-Ciocalteu solution. Then, we added 2 mL of 4% sodium carbonate solution.

We shook the tubes and allowed them in rest for 2 hours, sheltered from light. We measured spectrophotometrically the blue color, produced by the reduction of the Folin-Ciocalteu reagent by the phenolics, in the absorption range of 750 nm.

We made the calculation of the phenolic content from the equation of the straight line obtained from the standard curve of gallic acid. The results will be expressed in mg EAG.100g⁻¹.

Qualitative Analysis of Antioxidant Activity

A Central Composite Design (CCD) with k-factors and five levels requires $2^k + 2k + C$ experiments, where 2^k points are in the corners of the square, representing the amplitude (the domain) of the experiment. The axial points $2k$ are at a coded distance of ± 1.41 ($\pm \alpha$) from

the center of the design and measure the possibility of non-linearity in the obtained values of free radical sequestration and total phenolic as a function of the factors.

The C points in replicates in the center of the square (center point) are an estimate of the experimental error. Thus, considering two factors and four replicates of the central point, the planning involved 12 experiments represented (Table 2).

Table 2. Experimental conditions of the Central Composite (CCD) with axial points (factors with coded values).

Experiment (Theoretical sequence)	Factors (coded values)	Rotation Time (min)	Type of Solvent			
			Ethanol (85%)		Methanol (50%)/ Acetone (70%)	
			% of Free Radical Sequestration (DPPH)	Total Phenolics (mg EAG.g ⁻¹)	% of Free Radical Sequestration (DPPH)	Total Phenolics (mg EAG.g ⁻¹)
1	-1	-1	10,38	70,62	48,93	192,34
2	+1	-1	13,01	82,14	50,21	195,61
3	-1	+1	12,45	76,64	54,78	199,67
4	+1	+1	16,08	88,02	60,65	210,1
5	-1,41	0	10,50	71,05	42,98	192,34
6	+1,41	0	12,45	87,01	55,43	201,34
7	0	-1,41	10,89	75,83	51,00	197,56
8	0	+1,41	16,32	92,65	56,18	207,23
9	0	0	12,31	90,83	51,06	202,69
10	0	0	12,27	90,45	52,25	201,07
11	0	0	12,04	91,35	51,89	202,34
12	0	0	12,13	92,58	51,49	201,41

Source: Authors.

From Table 2, it is possible to identify the effectiveness of solvents and variables used in the sequestration of free radicals and in the determination of total phenolics present in bacaba.

Response Surface Analysis

Extraction can be statistically optimized if the value of total phenolic concentrations and free radical sequestration is known as a function of the experimental factors, generating a mathematical model. A graph of this function produces the response surface for the total phenolic and free radical sequestration, which is obtained by applying multiple linear regressions to the values obtained from their concentrations as a function of the experimental factors. The response surface can be represented by the following mathematical model described in the equation below:

$$\text{Equation 1: } Y = b_0 + b_1X + b_2Z + b_{11}X^2 + b_{22}Z^2 + b_{12}XZ$$

In equation 1, the response (y) was the concentrations obtained for total phenolic compounds and percentage of free radical sequestration by weight of the bacaba's sample. The b values are the estimates of the polynomial coefficients, the X and Z values represent the factors extraction time and rotation time.

The linear terms, b_1, X and b_2Z , are responsible for the main effects, the quadratic terms, b_{11}, X^2 and $b_{22}Z^2$, responsible for the curvature effects, and $b_{12}XZ$, is responsible for the interaction effects. We conducted a statistical F test to determine the significance of the different models obtained.

We conducted separately the complete statistical analysis defining the conditions of extraction interval and rotation time in the centrifuge for the determination of antioxidant compounds and total phenolics.

We submitted these results to the statistical treatment to assess if the difference between the results obtained was statistically significant, with the help of STATISTICA software, version 6.0. Thus, it was possible to analyze the results of each response variable, based on experimental evidence from the studied regions, to better understand the system, interaction effects, regression coefficients and the empirical models obtained (Pizato, 2013).

