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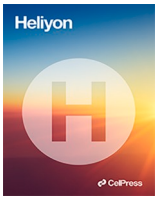

Research article

Evaluation of the extraction of antioxidant compounds from buriti pulp (*Mauritia flexuosa* L.) by response surface methodology

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A R T I C L E I N F O

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A B S T R A C T

Buriti is a fruit rich in antioxidant compounds, and hydroalcoholic extracts were produced from its pulp to investigate the extraction conditions that result in the highest concentrations of bioactive compounds and maximum antioxidant capacity. The results showed considerable variation in the bioactive compound contents in the pulp: total phenolics (104.64–270.61 mg GAE 100 g-1), total flavonoids (17.74–60.46 mg EC 100 g^{-1}), and total carotenoids (1.30–3.92 mg kg^{-1}). The antioxidant capacity of the extracts, evaluated by DPPH, ABTS, and FRAP methods, ranged from 1.23 to 3.47 μmol ET g^{-1}, 6.34–15.86 μmol ET g^{-1}, and 4.74–11.95 μmol ET g^{-1}, respectively, highlighting the broad diversity of antioxidant activities present in the extracts. Data modeling generated statistically significant mathematical models and contour graphs, which allowed the identification of the optimal extraction conditions for the compounds. Total carotenoids were most effectively extracted with 20 % buriti pulp, intermediate ethanol concentrations, and temperatures around 70 °C. All other bioactive compounds were more efficiently extracted with 5 % buriti pulp, intermediate ethanol concentrations, and temperatures close to 30 °C. The results confirmed the efficiency of the extractions and highlighted the excellent antioxidant potential of buriti pulp. Additionally, the analysis of the optimal regions for extracting antioxidant compounds from the pulp can be applied in future studies to optimize the extraction process, providing a solid foundation for using this fruit in industrial processes, such as the production of functional ingredients.

1. Introduction

The global increase in life expectancy has brought significant attention to the role of diet in promoting health and preventing diseases. This shift has driven a growing demand for functional foods that mitigate chronic and degenerative conditions [1]. Diet is

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recognized for its role in maintaining overall health and its potential to deliver bioactive compounds that act as antioxidants or stimulate the body's endogenous defense mechanisms to combat oxidative stress [2]. Oxidative stress, closely linked to aging and chronic, inflammatory, and degenerative disease progression, can be countered by antioxidants such as vitamins, minerals, phenolic compounds, and carotenoids [3]. These compounds, prevalent in fruits and vegetables, have been extensively studied for their protective health benefits.

Brazil, known for its rich biodiversity and favorable climatic conditions, is home to many native and exotic plant species, many of which are underexplored yet possess valuable bioactive properties. Among these species, the Buritizeiro (*Mauritia flexuosa* L.), a palm tree native to the Brazilian Cerrado and Amazon rainforest, stands out for its nutritional and functional potential. The buriti fruit is particularly rich in dietary fiber, phenolic compounds, and carotenoids, including β-carotene and lycopene, contributing to its potent antioxidant capacity [4,5]. This unique composition positions buriti as a promising candidate for functional foods, nutraceuticals, and cosmeceuticals applications. Furthermore, its ability to thrive in arid climates highlights its ecological resilience and importance in promoting biodiversity in the Cerrado.

The potential applications of buriti pulp extend across multiple industries. In the food sector, buriti-derived products could enhance formulations' nutritional value and shelf life through their antioxidant properties. In the pharmaceutical industry, the bioactive compounds in buriti may serve as natural alternatives for managing oxidative stress-related conditions, including cardiovascular diseases and neurodegenerative disorders. Additionally, buriti oil is already celebrated in cosmetics for its skin-nourishing and anti-aging properties, underscoring the broad utility of this fruit [4,6,7].

Despite its recognized chemical profile and antioxidant potential, research on the optimal extraction of bioactive compounds from buriti pulp remains limited. Existing studies have detailed the presence of phenolic acids (e.g., p-coumaric, ferulic, and chlorogenic acids), flavonoids (e.g., catechin, epicatechin, and quercetin), and carotenoids [5,6]. However, most have focused on characterization rather than refining extraction methods. Conventional solvent extraction is the predominant approach, but it often lacks efficiency in isolating and preserving sensitive bioactive compounds. Alternative techniques such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE) offer enhanced yields and align with sustainable processing principles. Yet, their application to buriti pulp is scarcely reported in the literature [8]. These gaps underline the need for more targeted studies to optimize extraction conditions, ensuring the maximum recovery of antioxidants while preserving their functionality.

To address this challenge, statistical tools like central composite design (CCD) and a response surface methodology (RSM) approach offer valuable insights into the optimization process. CCD minimizes the number of experiments needed to evaluate multiple variables, such as solvent composition, temperature, and extraction time while identifying the interactions between these factors [9,10]. Although RSM has been successfully applied to optimize bioactive compound extraction from various fruits, its utilization for buriti pulp extraction remains underexplored, presenting an opportunity for innovation.

This study seeks to bridge these gaps by investigating the optimal conditions for extracting bioactive compounds from buriti pulp using hydroalcoholic solvents. The research aims to enhance extraction efficiency and antioxidant capacity by employing CCD and RSM, contributing to developing functional ingredients with broad applications. Ultimately, this work not only leverages the unique chemical profile of buriti but also aligns with global trends toward sustainable extraction practices and the valorization of underutilized plant species. These findings could pave the way for advancements in the functional food, nutraceutical, and cosmeceutical industries while promoting biodiversity and innovation in food systems.

2. Material and methods

2.1. Material

The solvents and reagents used in the extraction and analytical determinations were: water purified by the reverse osmosis system, 92.8 % ethanol, 99.8 % pure methanol, sodium hydroxide PA, aqueous sodium carbonate, hydrochloric acid PA, chloride ferric PA, sodium nitrite PA, aluminum chloride PA and VETEC brand potassium persulfate, Folin-Ciocaulteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 98 % hydrated catechin, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman SIGMA brand -2-carboxylic acid (Trolox), SYNTH brand Tween 80 0.01 %.

