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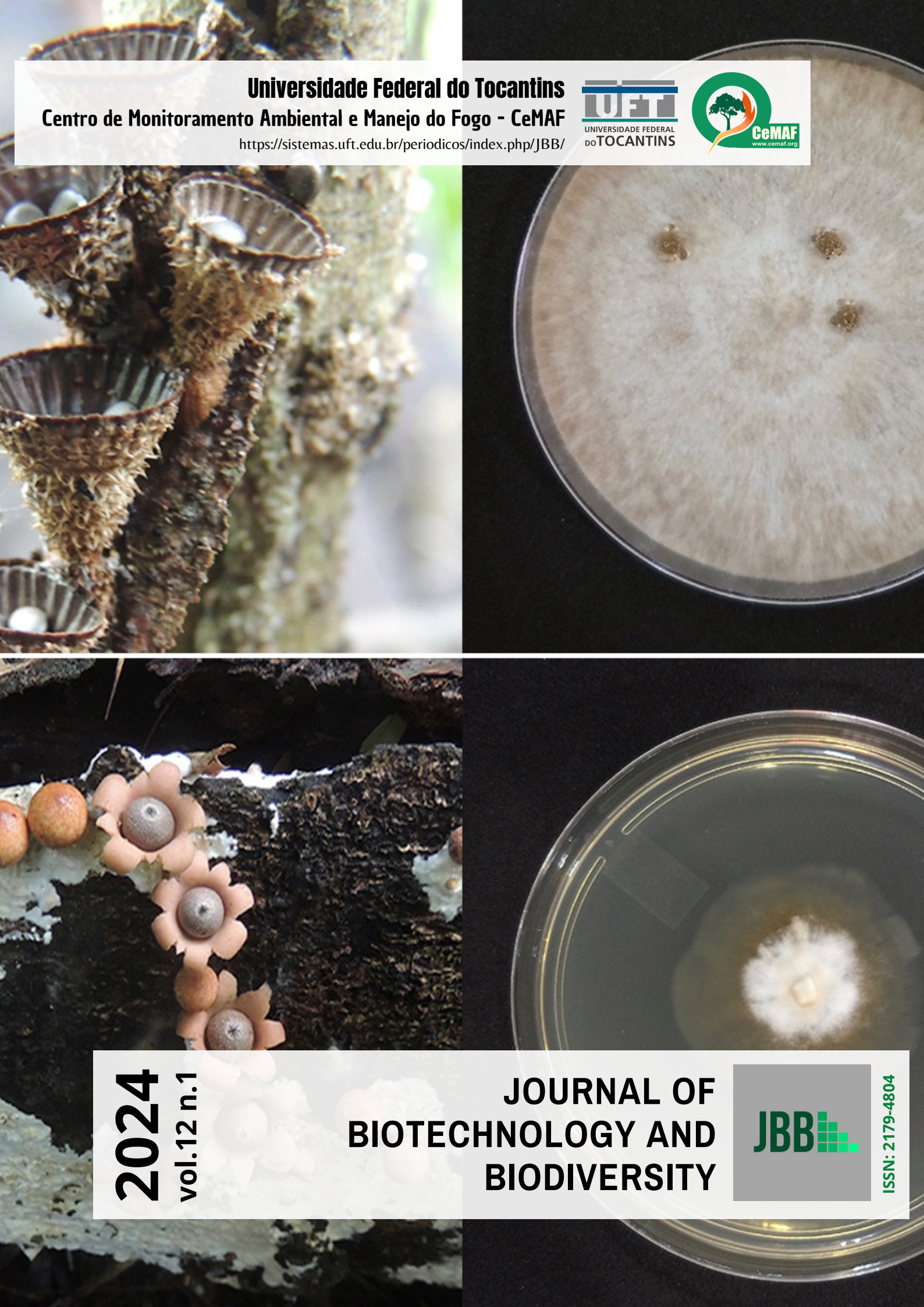

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Antioxidant potential of the sap of the *Croton lechleri* Müll. Arg. stem (Euphorbiaceae)

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

Keywords
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.

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

RESUMO

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.

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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.

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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'-Azino-bis (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.

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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,

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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 ($p<0.05$).

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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.

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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.