3. Results and Discussion

The concentrations of total phenolics in mg EAG.100g⁻¹, and the percentage of free radical sequestration from the DPPH method, obtained under different conditions of CCD are shown in Table 3.

Table 3. Estimates of the quadratic polynomial regression coefficients, pure error and significance (p), for the response of% of free radical sequestration and total phenol content mg/EAG.g⁻¹) with the solvents Ethanol and Methanol Acetone.

Solvent – Ethanol						
Coefficient	% FRS (DPPH)			Total Phenolics (mg/EAG.g ⁻¹)		
	Polynomial coefficient estimation	Standard error	p	Polynomial coefficient estimation	Standard error	p
b ₀	12,963	0,0062	0,0002	0,348	10,46	0,0097
b ₁	0,0410	0,0092	0,0206	1,054	0,15	0,0004
b ₂	-0,7520	0,0657	0,0014	6,160	0,00	0,0001
b ₁₁	-0,0002	0,0000	0,0153	-0,007	1,09	0,0013
b ₂₂	0,0334	0,0020	0,0004	0,165	0,03	0,0025
b ₁₂	0,0016	0,0004	0,0280	0,002	0,00	0,0097

Solvent – Methanol/Acetone						
Coefficient	% FRS (DPPH)			Total Phenolics (mg/EAG.g ⁻¹)		
	Polynomial coefficient estimation	Standard error	p	Polynomial coefficient estimation	Standard error	p
b ₀	55,940	2,580	0,0002	185,22	3,8430	0,0000
b ₁	0,1169	0,038	0,0054	0,3135	0,0565	0,0115
b ₂	-1,971	0,269	0,0052	-0,3009	0,4009	0,0507

b ₁₁	-0,0007	0,0002	0,0465	-0,0029	0,0003	0,0033
b ₂₂	0,0625	0,0082	0,0047	0,0091	0,0122	0,0511
b ₁₂	0,0076	0,0017	0,0208	0,0119	0,0025	0,0182

Source: Authors.

The results presented by the ethanol solvent show that all the factors were significant for the percentage of free radical sequestration and for the determination of total phenolics, the same was applied to the methanol/acetone solvent since this solvent also presented all the significant results for sequestration of free radicals and for total phenolics ($p \leq 0.05$).

In order to assess how much the proposed quadratic polynomial model represents fidelity of the response obtained in relation to the dependent variable under discussion, we made a variance analysis (ANOVA) (Table 4).

Table 4. Variance analysis (ANOVA) for% of Free Radical Sequestration and Total Phenolic (mg/EAG.g⁻¹) in bacaba's samples.

Solvent – Ethanol						
Dependent Variables	Source of variation	Sum of squares	Degrees of freedom	Means quarte	F calculated	F tabulated
% FRS	Regression	37,77	5	7,5554	27,57	4,39
	Residue	1,37	6	0,274		
	Lack of adjustment	1,33	3			
	Pure error	0,046	3			
	Total	39,140	11			
Total Phenolics (mg/EAG.g ⁻¹)	Regression	760,879	5	152,175	35,26	4,39
	Residue	25,896	6	4,316		
	Lack of adjustment	23,312	3			
	Pure error	2,584	3			
	Total	786,775	11			
Solvent Methanol/Acetone						
% FRS	Regression	196,322	5	39,26	21,43	4,39
	Residue	10,997	6	1,83		
	Lack of adjustment	10,208	3			
	Pure error	0,789	3			
	Total	207,319	11			
Total Phenolics (mg/EAG.g ⁻¹)	Regression	301,743	5	60,348	30,88	4,39
	Residue	11,725	6	1,954		
	Lack of adjustment	9,98	3			
	Pure error	1,744	3			
	Total	313,468	11			

Source: Authors.

Table 3 demonstrates how significant the results were at a 95% confidence level, we can determine that all factors evaluated were significant in this research.

