CAGAITA A STUDY USING THE RESPONSE METHODOLOGY TO DEFINE THE BEST EXTRACTION PARAMETERS

CAGAITA UM ESTUDO UTILIZANDO A METODOLOGIA DE RESPOSTA PARA DEFINIR OS MELHORES PARÂMETROS DE EXTRAÇÃO

CAGAITA UN ESTUDIO QUE UTILIZA LA METODOLOGÍA DE RESPUESTA PARA DEFINIR LOS MEJORES PARÁMETROS DE EXTRACCIÓN

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ABSTRACT
The Brazilian Cerrado is a biome with great territorial extension and consequently numerous native fruits that have a great technological potential, mainly due to its nutritional composition and bioactive compounds. Thus, this work aims to use the response surface methodology for optimization in the process of extraction of bioactive compounds for Cagaita fruit. The extraction temperature (60 °C) and the solvent (water) used were those that presented the greatest emphasis for optimization in the extraction of bioactive compounds. This result allows subsequent developments by characterizing the extract more widely, application of other technologies such as evaporation, microencapsulation and nanoemulsion, promoting greater technological development and a greater knowledge about the fruit by the population and the different regions.

Keywords: Bioactive compounds. Eugenia Dysenterica. Brazilian Cerrado. Technological innovation.

RESUMO
O Cerrado brasileiro é um bioma com grande extensão territorial e por consequência inúmeras frutas nativas que possuem um grande potencial tecnológico, principalmente devido sua composição nutricional e compostos bioativos. Assim, este trabalho tem como objetivo a utilização da metodologia de superfície de resposta para a otimização no processo de extração dos compostos bioativos para o fruto da Cagaita. A temperatura de extração (60 °C) e o solvente (água) utilizados forma os que apresentaram maior destaque para a otimização na extração dos compostos bioativos. Este resultado possibilita a desenvolvimentos subsequentes caracterizando de forma mais ampla o extrato, aplicação de outras tecnologias como evaporação, microencapsulação e nanoemulção, promovendo um maior desenvolvimento tecnológico e um maior conhecimento sobre o fruto pela população e pelas diferentes regiões.

Palavras-chave: Compostos bioativos. Eugenia Dysenterica. Cerrado brasileiro. Inovação tecnológica.

RESUMEN
El Cerrado brasileño es un bioma con una gran extensión territorial y en consecuencia numerosos frutos nativos que poseen gran potencial tecnológico, principalmente por su composición nutricional y compuestos bioactivos. Así, este trabajo tiene como objetivo utilizar la metodología de superficie de respuesta para optimizar el proceso de extracción de compuestos bioactivos del fruto de Cagaita. La temperatura de extracción (60 °C) y el solvente (agua) utilizados fueron los que presentaron mayor énfasis en optimizar la extracción de compuestos bioactivos. Este resultado permite desarrollos posteriores caracterizando más ampliamente el extracto, aplicando otras tecnologías como la evaporación, la microencapsulación y la nanoemulsión, promoviendo un mayor desarrollo tecnológico y un mayor conocimiento del fruto por parte de la población y de las diferentes regiones.

Palabras clave: Compuestos bioactivos. Eugenia disenterica. Sabana brasileña. Innovación tecnológica.

INTRODUCTION

The Brazilian Cerrado is described as the largest tropical savannah in the world, the largest and richest in the world and hosts a wide diversity of fruits with significant nutritional and functional potential (bioactive properties such as anti-inflammatory, anticancer and antimicrobial effects), but receives less attention and conservation resources, prevent deforestation and forest degradation as this is essential to maintain ecosystem services (Almada et al., 2024; Carvalho et al., 2025; Teruko Shirai et al., 2024). In recent decades, the intensification of human activities, directly influencing changes in use and coverage, has caused severe environmental problems (Vick et al., 2024). The potential of native fruits for sustainable applications in the food and pharmaceutical industries, benefits and ensure the conservation of Cerrado biodiversity (Carvalho et al., 2025).

Cagaita (*Eugenia dysenterica* DC) is a tree of the Brazilian Cerrado biome belonging to the family Myrtaceae. Fruits and leaves are used as alternative medicine by local communities to treat diarrhea, diabetes and jaundice, the globular-shaped fruit, slightly flattened, with thin bark and light yellow color (da Silva et al., 2020) and is consumed mainly in the form of juices and pulps (Santos et al., 2024). This fruit appears to be a promising candidate for an adjuvant in glucose regulation in healthy individuals (Araujo et al., 2021). Phenolic compounds of the cacaita fruit demonstrated antioxidant potential in vitro and inhibitory actions regarding the activity of enzymes involved in carbohydrate metabolism (Donado-Pestana et al., 2015).