The buritis were purchased from retailers in Itapecuru Mirim, MA, Brazil, and transported frozen, stored with ice cubes in a Styrofoam box. The fruit pulp was manually separated from the peel and seed for the research and then pressed through a sieve (BERTEL, Mesh 16, Brazil) to obtain a homogenized mass. Subsequently, the pulp was vacuum packed in plastic bags (SULPACK, SV460, Brazil) measuring 15.7 × 20 cm and 10 μm thick and stored at −20 °C for 30 days until extraction. Buriti pulp is known for its rich bioactive composition, including high levels of carotenoids such as β-carotene and lycopene, phenolic compounds, dietary fibers, and essential vitamins like vitamins A and C. It also contains unsaturated fatty acids and essential minerals such as potassium, calcium, and phosphorus, making it suitable for various food, pharmaceutical, and cosmetic applications.

2.2. Methods

2.2.1. Experimental design

The experimental design used the DCCR in the Statistica® 12.0 program to carry out the extractions under different conditions (StatSoft Incorporation, USA, 2011). The DCCR was composed of 19 experiments and 5 repetitions of the central point, and each

variable was investigated in 5 levels: α, −1, 0, +1, and +α.

Three factors (independent variables) were considered in the DCCR: percentage solid/liquid ratio (pulp/extractor solution), percentage liquid/liquid ratio (ethanol/water), and temperature. The ranges used in the extraction tests for pulp percentage, ethanol percentage, and temperature were 5–20 %, 15–65 %, and 30–70 %, respectively, based on the studies by Chew et al. [11] and Yi et al. [12].

Initially, screening tests were conducted with a pulp percentage between 5 % and 25 %. Still, the mechanical stirrer did not support the number of solids, resulting in difficulty mixing and homogenizing with the extracting solution, which conditioned the work of this variable to a smaller range.

Table 1 presents the DCCR for buriti pulp extractions with the conditions determined by the independent variables.

2.2.2. Extraction method

The extracts derived from buriti pulp were based on the studies by Chew et al. [11] and Yim et al. [12]. The pulp temperature was controlled between 20 and 22 °C for weighing, and the extracting solution (ethanol/water) was kept in a thermostatic bath (TECNAL, TE184, Brazil) until reaching the established temperature. Then, the pulp was weighed inside a reactor on an analytical balance (SHIMADZU, AY220, Brazil), and 50 mL of extracting solution was added at the desired temperature. Using a mechanical stirrer with a propeller (FISATOM, 713D, Brazil) over the reactor, the slurry and solution were stirred at 4000 rpm for 1 h. The extraction process was conducted with the reactor inside the thermostatic bath to control the temperature in each test.

After extraction, the mixture was centrifuged (NOVATECNICA, NT825, Brazil) at 6000 rpm for 15 min, resulting in the extract shown in Figure A (Appendices).

The supernatant extracts were collected, having their ethanol fraction evaporated at low pressure in a rotary evaporator (TECNAL, TE-211, Brazil) at 35 °C and then frozen at −85 °C in an ultra freezer (LIOTOP, 0FR30, Brazil).

The extracts were lyophilized (LIOTOP, L101, Brazil) for 48 h and suspended with 15 mL of water/0.01 % Tween 80 solution when removed from the ultra freezer.

Rectoevaporation of ethanol and lyophilization were necessary for the extracts to be applied in another research involving the cultivation of microorganisms. The resuspension in an aqueous Tween solution was performed to guarantee the homogenization of the extracts in all analyses.

All containers used were covered with aluminum foil to avoid hydrolysis, oxidation, and/or isomerization reactions in the presence of light.

2.2.3. Analytical determinations

The suspended extracts were analyzed for total phenolics, flavonoids, carotenoids, and antioxidant capacity by DPPH, ABTS, and FRAP methods. All analyses were performed in triplicate and in a practically dark room. To read absorbances in all analyses, a UV–Vis spectrophotometer (OCEAN OPTICS, USB-650 RED TIDE, Brazil) was used together with the SpectraSuite® 2.0 program (OCEAN OPTICS, USA, 2009).

2.2.4. Total phenolics

The resuspended hydroethanolic extracts were analyzed for total phenolic content using the Folin-Ciocaulteau method. For its determination, 30 μL of extract, 2370 μL of water, and 150 μL of Folin-Ciocalteau reagent were used. The solution was homogenized, and after 2 min, 450 μL of 15 % sodium carbonate (aq) was added. After 2 h, the absorbance of the samples was read at 765 nm [13]. To quantify the total phenolic content, a standard curve of gallic acid (GA) shown in Figure B (Appendices) was used, with concentration values of 30, 177, 324, 618, 912, 1206, and 1500 mg L^{-1} (Abs = 0.0011 GA - 0.0088; $R^2 = 0.9994$) and the results were expressed in equivalent milligrams of gallic acid per 100 g of buriti pulp (mg GAE 100 g^{-1}).

2.2.5. Flavonoids

250 μL of resuspended hydroethanolic extract, 1000 μL of water, and 75 μL of 5 % sodium nitrite (aq) were used to analyze the flavonoid content. The solution was centrifuged; after 5 min, 75 μL of 10 % aluminum chloride (aq) was added, and after 6 min, 500 μL of 1 mol L^{-1} sodium hydroxide (aq) was added. The absorbance reading of the samples was performed at 510 nm (Annegowda et al. [13]). A standard catechin curve (C) was used, shown in Figure C (Appendices), with concentration values of 15, 100, 200, 300, 375, and 450 mg L^{-1} (Abs = 0.0043C + 0.0407; $R^2 = 0.9987$) and the results were expressed in equivalent milligrams of catechin per hundred grams of buriti pulp sample (mg EC 100 g^{-1}).

2.2.6. Carotenoids

The determination of the total carotenoid content was performed using adaptations of the method described by Official Methods of Analysis of AOAC [14] in which 250 μL of resuspended hydroethanolic extract, 1.5 mL of isopropyl alcohol and 500 μL of hexane were used, the which were vortexed (BIOMIXER, QL901, Brazil) for 1 min. Homogenates were added with 4.25 mL of distilled water, vortexed for 30 s, and centrifuged at 4000 rpm for 5 min. The supernatant was collected (organic phase), inserted into a new tube, and washed twice more with 4.25 mL of water and a centrifugation process. The upper phase obtained in the last centrifugation was removed and inserted into a 10 mL volumetric glassware that was completed to a level of 2,5 mL with 10 % acetone with hexane and stirred. The absorbance reading was performed at 450 nm [14]. Equation (1) was used to obtain the results expressed in milligrams per hundred grams of sample (mg 100 g^{-1}), which were later converted to be expressed in milligrams per kilogram of buriti pulp sample (mg kg^{-1}) [12].

Table 1
Experimental conditions and responses of the levels of bioactive compounds and antioxidant capacity of buriti in the weight of pulp.

