Journal of Biotechnology and Biodiversity

journal homepage: https://sistemas.uft.edu.br/periodicos/index.php/JBB/index

Antioxidant potential of the sap of the *Croton lechleri* Müll. Arg. stem (Euphorbiaceae)

Simone Letícia Perín [a], Letícia Thaís Chendynski [b*], Sandra Inês Adams Angnes Gomes [a], Edneia Durli Giusti [a]

[a] Instituto Federal do Paraná - Campus Palmas, Brasil
[b] Instituto Federal do Paraná - Campus Ivaiporã, Brasil
[*] Autor correspondente (leticia.chendynski@ifpr.edu.br)

INFO

Keyworks
experimental design
extraction
blood dragon
total phenolic compounds

ABSTRACT

The *Croton lechleri* plant is used by the indigenous and riverside population for its healing and anesthetic power. The objective of this research was to evaluate the amount of total phenolic compounds and the antioxidant properties of the sap extracted from the stem of the Blood Dragon (*Croton lechleri*) to use the natural extract as a renewable alternative to the synthetic antioxidants present in the food and biodiesel industry. The extraction conditions were optimized and the antioxidant activity of these bioactive compounds through fractional factorial design. The variations were: solvent (pure water, 50% ethanol, 100% ethanol), temperature (50 °C, 70 °C and 90 °C) and time (30 min, 60 min, 90 min). Nine assays were evaluated with the software Statist 7®. The total phenolic compounds content ranged from 92.22 ± 1.35 to 193.29 ± 1.93 mg of EAG/g of sap and antioxidant activity by the ABTS method from 15.17 ± 0.08 to 26.80 ± 0.04 mM TE/g sap. The analysis of the global response and its equations made it possible to verify that ethanol 50% (v/v) was more effective in extracting total phenolic compounds and antioxidant activity under conditions of 90°C and shorter extraction time, 30 minutes.

RESUMO

Palavras-chaves
delineamento experimental
extração
sangue do dragão
compostos fenólicos totais

Potencial antioxidante da seiva do caule da planta Croton lechleri Müll. Arg. (Euphorbiaceae)
A planta *Croton lechleri* é utilizada pela população indígena e ribeirinha devido seu poder curativo e anestésico. O objetivo da pesquisa foi avaliar a quantidade de compostos fenólicos totais e as propriedades antioxidantes da seiva extraída do caule da planta conhecida como Sangue de Dragão (*Croton lechleri*) para utilizar o extrato natural como alternativa renovável aos antioxidantes sintéticos presentes na indústria de alimentos e de biodiesel. As condições de extração e a atividade antioxidante destes compostos bioativos foram otimizadas através de planejamento fatorial fracionado. As variações foram: solvente (água pura, etanol 50%, etanol 100%), temperatura (50 °C, 70 °C e 90 °C) e tempo (30 min, 60 min, 90 min). Nove ensaios foram avaliados com o software Statist 12®. O teor de compostos fenólicos totais variou de 92,22 ± 1,35 a 193,29 ± 1,93 mg de EAG/g de seiva e a atividade antioxidante pelo método ABTS de 15,17 ± 0,08 a 26,80 ± 0,04 mM TE/g de seiva. A análise da resposta global e suas equações permitiu verificar que o etanol 50% (v/v) foi mais eficaz na extração de compostos fenólicos totais e atividade antioxidante nas condições de extração a 90°C e o menor tempo de 30 minutos.

Received 04 November 2023; Received in revised from 16 January 2024; Accepted 28 January 2024

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n1.17403

INTRODUCTION

Plants produce their own nutrients through primary metabolism, using them for survival. In addition, plants produce compounds known as secondary metabolites, which are divided into three classes: phenolic compounds, terpenes and alkaloids. These specialized metabolites are produced in different situations, ranging their composition among different species [1-2]. Some of these compounds have been associated with various benefits to human health, such as their inclusion in herbal medicines with fewer side effects and because they have a natural composition, making the medicine less aggressive to the human body and contributing to the improvement of various pathologies[3-4].

The purpose of carrying out studies on plants is usually related to the investigation of their biological constituents and the search for efficient and safe extraction and identification methods. Plant extracts with antioxidant properties have been widely studied as an alternative to synthetic antioxidant additives, presenting phenolic compounds in their composition and low toxicity. Extracts of leaves (rosemary, oregano, basil, senna, black tea, green tea), flowers (hibiscus and chamomile), fruits (jabuticaba, plum, bacuri, araçá, blackberry, grape, pepper), tubers (ginger and saffron), spices (cloves, cinnamon) are examples of natural species with substances that have antioxidant properties [5-13].

The most common additives are synthetic antioxidants, however, most of them have low biodegradability and high toxicity. The interest in natural extracts has been growing due to their role as inhibitors of the oxidation reaction in industrialized products and biodiesel, with greater availability, biodegradability and lower toxicity. Phenolic compounds act as free radical scavengers and inhibitors, generating thermodynamically more stable products[14].

The organic compounds extraction from plants can be done by different methods, and the type of solvent, agitation, temperature and time can change the final composition of the extract. In this sense, it is essential to establish experimental conditions that allow an exclusive and efficient extraction of the compounds of interest[15]. For this, there is experimental design, group of statistical techniques, which facilitate optimization. And so, it allows precise conclusions with different variables, without repetition, facilitating the process of carrying out the analyzes, reducing the number of experiments and costs with materials and reagents[16].

The most used methods to determine the antioxidant activity and compounds “in vitro” for evaluation of antioxidant compounds in plants are: the free radical scavenging method 2,2-diphenyl-1-picrylhydrazyl (DPPH, the capture of free radical 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and the erric Reducing Antioxidant Power (FRAP)[17].

Within this context, this research performed the optimization of the extraction conditions of total phenolic compounds and the evaluation of the antioxidant activity of *Croton lechleri* sap through fractional factorial planning using the Statistica 7® software. The objective of this research was to evaluate the amount of total phenolic compounds and the antioxidant properties of the sap extracted from the stem of the Blood Dragon (*Croton lechleri)* to use the natural extract as a renewable alternative to the synthetic antioxidants present in the food and biodiesel industry.

MATERIALS AND METHODS

The research took place in Pato Branco - Paraná in partnership with the Federal Technological University of Paraná (UTFPR) *Campus* Pato Branco. *Croton lechleri* sap was collected from the stem in the Amazon Rainforest and provided by Schwaab Company - Research Industry of Commerce and Export and Import of Products from the Amazon Ltda., in Porto Velho, Rondônia - Brazil. *Croton lechleri* sap samples were lyophilized in a lyophilizer (Liotop - L1019, São Paulo, Brazil) and stored in a freezer at -12°C for further analysis.

Extraction Conditions Optimization

In order to obtain the best method for extracting total phenolic compounds present in *Croton lechleri* sap, the effect of the following experimental variables was evaluated using fractional factorial design$^{(3-1)}$ described in Table 1: solvent concentration (water, 50 % v/v ethanol:water and ethanol 99,5 %); temperature (50, 70 and 90°C) and time (30, 60, 90 min).

Croton lechleri sap extracts were prepared in Falcon tubes with 1 g of sample for each assay defined by the experimental design and 15 mL of the corresponding solvent were added, as preliminary tests demonstrated that it would be an adequate dilution for the four methodologies. After the tubes were shaken manually, the samples were heated in a water bath at the temperature and for the time described in Table 1.