Optimization is commonly performed using the response surface methodology (MSR), where a function is adjusted to the data to describe the design space of the effects of factors in the response variable to obtain the best operating conditions, is a type of statistical technique that evaluates and optimizes the process where there are several parameters, various reactions and their responses are designated (Dowlatshah et al., 2025; Pektezel & Ozdemir, 2025; Thakur et al., 2024). The use of this methodology is efficient for the description of the best way to extract bioactive compounds (Kirankumar et al., 2025).

Thus, this work aimed to use the surface response (MSR) methodology evaluating different parameters of extraction of antioxidant compounds for the fruits of cagaita.

MATERIALS AND METHODS

MATERIALS

Aproximately 500 g of samples were collected in the month of October at the Felicidade farm located in the municipality of Jussara, in the northwest of the state of Goiás, with the following geographic coordinates: Latitude: 15° 51' 31" South, Longitude: 50° 52' 9" West. The samples were sanitized and stored in polyethylene containers and frozen in horizontal freezer (Metalfrio) at freezing temperature of -18°C to -22°C. The samples were kept frozen and transported to the city of Maringá for analyses in a thermal box not allowing the fruit thawing so that there was no loss of the compounds present (Silva, Silva, et al., 2024).

COMPLETE EXPERIMENTAL PLANNING 2^4

To optimize the extraction conditions of antioxidants in this study, a surface response methodology was used, such as experimental planning, through the Central Composite Design (CCD) with four independent variables. This design made it possible to reduce the number of experimental points to be tested and allow the adjustment and testing of regression models, where it consisted of factor experiments (levels +1 and -1) totaling 16 treatments (Silva, Silva, et al., 2024).

The optimization of the extraction conditions of antioxidants from suckling milk in natura was performed using factorial experimental planning 2^4. The independent variables to be studied and changed were solvent concentration (ethanol), extraction temperature and time and use of ultrasonic bath. The dependent variables were antioxidant capacity and phenolic compounds of the extract. Data were analyzed using the response surface methodology. Factor planning was selected for each variable of the optimization process in two levels with sixteen experiments (Table 2). The index (-1) represents the lowest level and the index (+1) represents the highest level of the variable in question(Silva, da Silva, et al., 2024

In order to determine the best conditions to be used in the process of extraction of antioxidants, a factorial experimental planning was carried out 2^4, where some variables were tested in order to verify if they have significant influence on the process. The independent variables tested were extraction temperature, extraction time, ethanol concentration and use of ultrasonic bath equipment and the dependent variable was the antioxidant capacity quantified by DPPH radical sequestration methods, ABTS and FRAP and the content of phenolic compounds. Table 1 presents the real levels of

variables related to the coded levels used during the analysis planning stage, where -1 and +1 represent, respectively, lower level and upper level (Silva, da Silva, et al., 2024).

Table 1. Real and coded levels of variables.

Level	-1	+1
Extraction temperature (°C)	30	60
Extraction time (min)	30	60
Ethanol concentration (%)	0	100
Use of ultrasonic bath	No	Yes

Table 2 presents the experimental planning matrix according to the coded levels of each variable. The planning resulted in 16 experiments with different combinations of variables.

Table 2. Experimental planning matrix 2^4.

Ensaio	Temperature (°C)	Time (min)	Ethanol	Ultrasonic bath
1	-1	-1	-1	-1
2	+1	-1	-1	-1
3	+1	+1	-1	-1
4	+1	+1	+1	-1
5	+1	+1	+1	+1
6	+1	+1	-1	+1
7	+1	-1	+1	+1
8	-1	+1	+1	+1
9	-1	+1	+1	-1
10	-1	-1	-1	+1
11	-1	-1	+1	-1
12	-1	+1	-1	-1
13	-1	+1	-1	+1
14	+1	-1	+1	-1
15	+1	-1	-1	+1
16	-1	-1	+1	+1

QUANTIFICATION OF ANTIOXIDANT CAPACITY

ABTS radical

The cationic radical $ABTS^{\bullet+}$ is formed by the reaction of 5 mL of aqueous solution of $ABTS^{\bullet+}$. The radical cation $ABTS^{\bullet+}$ with 88 µL of potassium persulphate (PP) (140 mol/L), allowing the reaction to occur at room temperature, in the dark, for

16 hours before use (Rufino et al., 2009). Before the test, the $ABTS^{\bullet+}$ solution was diluted in ethyl alcohol PA, the analysis was done in triplicate. An aliquot of 30 µL of extract was added to the tubes, together with 3 mL of $ABTS^{\bullet+}$ reagent already diluted.