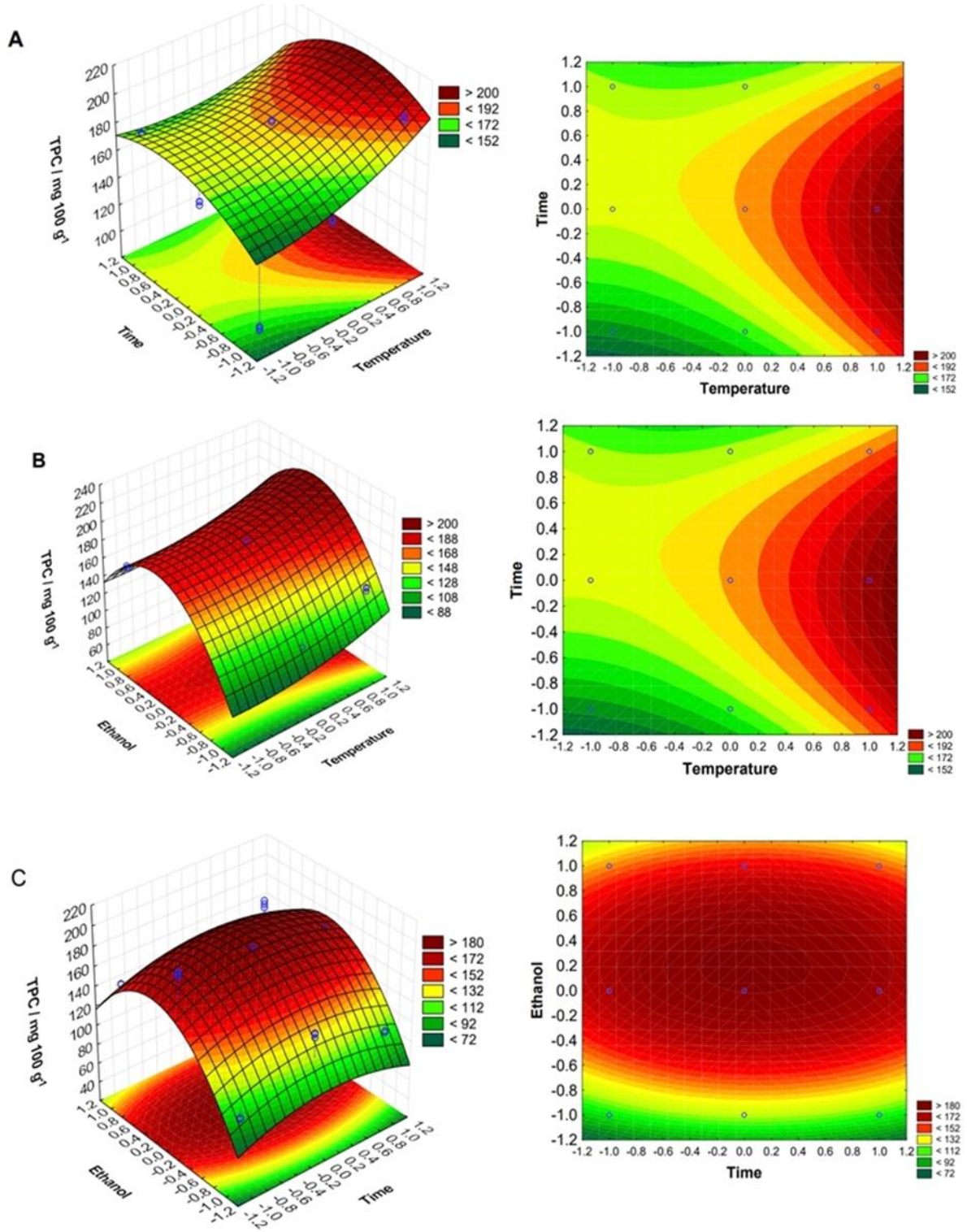

Figure 1 - Response surface (left) and contour plots (right) for total phenolic compound (TPC) extraction factors. A: Temperature x time; B: Ethanol x temperature and C: Ethanol x time

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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.