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n1.17403

Table 1 - Fractional experimental design $3^{(3-1)}$ applied to optimize the extraction conditions of total phenolic compounds in *Croton lechleri* sap

Assays	Solvent[1]	Temperature[2]	Time[3]
1	-1	-1	-1
2	1	-1	0
3	0	-1	1
4	1	0	-1
5	0	0	0
6	-1	0	1
7	0	1	-1
8	-1	1	0
9	1	1	1

[1]Solvent (1= ethanol 99.5%; 0= ethanol 50%; -1= pure water);
[2]Temperature (1= 90°C; 0= 70°C; -1=50°C) and
[3]Time (1= 90 min; 0= 60 min; -1=30 min).

The extracts were filtered through filter paper and used to determine the content of total phenolic compounds and antioxidant activity. All assays were performed in triplicate.

Determination of Total Phenolic Compounds (TPC)

The content of total phenolic compounds was determined according to the Singleton *et al.* (1999) spectrophotometric method of Folin-Ciocateau[9,18].

Antioxidant activity

Due to the complexity of bioactive compounds, it is necessary to evaluate the antioxidant activity by more than one method. Thus, in this study, the antioxidant capacity of the extracts obtained was determined by scavenging the radical DPPH (2,2-diphenyl-1-picryl-hydrazyl)[17,20], ABTS (,2'-Azinobis (3-ethylbenzthiazoline- 6-sulphonic acid)[19] and the Ferric Reducing Antioxidant Power (FRAP)[17], aiming at greater accuracy data.

Statistical analysis

The Statistica 7® software was used to analyze the results obtained in the execution of the experimental design. Data were evaluated by analysis of variance (ANOVA) ($p<0.05$), Tukey's test ($p<.05$), coefficient of determination, chi-square and lack of fit. Response surface and Pareto plots were also generated. The values obtained in the assays were presented as the mean value of the triplicates with their respective standard deviation. The equation of the global response (GR) was also used, as described in equation 1, aiming at the general optimization of the response variables in a single condition, according CORNELL (1990)[21].

$$GR = \frac{R(x1)}{MR(x1)} + \frac{R(x2)}{MR(x2)} \ldots + \frac{R(xn)}{MR(xn)}$$

Where $R(x_n)$ is the response for an element in a particular experiment and $MR(x_n)$ is the largest response in the set for that element

RESULTS AND DISCUSSION

Assays such as total phenolic compounds (TPC), ABTS, DPPH, FRAP are useful to evaluate the antioxidant potential in plant extracts. Statistical tools can be used in order to observe the factors that influence the extraction such as time, temperature and solvent concentration, obtaining the best extraction conditions. Table 2 presents the treatment of these data, in which the different letters associated with the averages indicate the significant difference between the samples.

The results (Table 2) for total phenolic compounds ranged from 92.22±1.35 to 193.29±1.93 mg 100 g^{-1}. For the analysis of antioxidant activity in the ABTS method, the variance was 15.17±0.08 to 26.80±0.04 mM TE g^{-1} and in the DPPH method it was 0.275±0.002 to 0.688±0.011 mM TE g^{-1}. Using the FRAP methodology, the variation ranged from 6.73±0.02 to 19.20±0.07 mM g^{-1}. All results are acceptable.

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n1.17403

Table 2 - Fractional Factorial Design (FFD) 3^{3-1} coded assays and responses obtained for *Croton lechleri sap*

Assays	TPC (mg 100 g^{-1})	ABTS (mM TE g^{-1})	DPPH (mM TE g^{-1})	FRAP (mM g^{-1})
1	92.22±1.35 h**	15.17±0.08 g	0.275±0.002 g	6.73±0.02 g
2	152.03±1.58 e	23.83±0.04 c	0.484±0.004 c	13.70±0.07 d
3	172.90±0.90 c	23.64±0.03 d	0.561±0.005 b	15.48±0.08 c
4	143.60±0.42 f	20.66±0.05 f	0,441±0.003 e	12.74±0.02 e
5	184.98±2.80 b	26.01±0.03 b	0.677±0.007 a	18.68±0.05 b
6	108.20±1.15 g	20.56±0.02 f	0.277±0.005 g	10.16±0.04 f
7	193.29±1.93 a	26.80±0.04 a	0.688±0.011 a	19.20±0.07 a
8	141.29±0.55 f	20.60±0.02 f	0.387±0.011 f	12.83±0.07 e
9	163.37±2.03 d	23.47±0.02 e	0.467±0.002 d	13.63±0.05 d

* Tukey's test, values followed by different letters in the same column represent a significant difference between the assays ($p<0.05$) and equal letters represent that there was no significant difference between the assays ($p<0.05$). Source: The authors (2022)

Thus, it was possible to identify that the highest extraction of total phenolic compounds was obtained in test 7, under the conditions of 50 % ethanol, with a time of 30 minutes at 90 °C. The test 1, with aqueous extract, showed the lowest extraction of phenolic compounds in 30 minutes and 50 °C. This shows that the aqueous extract, in this case, is not efficient due to the marked difference in polarity.

The values for the antioxidant activity tests showed that, for the ABTS method, the best condition was also in test 7 with 50 % ethanol conditions, with a time of 30 minutes and a temperature of 90 °C. Identically to the results of total phenolic compounds, the antioxidant activity was also lower for extraction 1 with the conditions of 0 % ethanol (purified water), with 30 minutes and 50 °C.

Similarly, for the DPPH method, the best condition was for test 7 in 50 % ethanol conditions, with a time of 30 minutes and a temperature of 90 °C. However, among the other column values, it can be observed that in test 5, under the conditions of 50% ethanol, with a time of 60 minutes at a temperature of 70°C, it also showed a good influence on the extraction. There was only a small variation in the means of trial 7 (0.688±0.011) and trial 5 (0.677±0.007).

Among the evaluated solvent concentrations, 50 % ethanol is more efficient in extraction with higher temperature and time variation. As in the other assays, the lowest extraction of the DPPH method was obtained in test 1 and test 6, which used deionized water as extractor solvent. In test 1 the experimental conditions were in a time of 30 minutes and a temperature of 50 °C and in test 6, at 90 minutes and 70 °C. Results varied in trial 1 (0.275±0.002) and trial 6 (0.277±0.005), showing that water does not maximize the extraction of compounds with antioxidant activity.

The FRAP method, like all the previous methods, showed the best result in test 7, under the same conditions, with 50 % ethanol, in a time of 30 minutes at 90 °C. Test 1 also agreed with the tests in other methods of quantification of antioxidant activity, not being effective in water extraction at 50 °C for 30 minutes.

In a comparative analysis of all tested methods, it can be seen that the DPPH method showed greater sensitivity to the extracted compounds, since in this case, two assays showed higher amounts of compounds with antioxidant properties: assay 5 under 50 % ethanol conditions , with a time of 60 minutes at a temperature of 70°C and test 7, for extraction with 50 % ethanol for 30 minutes at 90 °C. Despite having a small difference in extraction efficiency, the test of means did not show a significant difference in these assays (Table 2).

A similar study carried out with *Croton lechleri* to evaluate the antioxidant activity by the DPPH method using leaves, bark and sap residues, using water and ethanol as extracting solvent, with a time of 30 and 90 minutes and a temperature of 35 and 70 °C, resulted in phenolic compounds with the best extraction with water, at 70 °C for 30 min, while for compounds with antioxidant activity the best solvent was ethanol, at 70 °C for 90 minutes of extraction[22].

In other studies[22-23] , the antioxidant activity of botanical extracts from C*roton Heliotrpiifolius Kunth* known as Velame-da-caatinga and from *Croton blanchetianus Baill*, the white quince tree, was determined using the DPPH method and both extracts were capable of scavenging the DPPH free radical. However, the *Croton blanchetianus* extract showed greater antioxidant activity against *Croton Heliotrpiifolius Kunth*, compared to the synthetic antioxidant Butylhydroxytoluene (BHT). This difference in antioxidant activity can be attributed to the concentration and amount of phenolic compounds of each plant[24].