Experiments	Independent variables						Dependent variables					
	Encoded			Decoded			Bioactive compounds			Antioxidant capacity		
	x_1	x_2	x_3	Pulp (%, m v^{-1})	Ethanol (%, v v^{-1})	Temperature (°C)	Total Phenolics (mg GAE $100g^{-1}$)	Total Flavonoids (mg EC $100g^{-1}$)	Total Carotenoids (mg kg^{-1})	DPPH (μmol ET g^{-1})	ABTS (μmol ET g^{-1})	FRAP (μmol ET g^{-1})
1	−1.0000	−1.0000	−1.0000	8.04	25.13	38.11	225.16 ± 10.66	47.03 ± 2.17	2.37 ± 0.07	3.40 ± 0.15	13.95 ± 0.63	10.32 ± 0.44
2	−1.0000	−1.0000	1.0000	8.04	25.13	61.89	161.34 ± 4.36	30.48 ± 1.96	2.42 ± 0.06	1.59 ± 0.06	9.64 ± 0.34	7.18 ± 0.15
3	−1.0000	1.0000	−1.0000	8.04	54.87	38.11	270.61 ± 4.81	35.54 ± 1.58	2.50 ± 0.02	3.47 ± 0.08	15.61 ± 0.57	11.95 ± 0.57
4	−1.0000	1.0000	1.0000	8.04	54.87	61.89	221.47 ± 11.30	28.66 ± 0.61	2.71 ± 012	3.07 ± 0.12	14.24 ± 0.32	9.69 ± 0.58
5	1.0000	−1.0000	−1.0000	16.96	25.13	38.11	152.06 ± 9.27	27.01 ± 1.04	2.17 ± 0.03	1.67 ± 0.04	7.32 ± 0.10	6.74 ± 0.19
6	1.0000	−1.0000	1.0000	16.96	25.13	61.89	130.19 ± 2.86	23.98 ± 0.71	2.29 ± 0.07	1.52 ± 0.06	7.48 ± 0.05	5.59 ± 0.12
7	1.0000	1.0000	−1.0000	16.96	54.87	38.11	214.30 ± 1.61	29.81 ± 1.90	3.73 ± 0.02	2.28 ± 0.06	12.38 ± 0.44	9.82 ± 0.36
8	1.0000	1.0000	1.0000	16.96	54.87	61.89	185.19 ± 6.26	25.02 ± 0.84	3.92 ± 0.06	2.57 ± 0.06	9.80 ± 0.48	6.14 ± 0.22
9	−1.6818	0.0000	0.0000	5.00	40.00	50.00	266.33 ± 1.26	60.46 ± 2.90	2.37 ± 0.09	3.40 ± 0.15	15.96 ± 0.64	11.86 ± 0.19
10	1.6818	0.0000	0.0000	20.00	40.00	50.00	195.04 ± 5.83	23.15 ± 0.46	3.57 ± 0.09	2.06 ± 0.09	10.81 ± 0.41	8.65 ± 0.10
11	0.0000	−1.6818	0.0000	12.50	15.00	50.00	104.64 ± 12.52	24.80 ± 0.73	1.30 ± 0.05	1.23 ± 0.06	6.34 ± 0.19	4.74 ± 0.19
12	0.0000	1.6818	0.0000	12.50	65.00	50.00	148.64 ± 3.88	17.74 ± 0.44	2.56 ± 0.04	2.17 ± 0.11	8.45 ± 0.33	7.35 ± 0.13
13	0.0000	0.0000	−1.6818	12.50	40.00	30.00	206.95 ± 7.62	25.36 ± 1.77	2.72 ± 0.09	2.39 ± 0.06	10.79 ± 0.45	7.95 ± 0.21
14	0.0000	0.0000	1.6818	12.50	40.00	70.00	146.09 ± 3.93	22.17 ± 0.31	2.86 ± 0.10	1.87 ± 0.08	8.15 ± 0.33	5.53 ± 0.16
15	0.0000	0.0000	0.0000	12.50	40.00	50.00	180.86 ± 1.53	25.69 ± 1.19	3.16 ± 0.10	3.33 ± 0.16	9.46 ± 0.13	6.50 ± 0.31
16	0.0000	0.0000	0.0000	12.50	40.00	50.00	196.93 ± 3.17	27.81 ± 1.99	3.21 ± 0.13	3.38 ± 0.10	10.83 ± 0.05	7.48 ± 0.15
17	0.0000	0.0000	0.0000	12.50	40.00	50.00	199.48 ± 3.96	25.21 ± 0.67	2.75 ± 0.07	3.37 ± 0.11	10.67 ± 0.07	7.72 ± 0.06
18	0.0000	0.0000	0.0000	12.50	40.00	50.00	199.48 ± 16.68	24.85 ± 1.43	2.74 ± 0.06	3.09 ± 0.12	10.53 ± 0.05	7.55 ± 0.05
19	0.0000	0.0000	0.0000	12.50	40.00	50.00	166.46 ± 12.95	32.05 ± 1.08	2.68 ± 0.08	3.19 ± 0.15	10.69 ± 0.24	7.42 ± 0.03

Notes: Mean of the results obtained by the analyses described ± standard deviation.

$$\text{Total carotenoid content } (\text{mg } 100\ g^{-1}) = \frac{\text{Abs x } 100}{250 \text{ x L x W}} \quad (1)$$

where: Abs is the absorbance, L is the cuvette width, and W is the original quotient between the initial sample and the final dilution volume.

2.2.7. Antioxidant capacity

2.2.7.1. DPPH. To evaluate the extracts using this method, 100 μL of resuspended hydroethanolic extract and 3900 μL of DPPH 60 μmol L^{-1} solution were homogenized. After 30 min, absorbances were read at 515 nm (Brand-Williams et al. [15]). A trolox standard curve (T) shown in Figure D (Appendices) was used with concentration values of 50, 275, 500, 700, 850, and 1000 μmol L^{-1} (Abs = $-0.0005\ T + 0.6637$; $R^2 = 0.9996$) for conversion into results expressed in micromol trolox equivalents per gram of matrix buriti pulp (μmol ET g^{-1}).