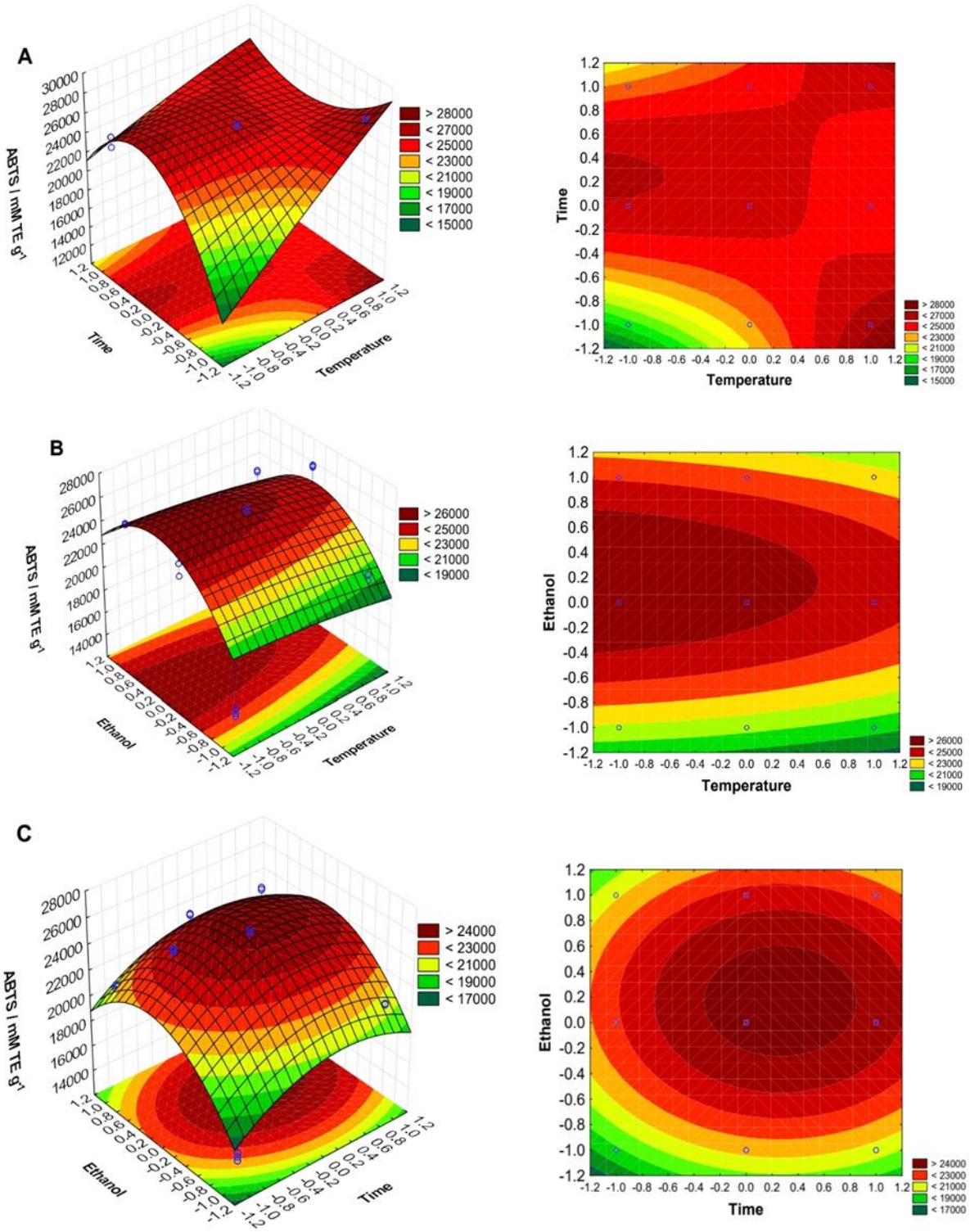

Figure 2 - Response surface (left) and contour plots (right) for extraction factors by the ABTS method. A: Temperature x time; B: Ethanol x temperature and C: Ethanol x time

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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.