The ratios between the mean square of the regression by the mean square of the residue

$$F_{CAL} = (MS_{REGRESSION} / MS_{RESIDUE}),$$

varied with the ethanol solvent of 21.57 and 35.26 and with the methanol/acetone solvent mixture the ratios presented values between 21.43 to 30.88 for the extraction time/rotation time equations in order to determine the percentage of free radical sequestration bacaba's total phenolic.

Both the ethanol solvent and the acetone/methanol solvent were assessed against the dependent variables (%FRS and total phenols) and presented values of $F_{CALCULATED}$ higher than the $F_{TABULATED}$ values ($F_{5,6} = 4.39$), at the 95% level of confidence, indicating significant regression equations, or the validity of the experimental model.

Repeatability of the experiment in the analysis of each factor studied can be verified due to the low pure error values obtained in the variance analysis of the bacaba's fruit regressions (Table 5).

Table 5. Regression equations in coded model representing the response surface of the experiments in percentage of free radical sequestration (% FRS) and total phenolic contents (mg/EAG.g⁻¹) present in bacaba.

Solvent – etanol				
Dependent variables	Equation Y = (% FRS) (Coded model)	r ²	F _{cal}	Pure error
% FRS	Y=12,963324964606+0,041582687128237X-0,00027883159587785X ² -0,75289300405448Z+,033431450303499Z ² +,0016666666666667XZ	0,96	27,57	0,04
Total phenolics (mg/EAG.g ⁻¹)	Y=0,34863894105553+1,0549215126687X-0,0075583737166838X ² +6,1609244633278Z-0,16577492318837Z ² -0,0002500000000011XZ	0,97	35,26	2,58
Solvent – methanol/Acetone				
% FRS	Y=55,943615471781+,11697572652814X-0,00074838778840791X ² -1,9717481973836Z+0,062547835535683Z ² +,0076499999999999XZ	0,95	21,43	0,79
Total phenolics (mg/EAG.g ⁻¹)	Y=185,22719694068+0,31345532894117X-0,0028965834089654X ² -0,30088204365237Z+0,009090344216022Z ² + 0,0119333333333333XZ	0,96	30,88	1,74

% FRS- Percentage of Free Radical Sequestration; X = extraction time; Z = Rotation time; r² = coefficient of determination; F_{cal} = (MS_{Regression} / MS_{Residue})

Values of pure errors for ethanol ranged from 0.046 to 2.584 for percentage of free radical sequestration and total phenolics respectively, and values with methanol/acetone solvents presented variation of 0.789 and 1.74 for percentage of free radical sequestration and total phenolics, respectively. The values of pure error were lower in relation to the variable percentage of free radical sequestration indicating the sensitivity of the DPPH method.

Table 5 represents the regression equations of the experimental model for free radicals sequestration/total phenolics extracted with the ethanol solvents and the methanol/acetone mixture of the bacaba's samples. The equations are based on the variables studied, extraction time in minutes (X) and rotation time in the centrifuge (Z), with R² being the coefficient of determination of these.

The coefficient of determination R^2 represents the predictive power of its regression line, that is, it indicates whether or not the model is adequate and the closer to 1 is the R^2 value, the greater the model's capacity to predict the data.

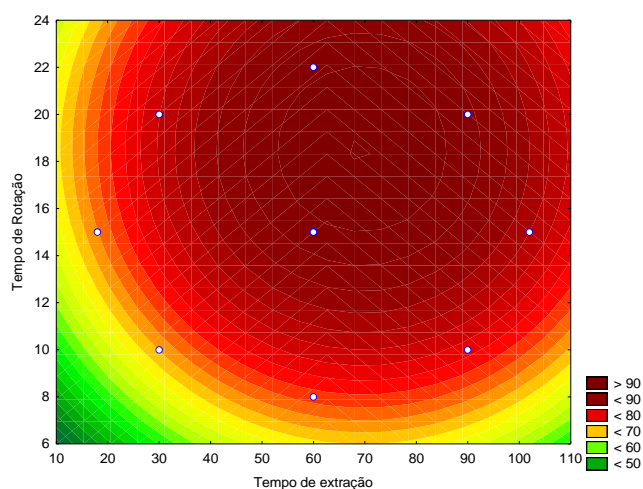
Applying the same analytical sequence by multiple regressions to the CCD data, it was possible to obtain a mathematical model for each experiment conducted, considering the linear, quadratic terms and their products. In the refined mathematical models obtained (Table 5), at 95% confidence level, the values of the determination coefficients (R) of each generated equation ranged from 0.96 to 0.97 for the ethanol solvent and from 0.95 to 0.96 for the mixture of methanol/acetone solvents.