2.2.7.2. ABTS. To evaluate the extracts using the ABTS method, the method by Arnao et al. [16] was modified by Thaipong et al. [17], in which two stock solutions were prepared. The first solution was 7.4 mM of ABTS, and the second was 2.6 mM of potassium persulfate with 20 mL of distilled water. The solutions were mixed to form the working solution, which reacted for 12 h in the dark at room temperature. The working solution was then diluted with 60 mL of methanol, and 2850 μL of this was mixed with 150 μL of resuspended hydroethanolic extract. The absorbance reading was performed after 2 h at 734 nm. A Trolox (T) standard curve, shown in Figure E (Appendices), was used with concentration values of 50, 140, 230, 320, 410, and 500 μmol L^{-1} (Abs = - 0.0017 T + 1. 0678; $R^2 = 0.9994$) and the results were expressed in micromol Trolox equivalents per gram of matrix buriti pulp (μmol ET g^{-1}).

2.2.7.3. FRAP. To carry out the analysis regarding the antioxidant capacity by the FRAP method, 100 μL of resuspended hydroethanolic extract, 300 μL of distilled water, and 3 mL of ferric complex previously prepared with TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine), ferric chloride and acetate buffer (1:1:10 v v^{-1}). The solution was kept in a thermal bath at 37 °C for 30 min, and the absorbances were read at 593 nm (Benzie & Strain [18]). A standard curve of Trolox (T) was used, as shown in Figure F (Appendices), with concentration values of 50, 250, 500, 750, and 1000 μmol L^{-1} (Abs = $0.0012\ T + 0.0008$; $R^2 = 0.9971$) and the results were expressed in micromol Trolox equivalents per gram of matrix buriti pulp (μmol ET g^{-1}).

2.2.8. Statistical modeling and analysis

Mathematical modeling was performed using the Statistica® 12.0 program (StatSoft Incorporation, USA, 2011). The mean values of responses were fitted to a second-order polynomial model whose regression coefficients were obtained by multiple linear regression. The coefficients were analyzed using the *t*-test and excluded from the model when they were insignificant ($p < 0.05$).

The models were evaluated by regression analysis (R^2 and R^2 adjusted) and variance analysis ($p < 0.05$).

The relationships between the independent variables were analyzed using contour graphs. The regions in shades of red, yellow, and green represent high, medium, and low levels of antioxidants, respectively.

3. Results

3.1. Response of the levels of bioactive compounds and antioxidant capacity

Table 1 presents the responses of the bioactive compounds and antioxidant capacity levels for each extraction condition in which the buriti pulp was submitted. The variations of bioactive compounds ranged from 104.64 to 270.61 mg GAE 100 g^{-1} for total phenolics, from 17.74 to 60.46 mg CE 100 g^{-1} for total flavonoids, and from 1.30 to 3.92 mg kg^{-1} for total carotenoids. The antioxidant capacity of the extracts, evaluated by the DPPH, ABTS, and FRAP methods, ranged from 1.23 to 3.47 μmol ET g^{-1}, 6.34–15.86 μmol ET g^{-1}, and 4.74–11.95 μmol ET g^{-1}, respectively. These results indicate a wide range of antioxidant activities among the extracts, reflecting the diversity of bioactive compounds in the buriti pulp.

A comparison with the findings of Cardoso et al. [19] for buriti fractions and pastes shows some similarities and differences in bioactive compound levels. Cardoso et al. [19] observed vitamin C concentrations ranging from 48.44 to 55.22 mg 100 g^{-1}, carotenoids from 6.05 to 21.03 mg 100 g^{-1}, total phenolics from 19.31 to 33.30 mg GAEq 100 g^{-1}, and antioxidant activity from 111.24 to 190.43 μmol TE g^{-1} DM in the fruit fractions. The highest values were observed in the peel, which aligns with our findings that the peel fraction of buriti is rich in bioactive compounds.

Our results, such as total phenolics ranging from 104.64 to 270.61 mg GAE 100 g^{-1}, and carotenoids from 1.30 to 3.92 mg kg^{-1}, are consistent with the findings of Santos et al. [20], who reported total phenolics in buriti pulp ranging from 100 to 150 mg GAE 100 g^{-1}. However, our study presents a broader range of values, indicating a more varied extraction efficiency across different conditions.

In contrast, the total flavonoid and carotenoid concentrations in our research were higher than those reported by Lima et al. [21], who documented flavonoids ranging from 10 to 40 mg CE 100 g^{-1} and carotenoids between 1.0 and 3.0 mg kg^{-1}. These discrepancies can be attributed to factors like plant genetic variation, fruit ripeness, environmental conditions, and the extraction methods used, which affect the concentration of bioactive compounds.

When we compare our results with other antioxidant-rich fruits, the antioxidant potential of buriti pulp remains competitive. Silva

et al. [22] and Costa et al. [23] have highlighted the high levels of phenolic compounds and flavonoids in açaí, but the antioxidant values in buriti pulp are comparable or even superior, reinforcing the potential of buriti as an antioxidant powerhouse. The rich composition of carotenoids such as β-carotene and lycopene in buriti pulp is a distinguishing feature, as these compounds are less prevalent in other tropical fruits.

Our findings also align with those of Silva et al. [24], who observed high extraction efficiency of bioactive compounds, including carotenoids and phenolic compounds, from tropical fruits using similar methods. The high extraction yields in our study further support the effectiveness of the extraction methods in capturing valuable bioactive compounds from buriti pulp.

3.2. Adjusted mathematical models

The statistically significant regression coefficients ($p < 0.05$) that form the adjusted mathematical models are presented in Table A (Appendices), where the values from $\widehat{A}$ to $\widehat{C}$, and $\widehat{D}$ to $\widehat{F}$ correspond to the extraction capacity of bioactive compounds and the extraction capacity of antioxidants per gram of matrix, respectively.

Most of the models presented have terms with a negative linear effect and a positive quadratic effect for the percentage of buriti pulp (x_1) that corresponds to the pulp percentage of the extraction. This indicates that the increase in solids content hinders the extraction of bioactive compounds and other antioxidants up to about 12.5 % (the central point of the design for this variable). From that point on, the negative effect becomes less intense. A significant quadratic effect implies that the linear effect is constrained, creating a maximum or minimum response for the independent variable. This critical insight highlights the non-linear nature of the extraction process, where an optimal percentage of pulp must be maintained to maximize efficiency. Ribeiro et al. [25] reinforce this interpretation, emphasizing that quadratic effects often temper or limit the influence of linear trends, thereby dictating precise operational windows for maximizing responses.