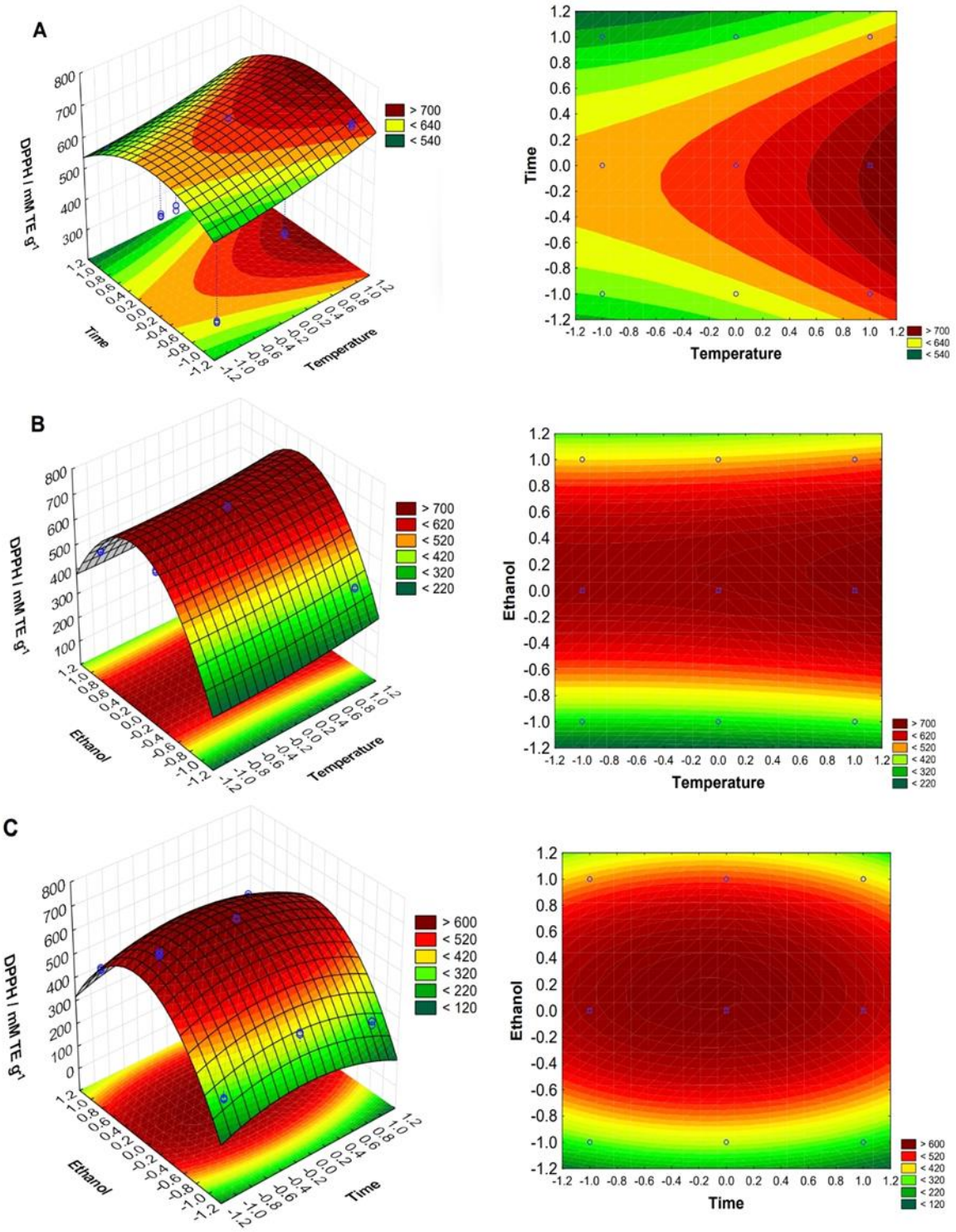

Figure 3 - Response surface (left) and contour plots (right) for extraction factors by the DPPH method. A: Temperature x time; B: Ethanol x temperature and C: Ethanol x time