Piovesan (2016) carried out investigations with blueberry bioactive compounds, aiming at a greater yield of antioxidant constituents using different parameters in the extractive process. The best results were obtained with the DPPH and FRAP methods, with conditions of a 60 % hydroethanolic solution,

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n1.17403

with 60 minutes at 30 °C and 80w of power, and the results obtained by the DPPH method did not vary with temperature. The best yield in the ABTS method was for the test at 60 % solvent, 120 minutes and 40 °C. In addition, the study confirmed the antioxidant capacity of blueberries for use in the preparation of other food products[25].

In another investigation of antioxidant activity carried out in onion peel with different extraction methods and conditions in relation to time, specifically 30 minutes, the DPPH method was favored[26,27]. However, in 240 minutes, the extraction was also proven effective by the ABTS method. Finally, according to the author's assessment, both methods were tested with ethanol solvent (60 % and 80 %) at a temperature of 70 °C[26]. The use of ethanol-solvent and higher temperatures, there is the best condition for extraction of antioxidant compounds, regardless of time. Based on the results achieved in the present study, it was possible to evaluate the estimate of effects and other information by ANOVA, showing how significant each dependent variant studied was or not.

For phenolic compounds (Table 3), it is observed that the ethanol concentration factor in linear and quadratic terms is more effective among the factors ($p<0.05$) and temperature and time also influenced the extraction, but in a linear, as $p<0.05$ is within the parameters, as well as the ethanol factor.

Table 3 - Linear, quadratic and interaction effects of the variables ethanol concentration (%), extraction temperature and extraction time, using fractional factorial design (FFD) 3^{3-1} on the response variables of total phenolic compound content (TPC)

TPC	SS	DF	MS	*F*	*p*
X_1L+Q	22041.76	2	11020.88	4399.258	0.000000
X_2L+Q	3551.67	2	1775.84	708.869	0.000000
X_3L+Q	328.77	2	164.39	65.619	0.000000
X_1L // X_2L	4480.13	4	1120.03	447.089	0.000000
X_1L // X_3L	3551.67	2	1775.84	708.869	0.000000
X_2L // X_3L	22041.76	2	11020.88	4399.258	0.000000
Error	45.09	18	2.51	-	-
Total SS	27065.62	26	-	-	-
Lack of fit	160.86	2	80.43	32.106	0.000001

Sum of squares (SS), degree of freedom (DF), mean square (MS), standard error, linear and quadratic (L and Q). Ethanol (X_1L and X_1L), Temperature (X_2L and X_2L) and Time (X_3L and X_3L).

Table 4 shows the results of the analysis using the ABTS methodology, showing that the ethanol concentration factor was significant together with the temperature in linear and quadratic terms ($p<0.05$). The time factor is variable, but significant with $p<0.05$.

Table 4 - Linear, quadratic and interaction effects of the variables ethanol concentration (%), extraction temperature and extraction time, using fractional factorial design (FFD) 3^{3-1} antioxidant activity by the ABTS method

ABTS	SS	DF	MS	*F*	*p*
X_1L+Q	204.2771	2	102.1386	62409.55	0.000000
X_2L+Q	33.9959	2	16.9979	10386.22	0.000000
X_3L+Q	1.5830	2	0.7915	483.64	0.000000
X_1L // X_2L	61.6503	4	15.4126	9417.53	0.000000
X_1L // X_3L	33.9959	2	16.9979	10386.22	0.000000
X_2L // X_3L	204.2771	2	102.1386	62409.55	0.000000
Error	0.0295	18	0.0016	-	-
Total SS	297.0255	26	-	-	-
Lack of fit	27.3213	2	13.6607	8347.05	0.000000

Sum of squares (SS), degree of freedom (DF), mean square (MS), standard error, linear and quadratic (L and Q). Ethanol (X_1L and X1L), Temperature (X_2L and X_2L) and Time (X_3L and X_3L).

In the DPPH analyzes as shown in Table 5, the solvent, temperature and time are significant, both as linear and quadratic, with values within the parameters ($p<0.05$).

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n1.17403

Table 5 - Linear, quadratic and interaction effects of the variables ethanol concentration (%), extraction temperature and extraction time, using fractional factorial design (FFD) 3^{3-1} antioxidant activity by the DPPH method

DPPH	SS	DF	MS	*F*	*p*
X_1L+Q	0.487062	2	0.243531	5822.870	0.000000
X_2L+Q	0.025492	2	0.012746	304.759	0.000000
X_3L+Q	0.018788	2	0.009394	224.609	0.000000
X_1L // X_2L	0.151212	4	0.037803	903.877	0.000000
X_1L // X_3L	0.025492	2	0.012746	304.759	0.000000
X_2L // X_3L	0.487062	2	0.243531	5822.870	0.000000
Error	0.000753	18	0.000042		
Total SS	0.545104	26			
Lack of fit	0.001774	2	0.000887	21.214	0.000018

Sum of squares (SS), degree of freedom (DF), mean square (MS), standard error, linear and quadratic (L and Q). Ethanol (X_1L and X1L), Temperature (X_2L and X_2L) and Time (X_3L and X_3L).

In the FRAP method (Table 6), the solvent was significant and the temperature also in quadratic and linear terms, along with time, with parameters of $p<0.05$, showing the influence of these factors on the extraction.

Table 6 - Linear, quadratic and interaction effects of the variables ethanol concentration (%), extraction temperature and extraction time, using fractional factorial design (FFD) 3^{3-1} antioxidant activity by the FRAP method

FRAP	SS	DF	MS	*F*	*p*
X_1L+Q	281.0508	2	140.5254	42064.90	0.000000
X_2L+Q	48.0258	2	24.0129	7188.03	0.000000
X_3L+Q	4.0027	2	2.0013	599.08	0.000000
X_1L // X_2L	85.2634	4	21.3159	6380.69	0.000000
X_1L // X_3L	48.0258	2	24.0129	7188.03	0.000000
X_2L // X_3L	281.0508	2	140.5254	42064.90	0.000000
Error	0.0601	18	0.0033		
Total SS	363.4856	26			
Lack of fit	8.1070	2	4.0535	1213.38	0.000000

Sum of squares (SS), degree of freedom (DF), mean square (MS), standard error, linear and quadratic (L and Q). Ethanol (X_1L and X1L), Temperature (X_2L and X_2L) and Time (X_3L and X_3L).

In general, considering all the tests, it was possible to notice that the factors solvent, temperature and time, in quadratic and linear terms in all analyzes significantly influenced the expected results for each extraction condition. It should be shown that the solvent in concentrations of 50 % ethanol is the most efficient for extraction, with a greater amount of extracted antioxidant compounds. The results are in agreement with the data obtained by Silva et al. (2021), who evaluated the phytochemical profile of ethanolic and methanolic extracts from Croton Blanchetianus, popularly known as quince tree or marmeleiro-do-mato. The extraction yield with ethanol was 3.37 %, higher than the yield of 2.43 % using methanol as extracting solvent, a fact possibly explained due to the greater similarity of polarity of the organic compounds present[27].

Also noteworthy in the same research, the assays of the ABTS method with variations of solvent x time, solvent x temperature with significant results so the temperature x time did not prove to be effective, that is, the time can still vary[22,27]. For the DPPH method, the solvent, time and temperature in quadratic terms were efficient and in linear terms they were not significant. In the FRAP test, temperature and solvent were efficient in linear terms, so the other conditions and interactions were not significant.