The samples were incubated for 6 min, protected from light at room temperature and the reading was made at a wavelength of 734 nm in FEMTO spectrophotometer (Cirrus 80MB). The antioxidant capacity of the succulent extract was determined from the standard curve of Trolox (2 mol/L) and expressed in mg Trolox/g sample. The extract was diluted at a concentration of 200 mg/mL so that the ABTS values were within the standard curve ($y = -0.0003x + 0.6603$ / $R^2 = 0.9953$).

DPPH radical

The assay to determine the antioxidant capacity of samples by capturing the radical DPPH was conducted according to Thaipong et al. (2006). Previously the DPPH solution diluted in methanol to obtain an absorbance of 1.1 at 515 nm in the spectrophotometer, then a 150 µL aliquot of the samples were added to 2.85 mL of a methanolic solution of DPPH, homogenized and kept for 1 hour under shelter from light. Then, the absorbance values were measured at 515 nm wavelength in FEMTO (Cirrus 80 MB) spectrophotometer, methanol solvent was used as white.

The analytical curve was prepared from ethanolic solutions of Trolox at concentrations ranging from 0 $\mu molL^{-1}$ to 900 $\mu molL^{-1}$. The response was expressed in mg equivalents of Trolox per gram of sample. The results were calculated using the standard Trolox curve and expressed in mg Trolox/g sample ($y = 0.1140x – 0.1125/R^2 = 0.9991$).

Determination of iron reducing antioxidant power (FRAP)

The FRAP assay was performed according to the methodology of Benzie and Strain (1999), the FRAP reagent was prepared using the combination of 0.3 mol/L acetate buffer solution TPTZ 10 mol/L ferric chloride 20 mol/L in the ratio 10:1:1 (v: v: v). The 300 mol/L sodium acetate buffer solution was performed by adding 3.1 g of anhydrous sodium acetate in 16 mL glacial acetic acid, completing with distilled water to a volume of 1000 mL. The preparation of 10 mol/L TPTZ solution (2, 4, 6 tripyridyl-s-triazine solution) was carried out by adding the mass of 0.312 g TPTZ in 5 mL HCl (hydrochloric acid) 40 mol/L. Following the preparation of 20 mol/L iron chloride solution.

The new working solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution and 2.5 mL of 20 mol/L iron chloride solution. An aliquot of 90 µL of sample extract, with 270 µL of distilled water and 2,7 mL of the FRAP solution by

incubation of 30 min sheltered from light at a temperature of 37°C. The FRAP reagent was used as white, the readings were then performed at a wavelength of 595 nm in spectrophotometer FEMTO Cirrus 80MB. The Trolox standard was used for the development of the calibration curve (0-700 µmol/L), the results were expressed in mg Trolox/g sample. For this analysis of FRAP, the extracts of mamacadela were diluted at a concentration of 200 mg/mL (y =0.0013x + 0.0059/ R^2 = 0.9995).

Determination of total phenolic compounds

The total phenolic compounds content was determined using the Folin-Ciocalteau method (Singleton & Rossi, 1965). In test tubes under shelter of light, 125 µL of extract was placed and 125 µL of Folin 50% was pipetted. Then 2250 µL of sodium carbonate (Na_2CO_3) was added. The same process was used for the blank, only replacing the sample with distilled water. The solutions were incubated in the dark, for 30 minutes for complete reaction of the reagent. The absorbance of the samples was determined by spectrophotometer at a wavelength of 725 nm. The analysis was performed in triplicate. Gallic acid was used for the development of the calibration curve (0-300 µmol/L), the results were expressed in mg of gallic acid equivalent per 100 g of sample on dry basis (mg EAG.100 g^{-1} dry base). For this analysis of phenolic compounds content, the extracts were diluted at a concentration of 200 mg/mL (y =0.0018x – 0.0182/ R^2 = 0.998).