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

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

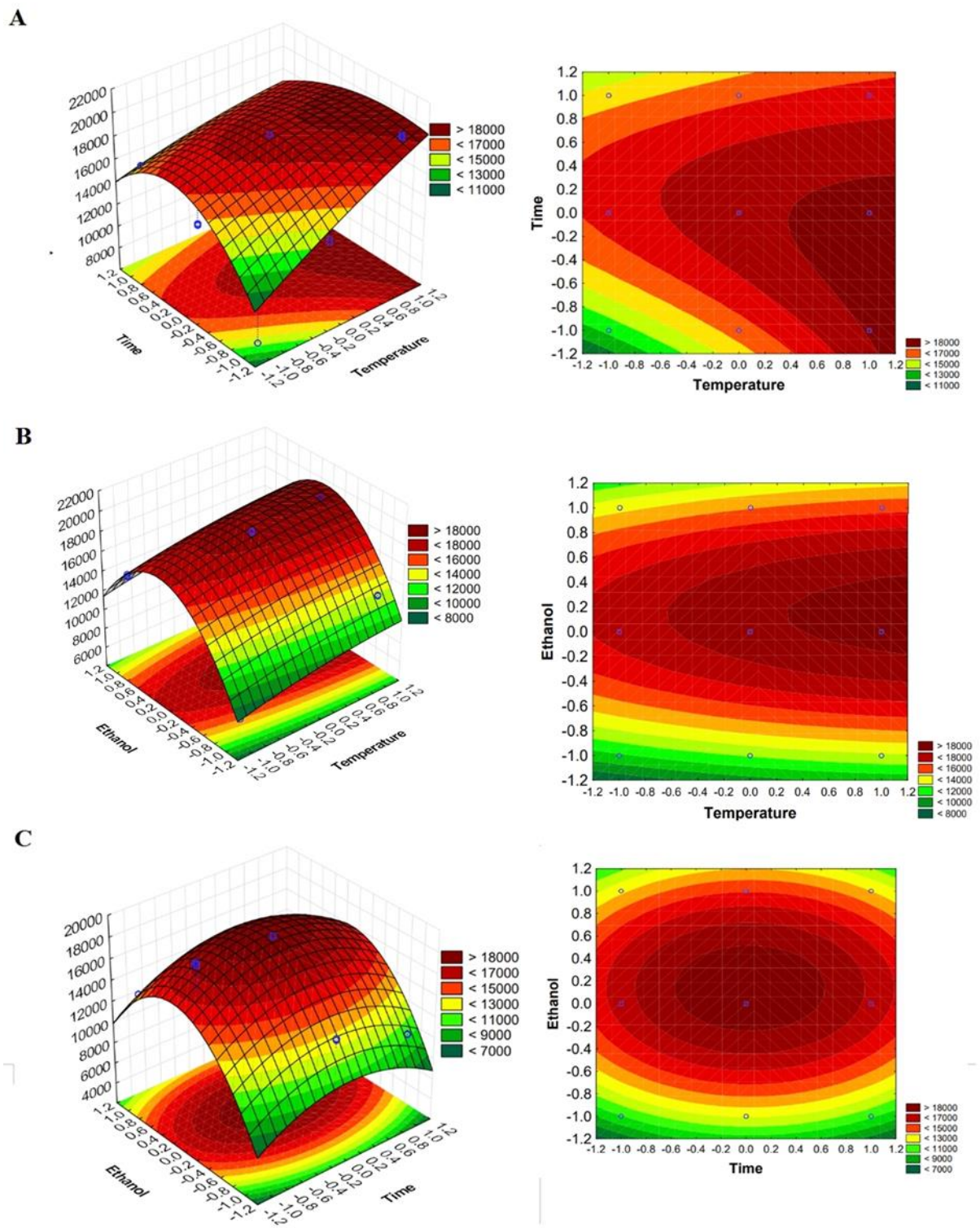

Figure 4 - Response surface (left) and contour plots (right) for extraction factors by the FRAP method. A: Temperature x time; B: Ethanol x temperature and C: Ethanol x time

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,

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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.