In this research, the response surface and contour graphs were included as a sequence of analyses, in which the influence of the independent variables solvent, temperature and time in relation to extraction is evaluated, with the dependent variables total phenolic compounds and antioxidant activity by ABTS, DPPH and FRAP methods. The response surface can be used for optimal formulations, evaluating the effects of interactions between independent variables. The response surface graphs are colored and curved, where the dark red region is more centralized and predominant near the center point, optimized with the best extractions.

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n1.17403

For total phenolic compounds, the temperature-time concentration factor, there may be a possibility of adjustment to be optimized, as it is outside the central point, so the solvent (% ethanol) exhibits an improved response along with time, closer to the central point , as shown in the graphs in Figure 1.

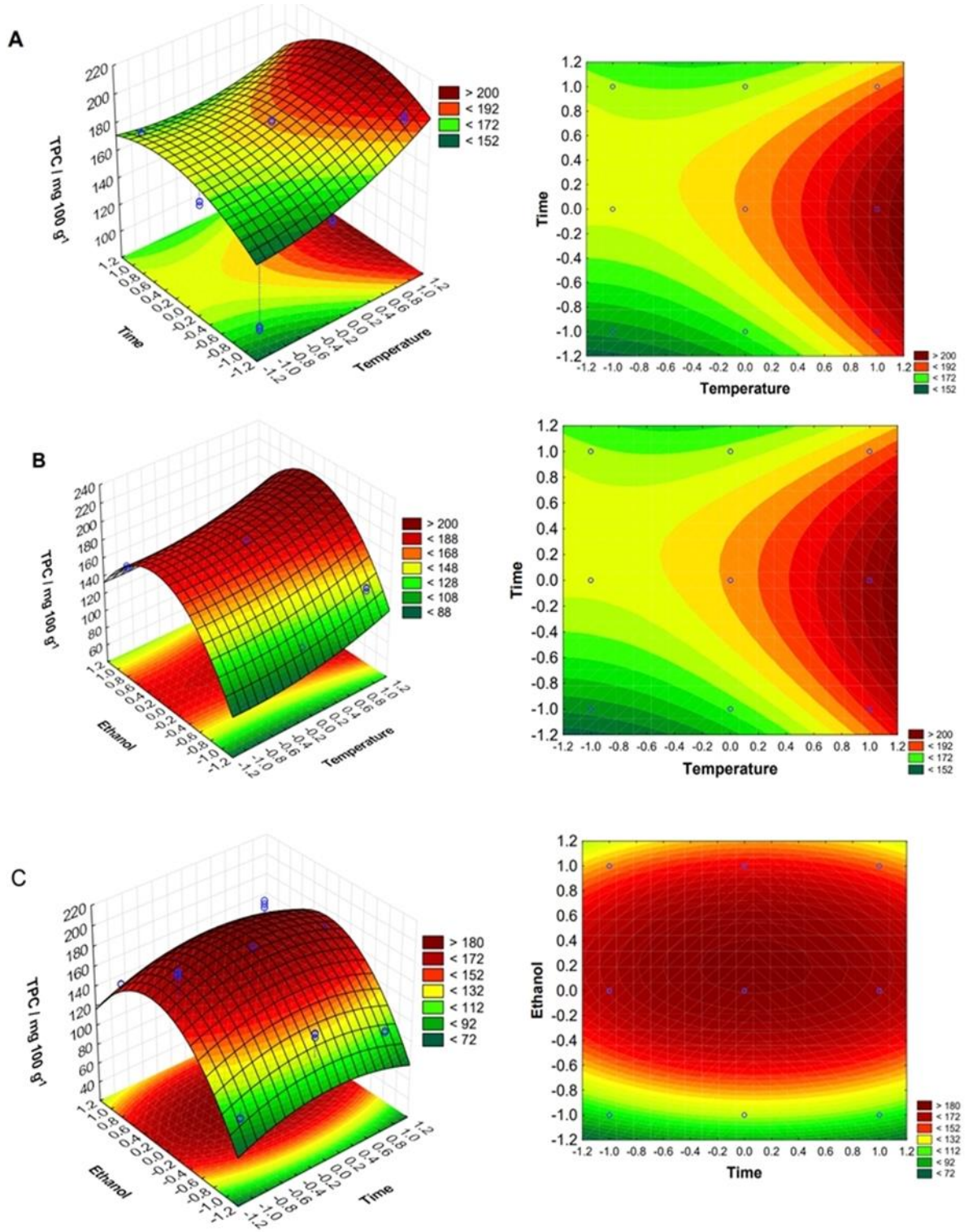

Figure 1 - Response surface (left) and contour plots (right) for total phenolic compound (TPC) extraction factors. A: Temperature x time; B: Ethanol x temperature and C: Ethanol x time

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n1.17403

In comparison with the information obtained in the experimental design and estimates of the effects described in Table 3, it is observed that the best extraction condition in the conditions of 50 % ethanol, higher temperature such as 90 °C and with a time of 30 minutes, time variable, but significant with ($p<0.05$), considering that the data are very similar to the graphs.

In the analyzes for the ABTS method (Figure 2), the solvent-time factors were more important in the optimization, therefore the temperature was less significant. On the other hand, it also had an influence on extraction. It can be said that higher temperatures significantly influence the extractions.