The decrease in the extraction of compounds per gram of pulp can be explained by the agitation speed of the particles, which likely dropped inside the reactor when the number of solids was higher, making contact between the solvent and the solids in the pulp more difficult. Additionally, increasing solids content saturated the solvent, decreasing the extraction capacity [26,27]. These combined effects highlight the necessity of controlling the solids-to-solvent ratio to optimize extraction efficiency.

The model for predicting the antioxidant capacity of the extracts evaluated by the DPPH method was particular because, in addition to the linear term, its quadratic term also negatively affected the percentage of buriti pulp. This result confirms the critical role of quadratic effects in moderating linear trends, as noted by Ribeiro et al. [25] illustrating how the increase in solids content initially decreases the antioxidant capacity yield of the extracts. Beyond a certain threshold, the high amount of solids interferes with the solid-solvent contact and saturates the solvent, as mentioned earlier. This underscores the importance of fine-tuning operational parameters to balance linear and quadratic effects for optimal extraction.

Contrary to what happened with most of the other compounds, the model that describes carotenoid extraction presented a positive linear term for the percentage of buriti pulp. This indicated that the increase in solids caused an increase in carotenoid extraction per gram of matrix. According to Norshazila et al. [27], who analyzed the effect of the solid-solvent ratio on the extraction of carotenoids in pumpkin extracts, the high solid-solvent ratio (percentage of pulp in the extracting solution) increases the concentration gradient of the solution and, therefore, enhances the diffusion rate, allowing greater extraction of carotenoids by the solvent. This linear behavior for carotenoids, untempered by a significant quadratic term, suggests a unique interaction mechanism compared to other bioactive compounds.

Regarding the effects of ethanol percentage (x_2), most of the adjusted forecast models showed a positive linear effect and a negative quadratic effect, indicating that the increase in ethanol concentration benefited the extraction of these compounds per gram of matrix up to about 40 % (the central point of the design for this variable), after which the extraction decreased non-linearly. Ribeiro et al. [25] suggest that such patterns arise when quadratic effects dominate beyond certain thresholds, limiting the positive impact of linear terms. According to Sridhar et al. [28], increasing the ethanol content allowed the partial solubilization of matrix cell membranes, improving access to bound antioxidants. However, at a certain point, ethanol began to dehydrate the membranes, making it difficult for water to interact with polar antioxidants linked to pulp cells. Although ethanol also has some apolar character (it has two carbons in the chain), increasing its content above 40 % did not yield higher antioxidant extraction, likely due to solvent saturation and limited solubility of certain compounds.

The model for predicting flavonoids, contrary to most models, showed a negative linear effect for the percentage of ethanol in the extracting solution, indicating that the increase in ethanol concentration caused a decrease in the extraction of flavonoids per gram of buriti pulp. According to Zhang et al. [29], this can be explained by the general principle of solvent extraction, which states that “like dissolves like”; the solvent only extracted similarly polar phytochemicals. Boeing et al. [30] also observed that ethanol is less efficient for extracting antioxidant compounds compared to other solvents, likely due to the low solvation promoted by the ethyl radical. The absence of a significant quadratic term for flavonoids suggests a more straightforward interaction mechanism with ethanol concentration.

The model for predicting the antioxidant capacity of the extracts evaluated by the FRAP method also showed a different trend, with a positive linear effect for the percentage of ethanol in the extracting solution. For this method, increasing the percentage of ethanol benefited the extraction of antioxidants per gram of matrix, potentially due to enhanced solubilization of compounds, as previously discussed.

For the effects of temperature, the models mostly showed a negative linear effect for temperature (x_3); that is, the increase in temperature caused a decrease in the extraction of compounds. According to Radojković et al. [31], generally above 40 °C, most

compounds' yield decreased due to thermal degradation. The significant quadratic term in certain models, such as the antioxidant capacity evaluated by the DPPH method, further underscores the interplay between linear and quadratic effects. Extraction capacity increased with temperature up to about 50 °C (the central point of the design for this variable), beyond which thermal degradation dominated. Ribeiro et al. [25] highlight how such quadratic effects create critical operational thresholds, defining maximum and minimum points for responses.

For carotenoids, however, temperature showed a positive linear effect; that is, carotenoid content increased with rising temperature. According to Sharmin et al. [32], increasing the temperature to a certain point enhances extraction efficiency, as heat makes matrix cell walls more permeable and increases compound solubility and diffusion. Strati and Oreopoulou [33] also confirm the increase in carotenoid yield with rising temperature due to these combined effects. The absence of a significant quadratic term for carotenoids suggests a relatively straightforward positive relationship between temperature and extraction efficiency.

The last two terms of the DPPH model are related to the interaction between the independent variables (x_1x_3 and x_2x_3). Due to the positive effect on both terms, simultaneous increases in pulp percentage and temperature or ethanol percentage and temperature resulted in higher antioxidant content due to synergistic relationships. Ribeiro et al. [25] discuss how interaction effects can mitigate the negative impacts of individual variables, providing pathways to optimize extraction conditions through coordinated parameter adjustments.

Tables B to G (Appendices) show the values used in the analysis of variance (ANOVA) referring to the adjusted models shown in Table A (Appendices). According to the analysis of variance, all mathematical models were significant for predicting the ability to extract bioactive compounds and antioxidants from buriti pulp extracts ($p < 0.05$) and did not show a considerable lack of fit ($p > 0.05$).

Table H (supplementary material) presents the determination coefficients (R^2) and adjusted determination coefficient (R^2 adjusted) for the mathematical models obtained. The regression analysis showed that the adjusted models for predicting the content of total phenolics, carotenoids, and antioxidants evaluated by the DPPH method were statistically significant. The flavonoid prediction model showed low significance, which may have been caused by experimental errors during the analytical determination of this compound.