These results illustrate that the quadratic model would be adequate to describe the conditions of extraction time/rotation time, although they do not reach the ideal value of 0.99, this result is considered a good coefficient for biological processes in which the influence exists of several factors during the analyzes, such as the complexity of the compound groups (Rocha, 2009).

In this way, the proposed models are valid, the regression equations allow predicting the effect of the two parameters studied on extraction time and rotation time.

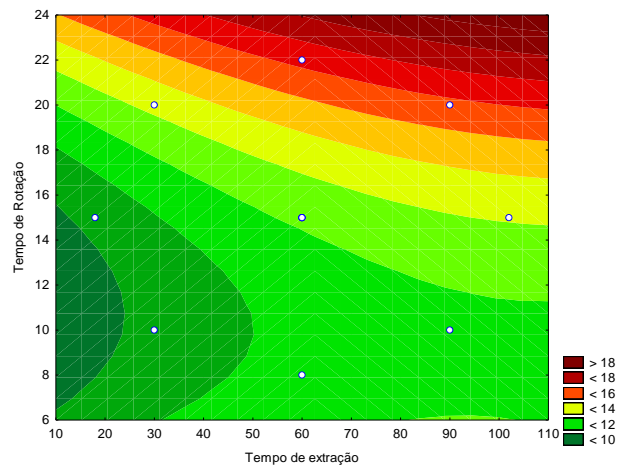
The relation between the independent and dependent variables is represented bimentionally by the response surface generated for the amount of total phenolics and percentage of free radical sequestration assessed in the samples, according to Figures 1 and 2 for the samples extracted with ethanol.

Figure 1. Response surfaces in the determination of total phenolics in a sample of bacaba depending on the rotation time and extraction time with the ethanol solvent:



Source: Authors.

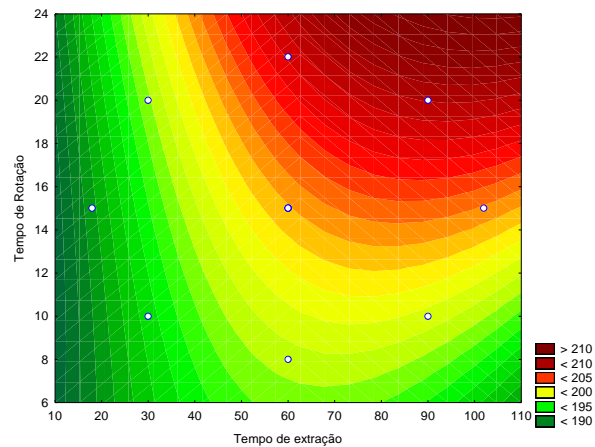
Figure 2. Response surfaces in the determination of % of Free Radical Sequestration in bacaba's sample in function of rotation time and extraction time with the ethanol solvent:



Source: Authors.

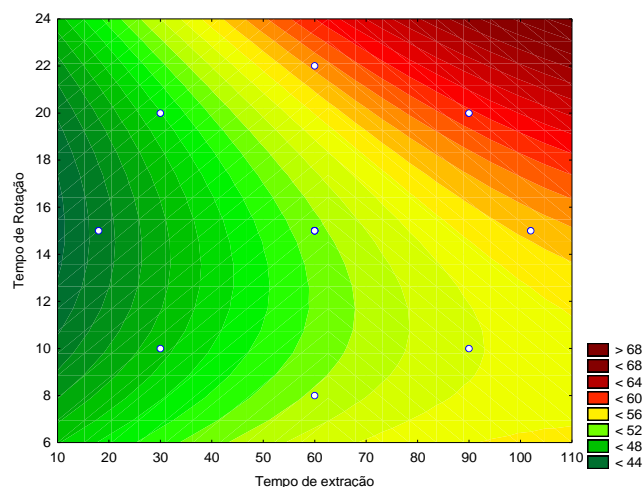
Figures 3 and 4 show information for samples extracted with the methanol/acetone solvent mixture respectively.