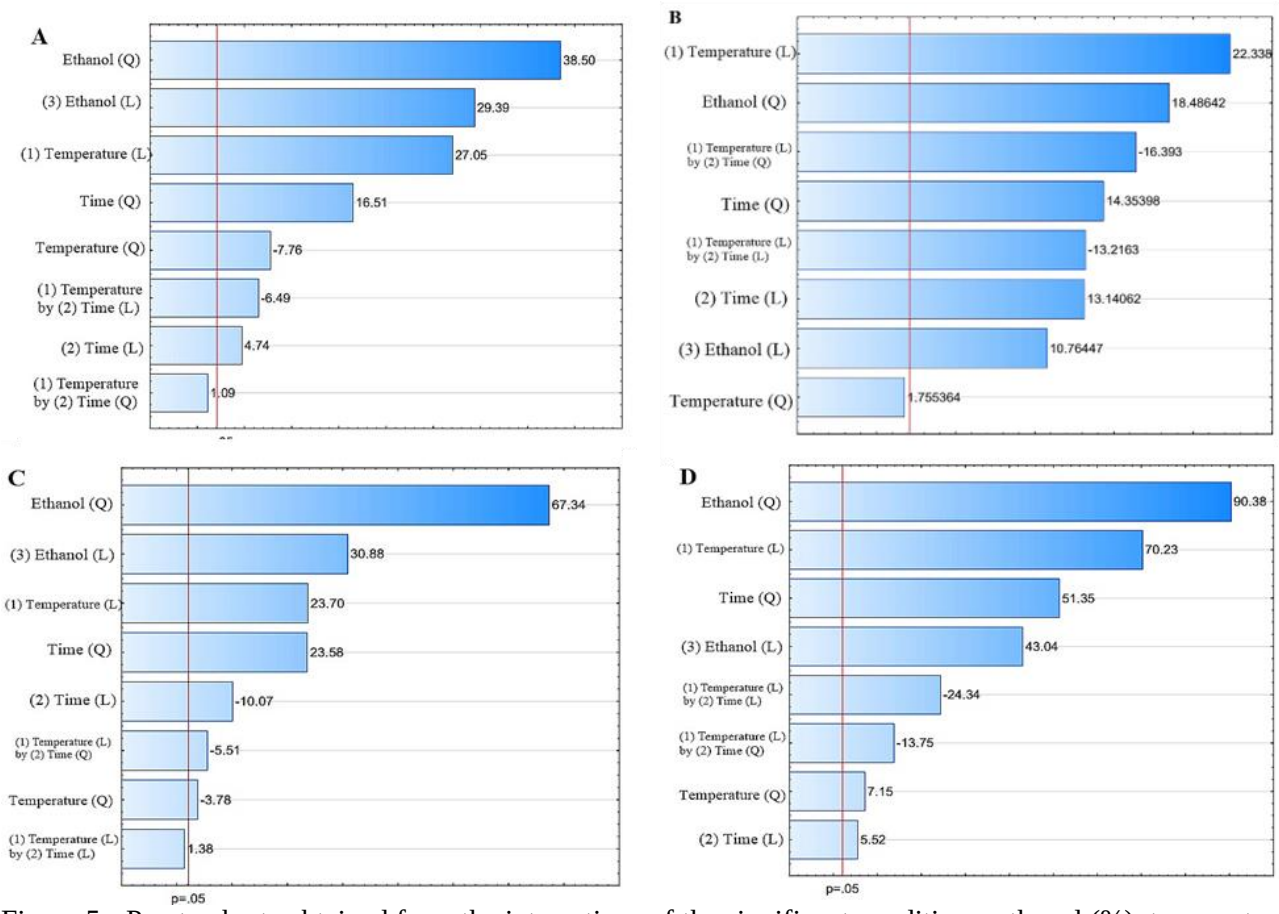

Figure 5 - Pareto charts obtained from the interactions of the significant conditions: ethanol (%), temperature (°C) and time (min) for each variable. (A) Total phenolic compounds (TPC) and Antioxidant activity by ABST (B), DPPH (C) and FRAP (D) methods by fractional factorial design (FFP) in *Croton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

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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

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(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.

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Estratégias de conservação e uso sustentável de áreas protegidas na região Amazônica: uma revisão sistemática

Carolina Wagner [a], Renato Abreu Lima [a*]

[a] Universidade Federal do Amazonas, Brasil
[*] Autor correspondente (renatoal@ufam.edu.br)

INFO

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ABSTRACT

Conservation and sustainable use strategies for protected areas in the amazon region: a systematic review
In the Amazon region, there is great degradation of protected areas through forest fires, deforestation, logging and mineral exploitation. It is observed that these areas are not being incorporated and little is being discussed to seek alternatives to these environmental changes. Thus, the objective of this present study was to discuss strategies for the conservation and sustainable use of Conservation Unit areas in the Amazon region. This is a systematic review article on dissertations, articles, books and official websites, available in the databases: Scielo, Web of Science and Google Scholar with an emphasis on presenting the proposed problem through 34 works, distributed between 2000 and 2023. As a result, the importance of generating innovations was highlighted to meet the demand for new practices and technologies, as well as to manage these natural resources without leading to their depletion. Therefore, it becomes necessary to seek new interactions and combinations to strengthen the knowledge network, highlighting the value of biodiversity, in order to contribute to better management planning, uses and enrichment of the Amazon region.

RESUMO

Palavras-chaves
biodiversidade
políticas públicas
unidades de conservação

Na região Amazônica ocorre uma grande degradação das áreas protegidas por meio dos incêndios florestais, desmatamento, exploração madeireira e de minérios. Observa-se que estas áreas não estão sendo incorporadas e pouco está sendo discutido para buscar alternativas para essas alterações do meio ambiente. Assim, o objetivo deste presente estudo foi discutir estratégias para a conservação e o uso sustentável das áreas de Unidades de Conservação na região Amazônica. Tratando-se de um artigo de revisão sistemática em dissertações, artigos, livros e sites oficiais, disponíveis nas bases de dados: Scielo, Web of Science e Google Acadêmico com ênfase em apresentar a problemática proposta por meio de 34 trabalhos, distribuidos entre 2000 a 2023. Como resultado, destacou-se a importância da geração de inovações para atender à demanda por novas práticas e tecnologias, bem como para realizar a gestão desses recursos naturais sem levar ao seu esgotamento. Dessa forma, torna-se necessário buscar novas interações e combinações para fortalecer a rede de conhecimento, evidenciando o valor da biodiversidade, a fim de contribuir para um melhor planejamento de manejo, usos e enriquecimento da região Amazônica.

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INTRODUÇÃO

A região Amazônica apresenta alta diversidade de ecossistemas, bem como um grande número de espécies endêmicas. É importante avaliar a fragilidade da rede de relações entre as espécies e o ambiente, que está presente nos diversos tipos de vegetação e habitats. Seu equilíbrio é essencial para a manutenção do clima, da qualidade da água, do solo, da reciclagem de nutrientes e de outros serviços ambientais. Sem esses recursos, tornam-se evidentes as ameaças a que estão submetidas (Veríssimo et al., 2011).