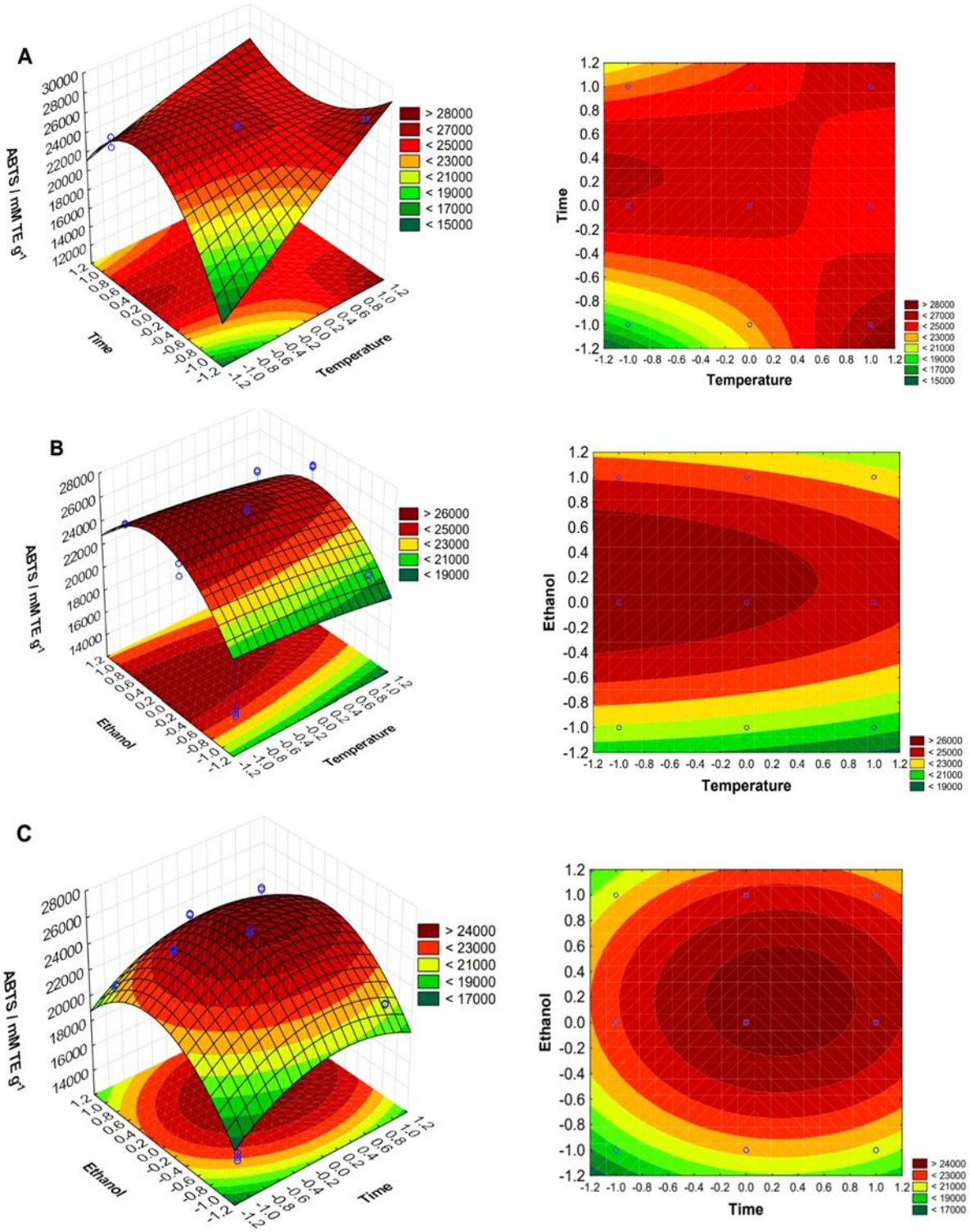

Figure 2 - Response surface (left) and contour plots (right) for extraction factors by the ABTS method. A: Temperature x time; B: Ethanol x temperature and C: Ethanol x time

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n1.17403

Figure 3 presents the results found for the DPPH method, showing the different interactions between solvent-time-temperature. The interaction between the time-temperature independent variables proved to be significant, indicating that the trend towards higher temperatures leads to better extractions, and may even alter the extraction time.

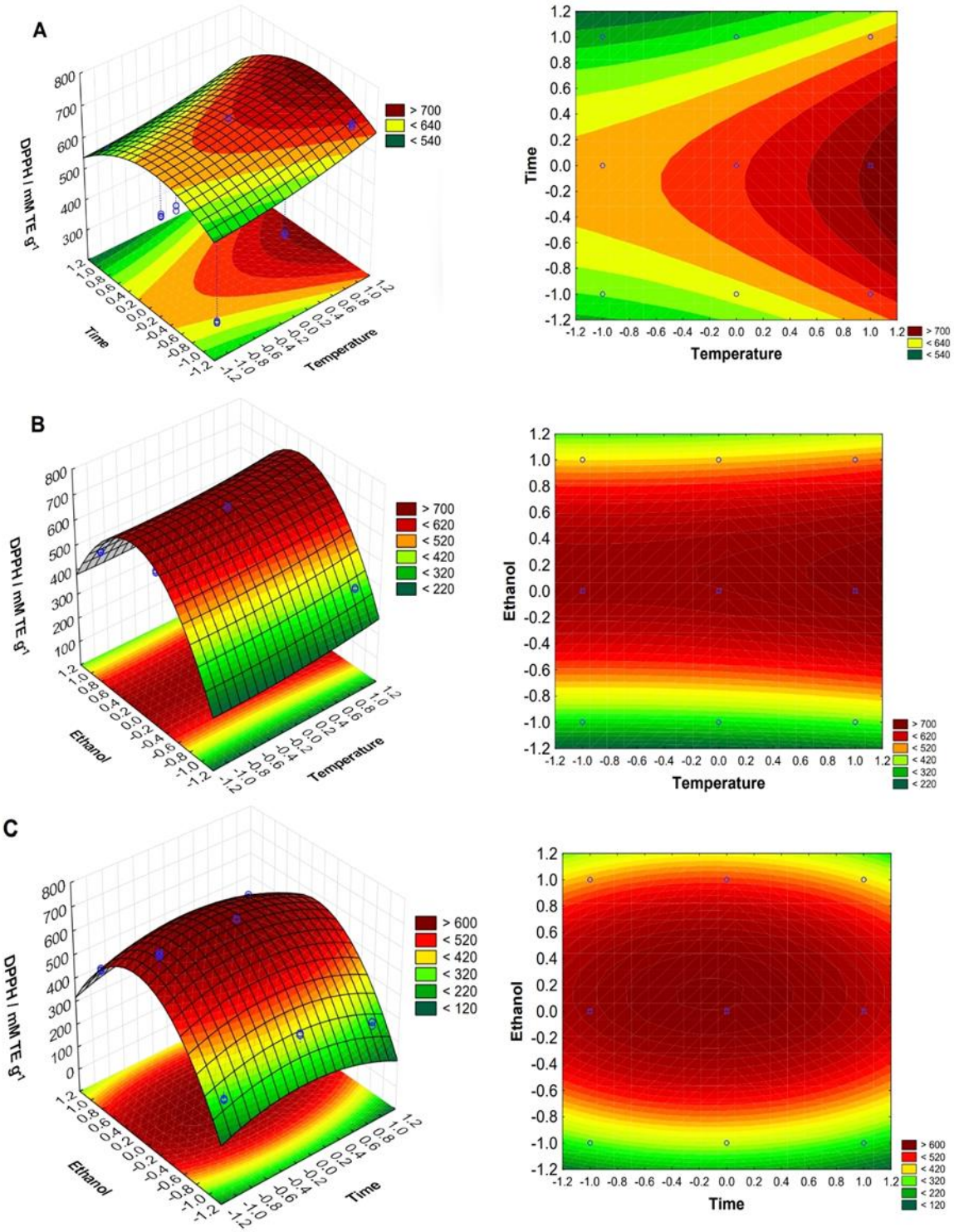

Figure 3 - Response surface (left) and contour plots (right) for extraction factors by the DPPH method. A: Temperature x time; B: Ethanol x temperature and C: Ethanol x time

For FRAP method (Figure 4), all evaluated dependents are located at the central point of the graph, that is, in this case, all of them influence the extraction in some way, compared to Table 1. It is

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n1.17403

noteworthy that at higher temperatures, results were obtained. the highest extraction in the shortest time with 50 % ethanol.