STATISTICAL ANALYSIS

The experimental values were expressed as mean standard deviation (n = 3). The results were analyzed by means of Analysis of Variance (ANOVA), followed by multiple comparisons with the Tukey test ($p < 0.05$) performed in the RStudio software (Posit, PBC; Boston, MA, USA).

RESULT AND DISCUSSION

For the potting, it was possible to observe that the solvent (C) (Figure 1), for both methods of quantification of antioxidant capacity showed a significance in the extraction process of the compounds of interest. This occurrence can be correlated to changes in the polarity of the compound that was responsible for the reduction in the quantification of antioxidant capacity. Relating this result to the polarity of the solvent used in the extraction, which directly affects not only the amount of total phenolic compounds, but also the composition and potency of phenolic compounds as antioxidants (Mustafa & Turner, 2011; Vajić et al., 2015). Consequently, this difference results in extracted

phenolic compounds and a varied antioxidant activity of the extracts (Zulkifli et al., 2020).

The development of new extraction techniques for food and pharmaceutical industries has attracted increasing interest due to safety concerns, demand to minimize energy costs, environmental restrictions and public health regulations (Carvalho et al., 2005; Chen & Liu, 2020; Nguyen et al., 2021).

Several factors have been shown to significantly affect the extraction yield, such as extraction methods, particle size, solvent types, solvent concentrations, solvent-solid ratios, extraction temperatures, extraction times and pH levels (Banik & Pandey, 2008; Pinelo et al., 2005; Silva et al., 2007).

The same behavior was observed by Lim & Murtijava (2007) and Wang et al. (2010), where the rise in temperature improved the performance of antioxidants in the extraction process, due to the fact that the higher temperature interrupted the matrix structure of the samples, weakening the phenolic matrix bonds, which increased the solubility of flavonoids (Krishnaswamy et al., 2013).

Figure 1. Main interactions between the parameters analyzed for quantification of antioxidant capacity DPPH (a), ABTS (b), FRAP (c) and phenolic compounds content (d).

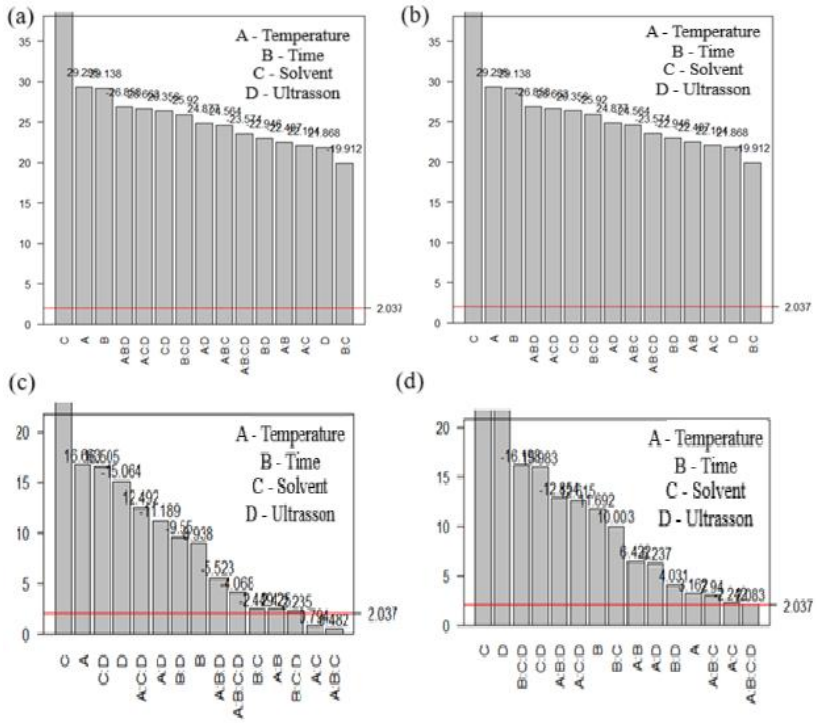
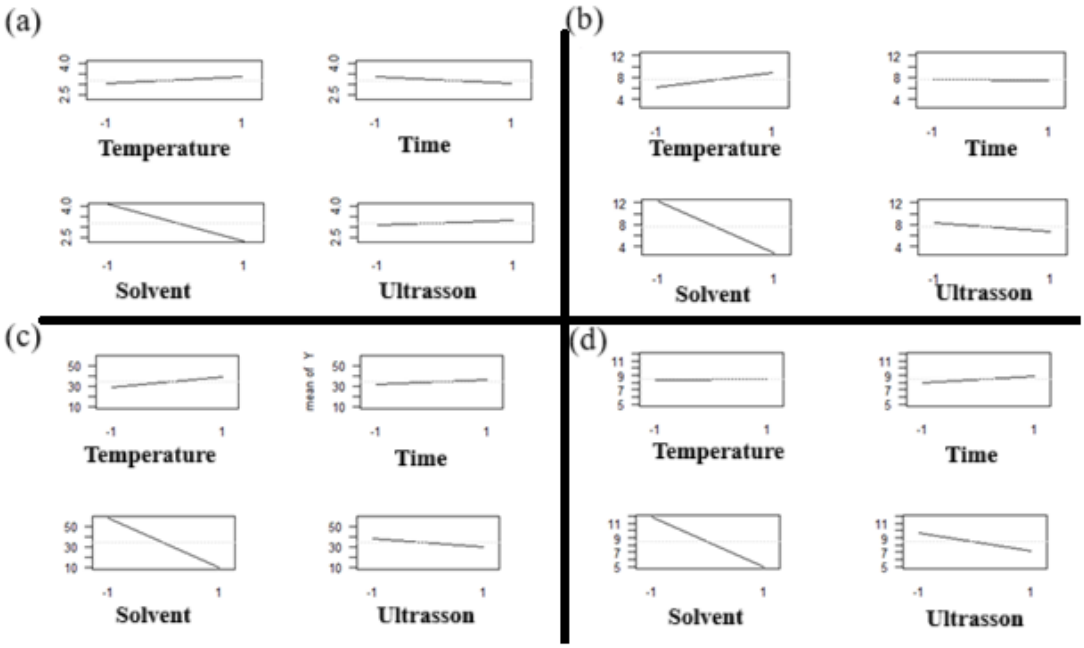