Os principais tipos de áreas protegidas presentes na região Amazônica são: unidades de conservação de uso sustentável e integral, tendo reservas extrativistas (RESEX), Floresta Estadual (FLOREST), Parques Estaduais (PAREST) e Nacionais (PARNA); Reserva Particular do Patrimônio Natural (RPPN); Área de Proteção Ambiental (APA); Reserva de Desenvolvimento Sustentável (RDS); Estação Ecológica (ESEC); Reserva Biológica (REBIO); Área de relevante interesse ecológico (ARIE); Refúgio de vida silvestre (REVIS) e Monumento Natural (MONA) (Brasil, 2022).

Essas áreas representam uma ferramenta altamente eficaz para preservar a integridade dos ecossistemas e os serviços ambientais que deles dependem. Esses serviços estão associados com a preservação do solo, das bacias hidrográficas, da polinização, da reciclagem de nutrientes e do equilíbrio climático. Além disso, a criação e implementação de AP também contribuem para garantir que as populações tradicionais e povos indígenas continuem nestes locais (Veríssimo et al., 2011).

Essas áreas têm potencializado e acelerado a reconstituição do ambiente. Atualmente existem várias críticas destinadas à política de criação de Unidades de Conservação no Amazonas, entretanto, os avanços têm sido historicamente significativos e essas atividades têm efeito positivo com relação a preservação e conservação da biodiversidade, além de fortalecer as organizações sociais dos povos tradicionais, e a contenção do desflorestamento (Fraxe et al., 2017).

A distribuição da população dentro e no entorno das Unidades de Conservação da região Amazônica desempenha um papel crucial na preservação dos objetivos originais dessas unidades e na implementação de práticas de manejo apropriadas. Em muitos casos, a delimitação dessas áreas ocorre sem um entendimento detalhado do volume, da distribuição e das características da população que vive ou circunda esses espaços, conforme apontado por Bueno e Dagnino (2011). Essa falta de conhecimento pode prejudicar a eficácia das medidas de conservação, destacando a importância de uma abordagem mais cuidadosa na definição das fronteiras das UCs e no desenvolvimento de estratégias de gestão.

As críticas se baseiam em vários motivos, refletindo desafios e complexidades associadas à implementação dessas políticas de conservação. A criação de UCs muitas vezes gera conflitos sociais, especialmente quando áreas tradicionalmente utilizadas por comunidades locais são convertidas em áreas protegidas. Esses conflitos podem surgir devido à restrição no acesso aos recursos naturais, como a coleta de madeira, pesca e agricultura de subsistência e com o deslocamento de comunidades. Outros motivos que podem ser exemplificados estão relacionados com a falta de participação e consulta, fragilidade institucional, pressões externas e desmatamento irregular, e falta de incentivos econômicos sustentáveis.

Ao considerar os desafios enfrentados na preservação da região Amazônica e a implementação de Áreas Protegidas (AP), é fundamental reconhecer que, apesar dos esforços empreendidos, a região ainda enfrenta significativa degradação, de acordo com (Maciel, Leite e Colares et al., 2019) esses fenômenos estão associados ao manejo inadequado dos solos. Essa degradação ocorre tanto por incêndios florestais, quanto pelo desmatamento, exploração madeireira, de minérios, cultivo de soja, algodão, gado, expansão humana e a ocupação irregular de terras (Ferreira et al., 2005). Por meio do desflorestamento ocorre a perda de biodiversidade e esta é totalmente irreversível (Vieira, Silva e Toledo, 2005).

O desmatamento surge com a abertura oficial ou clandestina de estradas que permitem a expansão humana e a ocupação irregular de terras à exploração predatória de madeiras nobres (Ferreira et al., 2005). Tendo uma relação direta entre a economia, com o mercado de exportação e o avanço da fronteira na região Amazônica.

Diante das preocupantes taxas de desmatamento e da crescente apreensão em escala global acerca do impacto da remoção da cobertura florestal nas emissões de gases de efeito estufa, tem-se o início de um processo de formulação de políticas governamentais. Essas políticas estão direcionadas ao controle, fiscalização e combate do desmatamento, refletindo a urgência em abordar e mitigar os efeitos negativos associados à perda de vegetação (Maurano, Escada e Renno, 2019).

A humanidade chegou a um ponto de saturação em relação aos recursos disponíveis tanto localmente quanto globalmente, e o modelo de desenvolvimento a ser adotado para as futuras gerações depende, em grande parte, dos serviços ecossistêmicos prestados pelo sistema natural, tais como o clima, o solo e a ecologia. Pode-se enfatizar que a conservação e a preservação da diversidade bioló-

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gica são fundamentais para assegurar a sobrevivência do planeta como um todo (Silva et al., 2017).