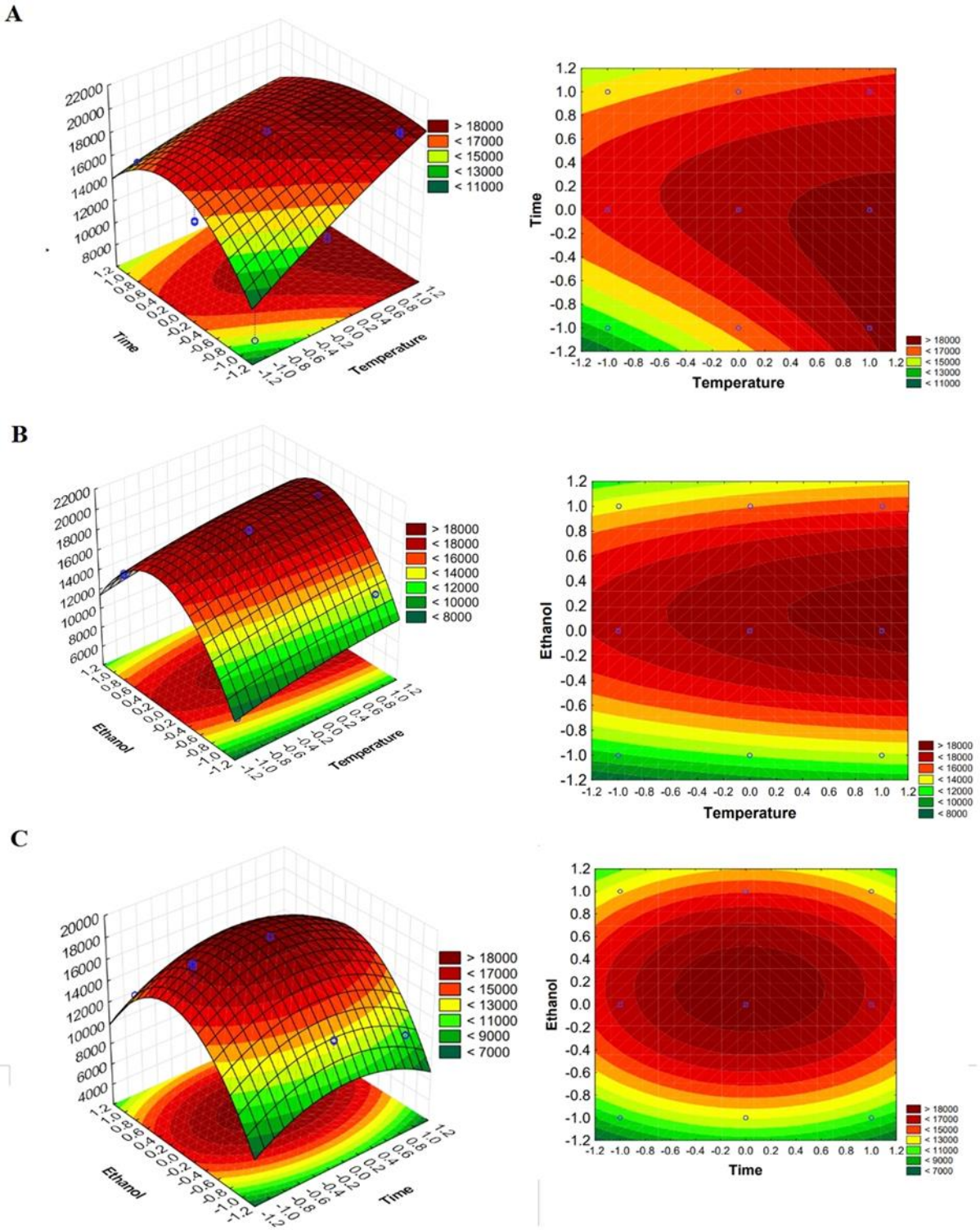

Figure 4 - Response surface (left) and contour plots (right) for extraction factors by the FRAP method. A: Temperature x time; B: Ethanol x temperature and C: Ethanol x time

The evaluation of the results by the contact surface method proved to be an important tool for data analysis, as it effectively contributed to the understanding and visualization of the results. The surface and contour graphs for the four variables also showed similar behavior to the others, where the central point region is in evidence in all analyses,

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n1.17403

indicating that the extraction optimization is using higher concentrations of ethanol, higher temperature and shorter time. of extraction.

The solvent-temperature interaction was the closest to an optimized condition, possibly being found between the solvent (50% ethanol), which had a great influence on all methods, as it was the one that showed the most significant effect through the analysis of each independent variable and their effect interactions, showing that aqueous extracts with purified water are less effective for extracting phenolic compounds and compounds with antioxidant potential[23]. Therefore, the temperature showed oscillations, as it is observed that at higher temperatures there were better results, greater extraction, as it facilitates the passage of cell walls, increasing the solubility of the components to be extracted. Despite this, evaporation of the solvent or even degradation of the compounds may occur due to oxidation. It is possible to observe that, in this study, the degradation of phenolic compounds by temperatures, which reached a maximum of 90 °C, did not occur.

In a study carried out by Fontoura (2021), on the antioxidant potential of chestnut tree peels, for total phenolic compounds, and antioxidant activity by the ABTS, DPPH and FRAP methods, using the same methodology with the independent variables of ethanol (0, 20, 50, 80 and 100 %), temperature (40, 50, 60, 70 and 80 °C) and time (30, 50, 75, 100 and 120 min), it was found that the solvent-ethanol factor had a greater influence, indicating a higher concentration in the central point between 50 and 60% being the most efficiently optimized extraction, and together with higher temperatures it proved to be more extractive, where the ideal temperature was close to 60°C, and in longer extraction times it had better optimization[21]. However, the time of 30 minutes, compared to the extraction of *Croton lechleri* which proved to be efficient with the solvent-ethanol at 50%, higher temperatures between (70 and 90 °C) with a time of close to 30 minutes had similar results. To complement the ANOVA, evaluating the results, there are Pareto charts (Fig. 5), as an aid in the visual understanding of the best extraction parameter for each method.