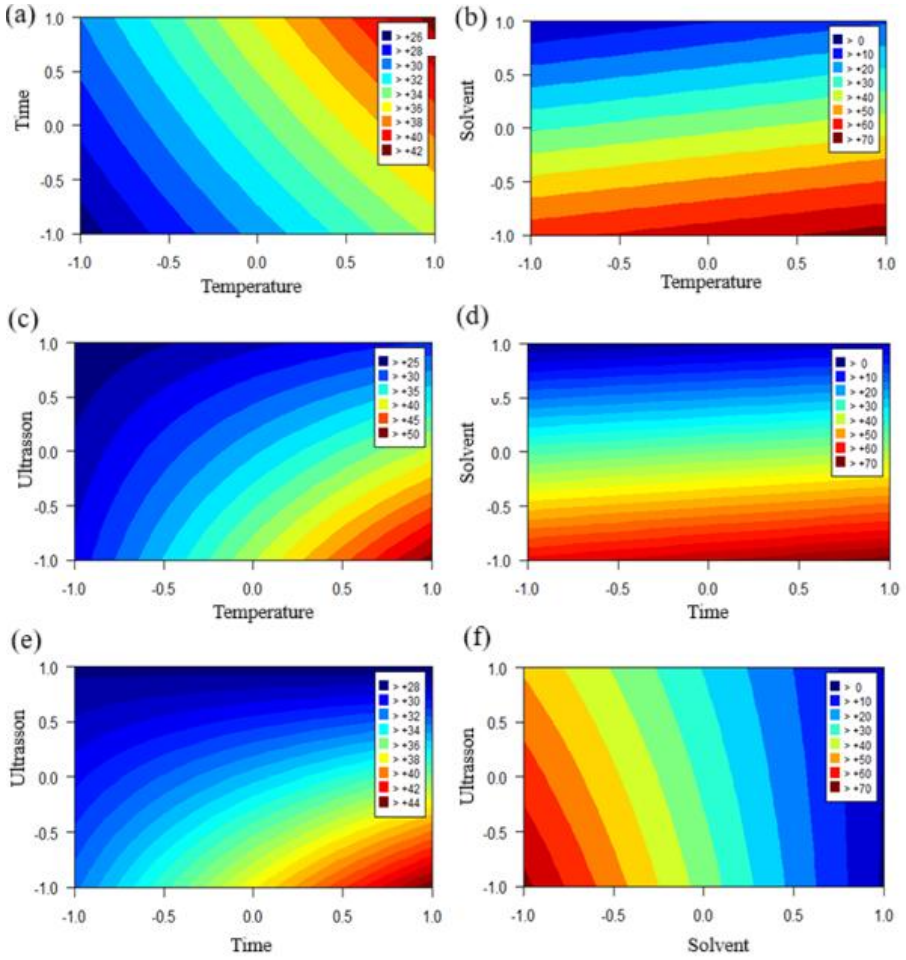
Figure 2. Effects for the extraction parameters A (extraction temperature), B (extraction time), C (solvent used: water or ethanol) and D (ultrasound use) for quantification of DPPH (a), ABTS (b), FRAP (c) and phenolic compounds content (d).