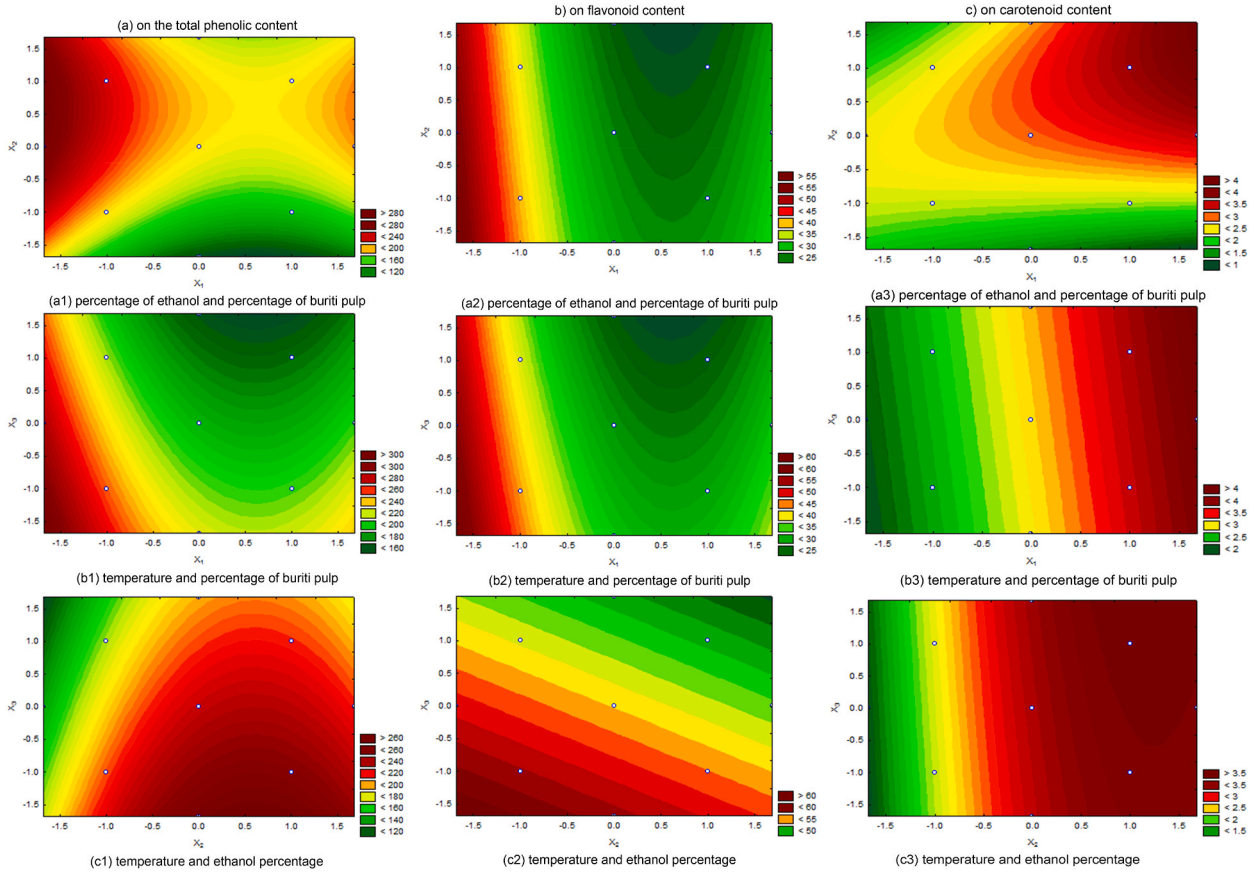


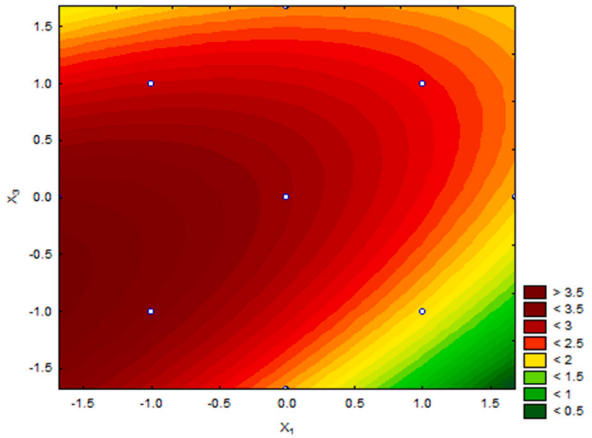
Fig. 1. Contour plot of the relationship with effect.

3.3. Analysis of contour graphs for the content of bioactive compounds and antioxidant capacity of buriti pulp extracts

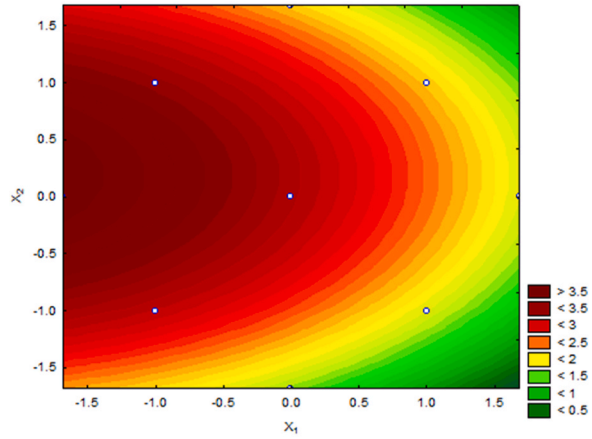
3.3.1. Conditions for total phenolics

Fig. 1 (a) shows the conditions for obtaining the total phenolic contents relating to the percentage of ethanol and percentage of buriti pulp (a1), temperature and percentage of pulp (b1), and temperature and percentage of ethanol (c1).

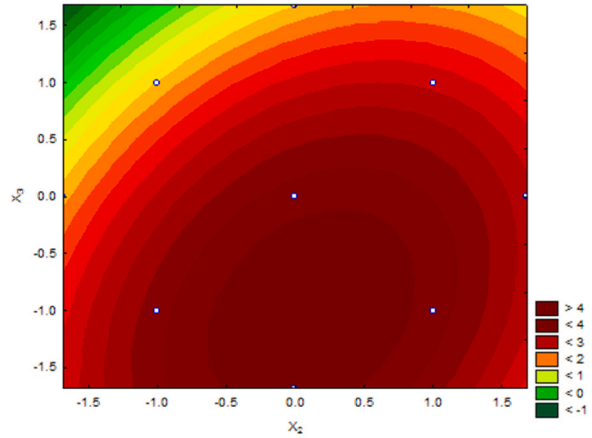
As can be seen in the contour graphs with coded axes, to obtain the highest possible content of this compound, the lowest percentage of buriti pulp was required (minimum of 5 % in the experimental design), while the percentage of ethanol was in the range



(a) temperature and percentage of buriti pulp



(b) percentage of ethanol and percentage of buriti pulp



(c) temperature and ethanol percentage

Fig. 2. Contour plot of the relationship with effect on the antioxidant extraction capacity evaluated by the DPPH method.

between 40 % and 54.87 %. The lowest percentage of buriti pulp was also required while the temperature was between 30 °C and 38.11 °C. Finally, the lowest possible temperature was needed (minimum of 30 °C), while the percentage of ethanol was between 40 % and 54.87 %.