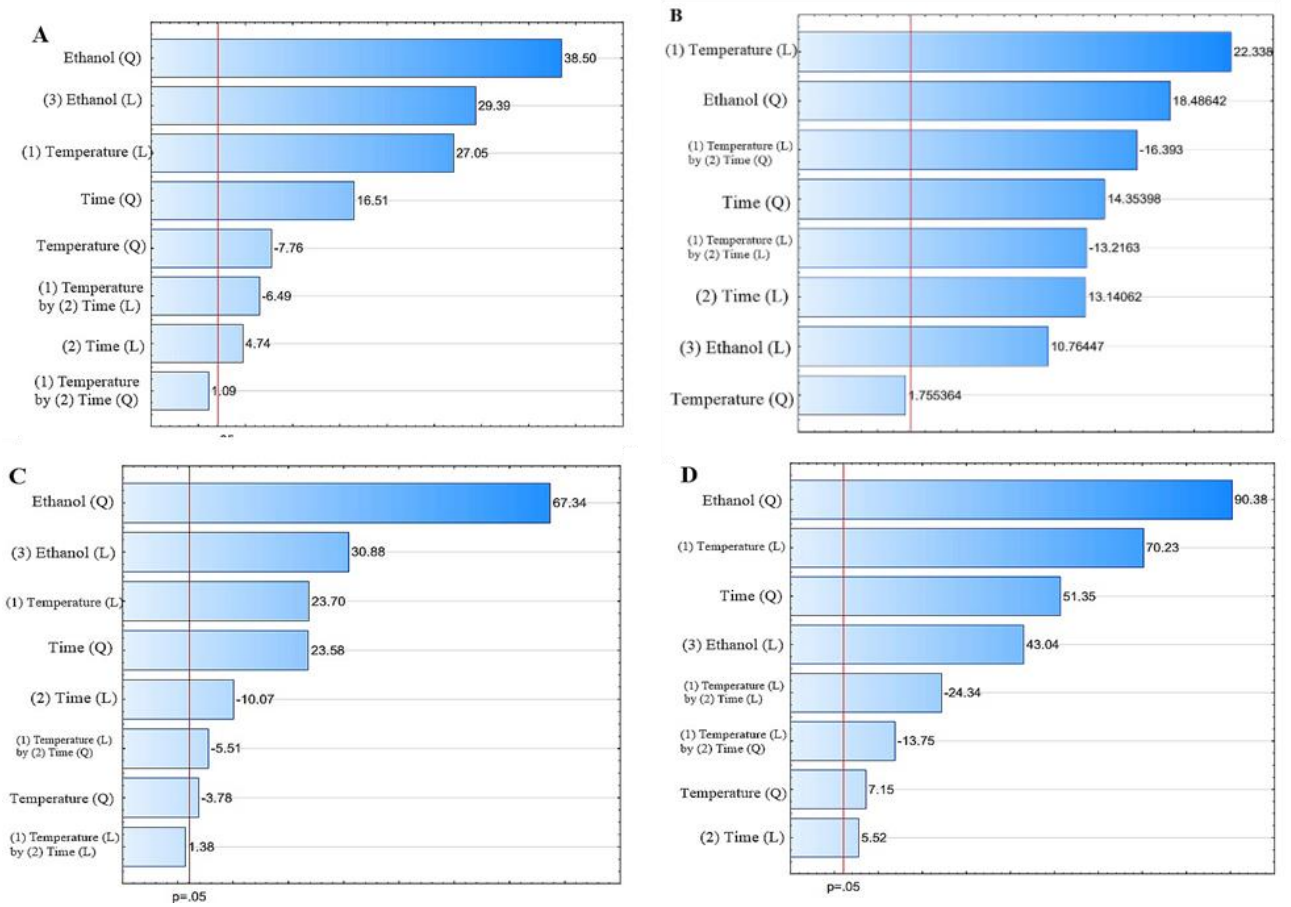

Figure 5 - Pareto charts obtained from the interactions of the significant conditions: ethanol (%), temperature (°C) and time (min) for each variable. (A) Total phenolic compounds (TPC) and Antioxidant activity by ABST (B), DPPH (C) and FRAP (D) methods by fractional factorial design (FFP) in *Croton lechleri* sap

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n1.17403

In the graph, the red dashed band is analyzed, meaning that all horizontal bars exceed the vertical red band, which has a significant effect on extraction. In the analysis, it can be said that the indexes of TPC and antioxidant activity (ABTS, DPPH and FRAP), in all the solvent (Q), had a greater influence. Subsequently, all other methods proved to be significant. That is, the Pareto chart for TPC showed significance for all factors, with the best conditions tending towards solvent-ethanol, the higher the concentration, the more significant for the extraction, so the higher the temperature, the better quadratic(Q) and the lower linear (L) and the time seems that the more time the more optimized.

For ABTS, the best conditions were with ethanol in higher concentration and higher temperature with shorter time. In DPPH and FRAP results were similar with ABTS. According to research by Fontoura (2021), the Pareto charts showed that the solvent concentration at lower levels had the most significant influence on the extraction in all tests performed, such as the time factor specifically for the extraction of phenolic compounds at lower levels, and temperatures closer to the central point, extraction can be more efficient.

The software used to carry out the STATISTICA 7 analyses, generated Table 7, that is, the regression coefficient, in which the conditions that are significant for the extraction were evaluated and a predictive equation was obtained. For calculation purposes, the partial derivative mathematical function is used, so that the variables of the equation are isolated, and thus discover the best level and real value, after being decoded by interpolation. The joint optimization, also known as global/general response, evaluates the minimum and maximum values achieved in each of the tests, and tries to find a variable or condition that is the best for everyone, either maximizing or minimizing the result obtained. All correlation coefficients of the equations (R^2) were equal 0.99 and p = 1.00 with 26 degrees of freedom.

Table 7 - Regression model equations used to determine the best extraction condition for each variable (total phenolic compounds (TPC), antioxidant methods ABTS, DPPH and FRAP) in fractional factorial design 3^{3-1} in *Croton lechleri sap*

Variables	Equations
TPC	$y = 184.98 + 20.97x_{et} - 20.97x_{et}^2 + 13.47x_T + 6.92x_T^2 + 3.27x_t - 8.80x_t^2 - 5.03x_{et}x_T$
ABTS	$y = 26.02 - 0.15x_{et} - 4.77x_{et}^2 + 1.37x_T - 0.16x_T^2 - 0.20x_t - 0.63x_t^2 - 1.12x_{et}x_T + 3.14x_{et}x_T^2$
DPPH	$y = 0.68 + 0.06x_{et} - 0.25x_{et}^2 + 0.04x_T + 0.01x_T^2 - 0.03x_t - 0.06x_t^2 + 0.03x_{et}x_T^2$
FRAP	$y = 18.68 + 1.05x_{et} - 6.16x_{et}^2 + 1.63x_T - 0.26x_T^2 - 0.24x_t - 1.08x_t^2 - 1.00x_{et}x_T + 1.01x_{et}x_T^2$

Ethanol (linear x_{et} and quadratic x_{et}^2), Temperature (linear x_T and quadratic x_T^2) and Time (linear x_t and quadratic x_t^2). Source: The authors (2022)

In order to determine a general condition, for the variables of the study carried out with the fractional factorial design, the value of corresponding to test 7 was obtained, shown in Table 1. Corresponding to the highest temperature (90°C), with the shortest time (30 min) and with 50% ethanol, which is considered the best global condition for extraction of active compounds, compared to all analyses. The results in Table 7 were evaluated using the regression coefficients and thus the regression coefficient (R2) was also generated, which tells how much the model was optimized on the standard curve, so how much (Table 7) were the results accurate with a regression coefficient in the analyzes of CPT and antioxidant activity (ABTS, DPPH and FRAP), are all significant with a coefficient of 0.99, that is 99% of variation with $p<0.05$, with a total number of experiments shown by the DF of 27 experiments. Therefore, the study model can be used for predictive purposes of extracting compounds with antioxidant properties from the studied plant.

When calculating the global response in the study, taking a general condition, the race represented with the number 7 of best extraction was in conditions with (50% ethanol, at 70°C and 30 min of extraction) and thus obtained the regression coefficients for statistical analysis. Similar results for total phenolic compounds the response occurred at 0.92% of variance by model and with ($p<0.05$). Thus, the author concludes that the result showed high efficiency and reliability for this variable, however for antioxidant activity (ABTS, DPPH and FRAP) the coefficient of determination was 0.68, 0.89 and 0.90, and o ($p<0.05$), concluding that they were significant[22].

CONCLUSIONS

The chemometric tools used in the study: the fractional experimental design, the response surface and the Pareto chart proved to be efficient in the global response of the results. Based on a set of variables, it was possible to optimize the extraction of total phenolic compounds with antioxidant potential from *Croton lechleri* sap extracts, with the best extracting condition being at a temperature of 90 °C, with the shortest extraction time evaluated

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n1.17403

(30 min) and ethanol (50% v/v) as solvent. In addition, these metrics also made it possible to reduce the number of tests, without reducing the effectiveness of the analysis, and with a reduction in costs, both for samples and for the solvents used.

Hydroalcoholic extracts from *Croton lechleri* sap have high levels of total phenolic compounds with significant antioxidant activity. In future studies, the characterization of the extracted constituents will be sought, mainly with a focus on the time of extraction and verification of the antioxidant performance in commercial products.

ACKNOWLEDGEMENTS

We thank Federal Institute of Paraná *Campus* Palmas and Federal Technological University of Paraná *Campus* Pato Branco. We thank Profa. Dra. Solange Teresinha Carpes and Profa. Dra. Ellen Cristina Perin for the samples and technical support.

REFERENCES

Alves CQ, David, JM, David, JP, Bahia, MV, Aguiar, RM. 2010. Methods for determining in vitro antioxidant activity in organic substrates. Química Nova 33 p. 2202-2210. http://dx.doi.org/10.1590/S0100-40422010001000033

Asolini FC, Tedesco AM, Carpes ST, Ferraz C, Alencar, SMD. 2006. Atividade antioxidante e antibacteriana dos compostos fenólicos dos extratos de plantas usadas como chás. Brazilian Journal of food technology 9: 206-215.