In both methods of quantification of antioxidant capacity, the increase in temperature had a positive influence (Figure 2), other authors also stated that under normal conditions, temperature has constructive effect on the extraction of phenolic compounds from plant sources (Bucić-Kojić et al., 2007; Harbourne et al., 2009; Spigno et al., 2007), because at higher temperatures there is greater solubility of phenolic compounds in the solvent used for extraction (Lim & Murtijaya, 2007; Yolmeh et al., 2014). The extraction time and the use of ultrasound did not significantly affect the.

The solvent showed a behavior of higher concentration of ethanol lower the antioxidants extracted, the same behavior was observed for Zulkifli et al. (2020) in its study that correlated to the changes of polarity of the compound as responsible for the reduction of the quantification of bioactive compounds.

Figure 3. Response surface for the analysis of antioxidant capacity (DPPH) of phenolic compounds contained in cagaita fruits. Interactions temperature versus time (a), temperature versus solvent (b), temperature versus ultrasound (c), time versus solvent (d), time versus ultrasound (e) and solvent versus ultrasound (f).



For the surface response analysis for the double interactions (Figure 3) of the extraction parameters that both methods of quantifying antioxidant capacity demonstrated the same behavior for the parameters established for this study. It was possible to observe for the temperature versus time (Figure 3a), that the highest values were obtained with the increase in temperature and the longest extraction time, this behavior observed for the temperature employed was the same for the other double interactions, where high temperature increases the diffusion of extracted molecules, reduces their viscosity and improves mass transfer (Prasad et al., 2009). In addition, high temperatures can increase the permeability of cell walls, breaking the interaction between phenolic compounds and macromolecules (proteins, polysaccharides) and thus facilitate the recovery of phenolic yield in the extract (Zulkifli et al., 2020).

The time of extraction had a negative behavior, where the greater the exposure of the sample, the lower the quantification of the response variable. Time is one of the

main factors that influence an extraction process, because it reduces the energy cost in the procedure with a shorter extraction period and also in the decomposition of active compounds, due to the long process. The extraction is described in two phases, the fast phase or slow phase, where the fast phase is related to solutes being present on surface sites of plant materials, and the slow phase to molecular diffusion of the solute being on internal sites, needing a longer time to perform this operation (Herodež et al., 2003; Salar et al., 2016; Spigno et al., 2007).

Ghasemzadeh et al. (2018) observed the same results in their study, i.e., an increase in flavonoid content with increasing extraction time, and attributed their results to the nature of the sample (seed, leaf, rhizome or bark), particle size, type of solvent and extraction approaches (Spigno et al., 2007; Wong & Kitts, 2006).

For both double interactions involving the solvent used, water was the one that showed a better extraction of antioxidants present in the analyzed sample. In general, the solvent used in the extraction is based on the law of similarity and intermiscibility "similar dissolves similar", which means that solvents extract phytochemicals with a polarity value close to the polarity of the solvent, because the polarity of the solvent influences the extraction of these compounds (Ribeiro et al., 2018; Zhang et al., 2007).

The efficiency of a solvent depends mainly on its ability to dissolve these compounds. Ethanol is a suitable solvent for dissolving flavonoid glycosides, while water is able to dissolve phenolic acid glycosides (Oreopoulou et al., 2019). It is important to note that ethanol and water are green solvents and have low toxicity, which expands to other applications of this extract. The use of ultrasound bath equipment was not significant for this study.

CONCLUSION

The study defined a better extraction of bioactive compounds when using water as an extractor solvent, together with the increase in temperature (60°C), promoting an improvement in the quantification of antioxidants, and thus improving the extraction process.

Thus, it is possible to observe that the fruits of the cerrado have numerous benefits to health and well-being and various technological applications, showing to be highly nutritious and high added value allowing applications asprime and greater variation of products found in the market.

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