3.3.2. *Conditions for flavonoids*

As can be seen in Fig. 1 (b) ((a2), (b2), and (c2)), the highest flavonoid contents were obtained, respectively, with the lowest

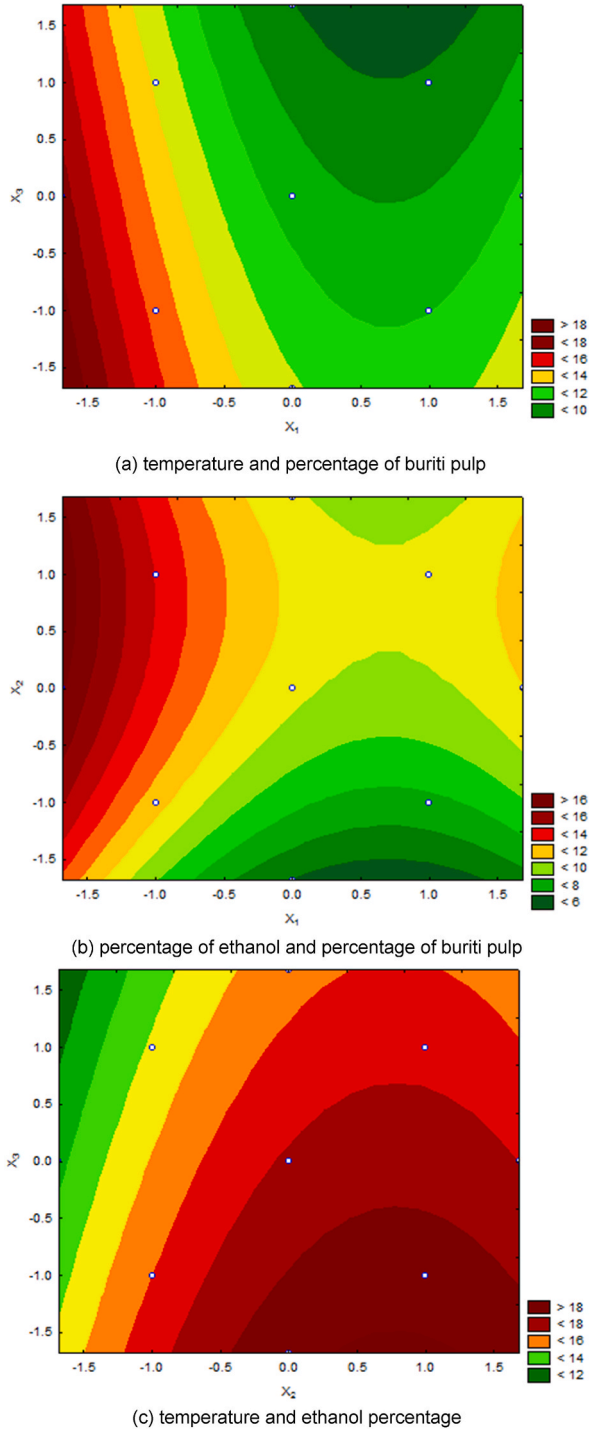


Fig. 3. Contour graph of the relationship with effect on the antioxidant extraction capacity evaluated by the ABTS method.

percentage of buriti pulp, while the percentage of ethanol was found in the range between 15 % and 25.13 %; the lowest percentage of buriti pulp while the temperature was in the range between 30 °C and 38.11 °C and the lowest temperature while the percentage of ethanol was at a maximum of 15 %.

3.3.3. Conditions for carotenoids

Fig. 1 (c), ((a3), (b3), and (c3)) shows the relationships of independent variables as effects on carotenoid content. Observing the

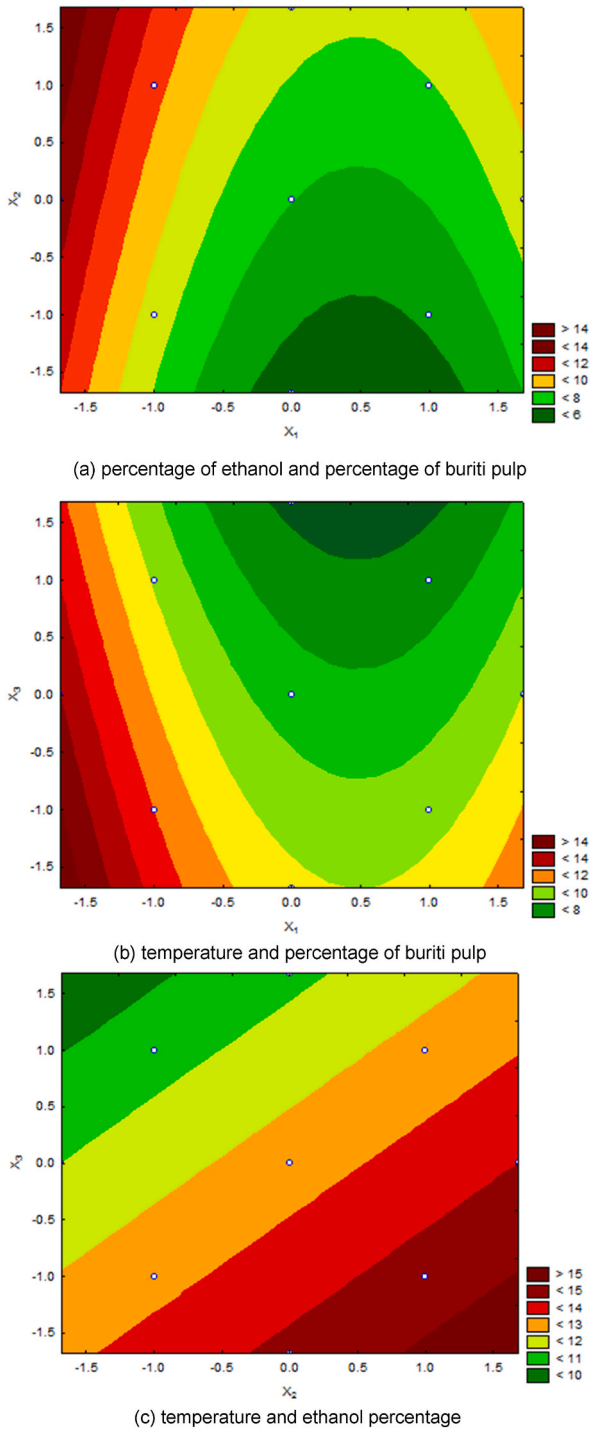


Fig. 4. Contour plot of the relationship with effect on the antioxidant extraction capacity evaluated by the FRAP method.

figures, it was possible to notice that the percentage of buriti pulp was more significant (maximum of 20 %). In comparison, the percentage of ethanol was between 54.87 % and 65 %, the more significant the content of extracted carotenoids. The higher the percentage of buriti pulp while the temperature was at a maximum of 70 °C, the higher the extraction rate of this compound. Furthermore, the higher the temperature, while the percentage of ethanol was between 54.87 % and 65 %, the greater the carotenoid extraction.