Figure 3. Response surfaces in the determination of total phenolics in bacabas sample in function of the rotation time and extraction time with the methanol/acetone solvent:



Source: Authors.

Figure 4. Response surfaces in determination of% of Free Radical Sequestration in bacaba's sample in function of rotation time and extraction time with the methanol/ Acetone solvent:



Source: Authors.

From the analysis of the response surface, it was possible to observe that the optimum range for the extraction of total phenolic compounds in bacaba using the ethanol solvent (85%) was in the 60 minute interval of extraction, and 15 minutes of rotation, and for the determination of % of free radical sequestration was in the range of 90 minutes of extraction, and 20 minutes of rotation, while regarding the use of the mixture of solvents Methanol (50%) and Acetone (70%) the best range for the determination of antioxidants and total phenolics was 90 minutes of extraction and 20 minutes of rotation.

The experiment conducted with the ethanol solvent showed values of 10.38% to 16.32% of free radical sequestration, and 70.62 mg EAG.g⁻¹ to 92.65 mg EAG.g⁻¹ of total phenolics, and values presented by the use of the methanol/acetone solvent mixtures ranged from 42.98% to 60.65% of free radical sequestration and 192.34 mg EAG.g⁻¹ and 210.1 mg EAG.g⁻¹ of total phenolics.

Finco et al. (2010) found values for total phenolics for bacaba of 1.759,27 mg EAG.100g⁻¹ sample, a result lower than the total phenolic value found in *pequi* (*Caryocarvillosum*) (4623.4 mg of EAG.100g⁻¹ sample) but higher than the total phenolics present in *buriti* (*Mauritiavinifera* Mart.) (108.1 mg EAG. 100g⁻¹ sample).

The result of total phenolics found by Neveset. al (2015) was 8.8 mg of EAG.100g⁻¹ of sample in bacaba pulp, a result lower than the values presented by this study.

Augusta et al. (2010) found values of 88% of free radical sequestration for pulp extract of the mature jamb by the DPPH method, values considerably higher than the percentages presented by the bacaba pulp.

Despite the abundant literature on phenolic content in foods, few studies describe adaptations of the extraction procedure in specific matrices and/or critical conditions of sample preparation for quantification. The optimization of the methodology of extraction of an analyte is essential, since small details may result in effects that compromise the reliability of the results (Souza et al., 2009).

The optimization of the bacaba antioxidant extraction and determination process can be affected by several variables, the control of conditions is a fundamental factor for the execution of this procedure. The basic literature showed results consistent with the values found in this study, maintaining that bacaba is a fruit with high antioxidant potential.

4. Conclusion

Through this work it was possible to optimize the process of extracting the antioxidants from bacaba.

Experimental design with two variables proved to be an important method to optimize and construct predictive models of the ideal range of time extraction and rotation for extraction of bacaba's antioxidants.

Results presented in this study indicate that for methanol/acetone solvent mixture was more efficient for the extraction of bacaba's antioxidants, with extraction time of 90 minutes and 20 minutes of rotation interval at 5,000 rpm, since their values were the most satisfactory within the parameters of analysis.

Bacaba is a fruit that has gradually conquered its space as a functional food, therefore it is necessary that the studies in the field of food sciences to deepen more and more in their chemical and biological properties.

In view of the results obtained, there is a great number of possibilities that can still be explored regarding the nutritional value of Amazonian fruits.

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Percentage of contribution of each author in the manuscript

Paula Beatriz Rocha Sampaio - 30%

Andressa Sousa Pereira - 20%

Caroline Roberta Freitas Pires -20%

Rodolfo Catilho Clemente -15%

Guilherme Nobre Lima do Nascimento - 5%