Boroski M, Aguiar AC, Boeing Jesus, Rotta EM, Wibby CL, Bonafé EG. *et al.* 2011. Enhancement of pasta antioxidant activity with oregano and carrot leaf. Food Chemistry 125:696-700. https://doi.org/10.1016/j.foodchem.2010.09.068

Brand-Williams W. *et al.* 1995. Use of a free radical method to evaluate antioxidant activity. Lwt - Food Science And Technology: LWT - Ciência e Tecnologia de Alimentos 28:25-30. http://dx.doi.org/10.1016/s0023-64389580008-5.

Carciochi RA, Manrique GD, Dimitrov K. 2015. Optimization of antioxidant phenolic compounds extraction from quinoa Chenopodium quinoa seeds. Journal of food science and technology 52: 4396-4404. https://doi.org/10.1007/s13197-014-1514-4

Chendynski LT, Cordeiro T, Messias GB, Mantovani ACG, Spacino KR, Zeraik ML, Borsato D. 2020. Evaluation and application of extracts of rosemary leaves, araçá pulp and peel of bacuri in the inhibition of the oxidation reaction of biodiesel. Fuel 261: 116379. https://doi.org/10.1016/j.fuel.2019.116379

Clemente MAJ, Marcheafave GG, Silva HHP, Branco IG, Canesin EA, Mantovani ACG, Borsato D. 2023. Addition of jabuticaba peel extract with antioxidant properties in biodiesel. Fuel Processing Technology 243:107678 https://doi.org/10.1016/j.fuproc.2023.107678

CORNELL, J. A. Experiments with mixtures - designs, models and the analysis of mixture data. 2. ed. New York: John Wiley and Sons, 1990. 632p

Correia IAS, Borsato D., Savada FY, Pauli ED, Mantovani ACG, Cremasco H, Chendynski LT. 2020. Inhibition of the biodiesel oxidation by alcoholic extracts of green and black tea leaves and plum pulp: Application of the simplex-centroid design. Renewable Energy 160: 288-296. https://doi.org/10.1016/j.renene.2020.06.118

Del Ré PV, Jorge, N. 2012. Spices as natural antioxidants: their application in food and implication for health. Revista Brasileira de Plantas Medicinais 14: 389 - 399. https://doi.org/10.1590/S1516-05722012000200021

Diedrich C et al. 2021. Bioactive compounds extraction of *Croton lechleri* barks from Amazon forest using chemometrics tools. Journal of King Saud University-Science 33: 101416 https://doi.org/10.1016/j.jksus.2021.101416

Fontoura BH. Determinação rápida e de baixo custo do potencial antioxidante em cascas de castanheira utilizando imagens digitais e modelo de regressão multivariada: determinação rápida e de baixo custo do potencial antioxidante em cascas de castanheira utilizando imagens digitais e modelo de regressão multivariada . Pato Branco: Departamento de Química, Universidade Tecnológica Federal do Paraná; 2021. 76 p.

Hussein RA, El-Anssary AA. 2019. Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. Herbal medicine 1: 3. http://dx.doi.org/10.5772/intechopen.76139

Justo OR, Moraes AM, Barreto GPM, Mercadante AZ, Vieira e Rosa PT. 2008. Evaluation of the antioxidant potential of plant extracts obtained by supercritical fluid extraction. Química Nova 31: 1699-1705. https://doi.org/10.1590/S0100-40422008000700019

Malaquias AV. *et al.* 2017. A utilização de plantas medicinais e fitoterápicos em prol da saúde. Única cadernos acadêmicos 3: 1.

Miranda KF, Neto MA, Sousa RP, Coelho RF. Manejo Florestal Sustentável em Áreas Protegidas de uso comunitário na Amazônia. Revista Sociedade & Natureza, v. 32| p.778-792, 2020. http://dx.doi.org/10.14393/SN-v32-2020-51621

Perin, EC. et al. 2020. RGB pattern of images allows rapid and efficient prediction of antioxidant potential in calycophyllum spruceanum barks. Arabian Journal of Chemistry 13: 7104-7114. https://doi.org/10.1016/j.arabjc.2020.07.015

Piovesan N. Influência de diferentes parâmetros em métodos de extração de compostos bioativos de mirtilo *Vaccinium ashei Reade* e atividade antioxidante e antimicrobiana. Ano de obtenção: 2016. 120 p. Tese Doutorado em Ciência e Tecnologia de Alimentos - Universidade Federal de Santa Maria, Santa Maria.

Prado ACC, Rangel EB, Souza HC, Messias MCTB. Etnobotânica como subsídio à gestão socioambiental de uma unidade de conservação de uso sustentável. Rodriguésia, v. 70, e02032017, p. 1-10, 2019. http://dx.doi.org/10.1590/2175-7860201970019

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n1.17403

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26:1231-1237. https://doi.org/10.1016/S0891-58499800315-3

Rodrigues OG, Angélico CE, Costa MGJ, Lucena MFA, Neto QV, Silva WW. 2016. Avaliação da atividade antioxidante dos extratos botânicos de *Croton Heliotrpiifolius Kunth*. e *Croton blanchetianus Baill*: Resultados Preliminares. Agropecuária Científica no Semiárido. 12:237-241. http://dx.doi.org/10.30969/acsa.v12i3.700

Romagnoli ES, Borsato D, Silva LRC, Chendynski LT, Angilelli KG, Canesin EA. 2018. Kinetic parameters of the oxidation reaction of commercial biodiesel with natural antioxidant additives. Industrial Crops and Products 125: 59-64. https://doi.org/10.1016/j.indcrop.2018.08.077

Shakya KA. 2016. Medicinal plants: Future source of new drugs. International Journalof Herbal Medicine 4:59-64.

Silva AB, Wiest JM, Carvalho HH. 2016. Chemicals and antioxidant activity analisys in *Hibiscus rosa-sinensis* L. mimo-de-venus and *Hibiscus syriacus* L. hibiscus-the-syrian. Brazilian Journal Food Technology 19: 2015074 https://doi.org/10.1590/1981-6723.7415

Silva AIB, Filho-Sa GF, Oliveira LC, Guzen P, Cavalcanti JRLP, Cavalcante SJ. 2021. Phytochemical profile of ethanolic and methanolic extracts from Croaton Blanchetianus. Brazilian Multidisciplinary Journal 24:1. https://doi.org/10.25061/2527-2675/ReBraM/2021.v24i1.1057

Singleton V, Libbey LM, Walradt JP. 1999. Analisis of Total Phenols and Other Oxidation Substrates and Anitoxidants by Means Of Folin-Ciocalteu Reagent. Lipids 3: 561. https://doi.org/10.1016/S0076-68799999017-1

Spacino KR, Borsato D, Buosi GM, Chendynski LT. 2015. Determination of kinetic and thermodynamic parameters of the B100 biodiesel oxidation process in mixtures with natural antioxidants. Fuel Processing Technology 137:366 - 370. https://doi.org/10.1016/j.fuproc.2015.05.006

Teles ARS et al. 2020. Optimization of ultrasover assisted extraction of the antioxidant compounds of the moringa leaf moringa oleifera lam using response surface methodology . Brazilian Journal of Development 6: 103983-103994. https://doi.org/10.34117/bjdv6n12-773

Twaij BM, Hasan MN. 2022. Bioactive secondary metabolites from plant sources: Types, synthesis, and their therapeutic uses. International Journal of Plant Biology 13: 4-14. https://doi.org/10.3390/ijpb13010003

Van den Berg R HG, Van den Berg H, Vander VW, Bast A. 2000. The predictive value of the antioxidant capacity of structurally related flavonoids using the trolox equivalent antioxidant capacity TEAC assay. Food Chemistry 703:391-395. https://doi.org/10.1016/S0308-81460000092-3

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n1.17403