3.3.4. Conditions for antioxidants (DPPH method)

The relationships between temperature and percentage of buriti pulp, percentage of ethanol, and percentage of pulp and temperature and percentage of ethanol to obtain the antioxidant content evaluated by the DPPH method are shown in Fig. 2 (a), (b), and (c), respectively.

To obtain the highest antioxidant content through this method, the lowest percentage of buriti pulp was required while the temperature was between 30 °C and 50 °C. It was necessary to work with the lowest buriti pulp percentage while the ethanol percentage was between 25.13 % and 54.87 %. The lowest possible temperature was also needed, while the percentage of ethanol was between 25.13 % and 54.87 %.

3.3.5. Conditions for antioxidants (ABTS method)

Fig. 3 (a), (b), and (c) shows the relationships between the independent variables to obtain the antioxidant content evaluated by the ABTS method. The lower the percentage of buriti pulp when the temperature was between 30 °C and 38.11 °C, the higher the antioxidant content obtained. Likewise, the lower the percentage of buriti pulp while the percentage of ethanol was between 40 % and 65 %, the higher the antioxidant content obtained. Finally, the antioxidant content increased when the temperature was the lowest possible, while the ethanol percentage was between 40 % and 65 %.

3.3.6. Conditions for antioxidants (FRAP method)

The relationships between the independent variables for obtaining antioxidants evaluated by the FRAP method are shown in Fig. 4 (a), (b), and (c). To get the highest possible antioxidant content, the lowest percentage of buriti pulp in the design was combined with a temperature range between 30 °C and 38.11 °C. The lowest percentage of buriti pulp was also combined with the percentage of ethanol found between 54.87 and 65 %. The lowest possible temperature was also combined while the percentage of ethanol was as high as possible (maximum 65 %).

3.3.7. Analysis of optimal regions

The regions where the best extraction conditions were found to obtain an extract with maximized response were differentiated for carotenoids and the rest of the analyzed compounds.

Total carotenoids tended to be better extracted with a percentage of buriti pulp around 20 %, intermediate percentages of ethanol, and temperatures around 70 °C. On the other hand, all other compounds showed better extraction capacity with percentages of 5 % of buriti pulp, intermediate percentages of ethanol, and temperatures around 30 °C.

This difference, as previously mentioned by Strati and Oreopoulou [33], may have occurred due to the structure of the carotenoid compounds, which, with increasing solid content and temperature, became more soluble and increased their diffusivity. However, the role of solvent saturation in this process was not considered, which could have influenced the extraction efficiency, especially given the typically low concentrations of carotenoids (micrograms per gram) in the sample.

Additionally, a comparative analysis of different solvents could provide valuable insights into the most effective conditions for extracting bioactive compounds from buriti pulp.

The selection of easily reproducible extraction variables, coupled with an investigation into solvent suitability for specific bioactives, remains a distinguishing factor in this study. Such an approach aims to contribute significantly to the chemical characterization and potential technological exploitation of buriti pulp.

Possible applications of the extract include its use as a natural colorant, antioxidant, and bioactive compound in the food, cosmetics, and pharmaceutical industries. These applications highlight the potential of buriti pulp as a sustainable and valuable resource.

While this study focused primarily on optimizing laboratory extraction methodologies, further research could explore scaling up the extraction process for industrial applications, taking into account factors such as solvent recovery, process efficiency, and cost-effectiveness. This would allow for the broader exploitation of buriti pulp in industrial bioproducts.

4. Conclusion

The results provide valuable insights into the extraction of antioxidant compounds from buriti pulp, corroborating previous studies highlighting the antioxidant capacity of this fruit. As seen, the variations of bioactive compounds in buriti pulp ranged from 104.64 to 270.61 mg GAE 100 g^{-1} for total phenolics, from 17.74 to 60.46 mg EC 100 g^{-1} for total flavonoids, and from 1.30 to 3.92 mg kg^{-1} for total carotenoids. The antioxidant capacity of the extracts evaluated by the DPPH, ABTS, and FRAP methods ranged from 1.23 to 3.47 μmol ET g^{-1}, 6.34–15.86 μmol ET g^{-1}, and 4.74–11.95 μmol ET g^{-1}, respectively. Thus, it was verified that there was efficiency in the extractions since the analytical determinations of all extracts showed responses of antioxidant compounds. Given these responses, the importance of buriti as a source of antioxidant compounds can also be confirmed.

The adjusted mathematical models established through the obtained responses were statistically significant and necessary for the projection of contour graphs that allowed the analysis of optimal regions. The regions where the best extraction conditions were found

to obtain an extract with maximized response were differentiated for carotenoids and the rest of the analyzed compounds, possibly due to differences in their chemical structure and temperature resistance.

While the carotenoid compounds required a percentage of buriti pulp at a maximum of 20 %, intermediate percentages of ethanol, and temperatures around 70 °C, all other compounds required a percentage of pulp at a minimum of 5 %, intermediate percentages of ethanol and temperatures around 30 °C.

The extracts obtained showed promising antioxidant activity, as assessed by the DPPH, ABTS, and FRAP methods, indicating the potential of buriti as a source of natural antioxidants. The results of the analysis of the optimal regions for the extraction of antioxidant compounds from buriti pulp will be used in future work on optimization in which the particle swarm algorithm will be used to determine an optimal point with the best extraction condition.

CRediT authorship contribution statement

Gabriela Mota Nogueira: Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Flávia Aparecida Reitz Cardoso:** Writing – review & editing, Writing – original draft. **Márcia Regina Ferreira Geraldo Perdoncini:** Validation, Methodology. **Marcos Vieira da Silva:** Validation, Methodology. **Eliane Sloboda Rigobello:** Supervision, Conceptualization.

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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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e41926.

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