Assim, atualmente as pesquisas estão visando a recuperação de áreas degradadas que tenham o foco principal na restauração da funcionalidade ambiental através da seleção e introdução de leguminosas arbóreas e arbustivas que podem crescer em condições adversas. O sucesso dessa abordagem depende da relação simbiótica entre as plantas, rizóbios e fungos micorrízicos, permitindo um crescimento rápido das espécies, independentemente da disponibilidade de nitrogênio no solo. Isso resulta em um aumento da quantidade de matéria orgânica disponível e da atividade biológica do solo através do acúmulo de material vegetal via serrapilheira (Embrapa, 2023).

Apesar das características ambientais adversas que compõem o ecossistema amazônico, a ocupação desta vasta região revela um potencial imensurável em termos de recursos naturais, como madeira de valor comercial, recursos medicinais e uma biodiversidade única. Essa riqueza coloca a Amazônia como um território estratégico, contudo, exige uma abordagem equilibrada para garantir a sustentabilidade. Notavelmente, ao se considerar o uso sustentável desses recursos, a região se alinha com o 12º Objetivo do Desenvolvimento Sustentável (ODS), que busca estabelecer padrões sustentáveis de produção e consumo. Esse ODS visa a eficiência no uso dos recursos naturais e a redução dos impactos ambientais e sociais ao longo de toda a cadeia produtiva. Diante desse contexto desafiador, o presente estudo tem como objetivo propor estratégias para a conservação e o uso sustentável das áreas de Unidades de Conservação na região Amazônica.

MATERIAL E MÉTODOS

O presente estudo consiste em uma revisão de literatura sistemática, relacionando à temática proposta com conteúdo pertinente ao objetivo desta pesquisa, utilizando levantamento de trabalhos no sentido de analisar as principais estratégias utilizadas para a conservação e uso sustentável das áreas protegidas que são encontradas na região Amazônica.

A metodologia do projeto contempla a pesquisa qualitativa e pesquisa sistemática. A pesquisa qualitativa estuda situações complexas ao invés de explicá-las, apresentando teorias e é uma pesquisa descritiva e empírica, tem a pretensão de ajudar na elucidação da compreensão sobre o mundo, sendo uma ferramenta epistemológica (Volpato, 2019) e a revisão sistemática pode ajudar a sintetizar a evidência disponível na literatura (Sampaio e Mancini, 2007).

Utilizou-se análise de conteúdo para obter as referências necessárias por meio da leitura de resumos, palavras-chave, descritores e índices, com o propósito de buscar informações de uma forma variável e de fácil acesso ao tema buscado, obtendo assim, a representação condensada da informação (Bardin, 2016).

Além disso, a análise de conteúdo pode seguir algumas etapas, sendo elas: pré-análise, exploração do material, tratamento dos resultados obtidos e interpretação (Deslandes et al., 2002). Para a revisão bibliográfica, a coleta de dados foi realizada em revistas indexadas de forma aleatória nos bancos de dados da Scielo, Web of science e Google Acadêmico. Tendo os seguintes termos descritores: estratégias de conservação, uso sustentável de áreas protegidas, áreas protegidas na Amazônia.

Assim, os critérios de inclusão foram: estudos que avaliam ou relatam estratégias de conservação e uso sustentável em áreas protegidas na região amazônica e trabalhos que apresentam alternativas para essa temática. Os critérios de exclusão estavam relacionados com estudos que não apresentam dados ou informações relevantes para a revisão e estudos que não foram publicados em periódicos científicos revisados por pares.

Dessa maneira, com a delimitação do tema foi possível acessar 34 trabalhos que estavam distribuídos em artigos, livros, sites oficiais e dissertações apresentados em uma linha de tempo entre 2000 e 2023 e que se enquadravam com os critérios estabelecidos, no entanto, deu-se ênfase a alguns trabalhos que evidenciavam as principais estratégias para combater a degradação da região Amazônica.

RESULTADOS E DISCUSSÃO

A região Amazônica abriga o maior bioma do Brasil e uma das maiores florestas tropicais do mundo (Figura 1). Desempenha um papel crucial na manutenção da biodiversidade global, no ciclo da água e na regulação do clima. Até o presente momento, na região Amazônica no território brasileiro existe aproximadamente 373 Unidades de Conservação, totalizando 1.219.965,03 km^2, o que corresponde a 28,94% desse território em relação aos outros biomas brasileiros (Brasil, 2022).

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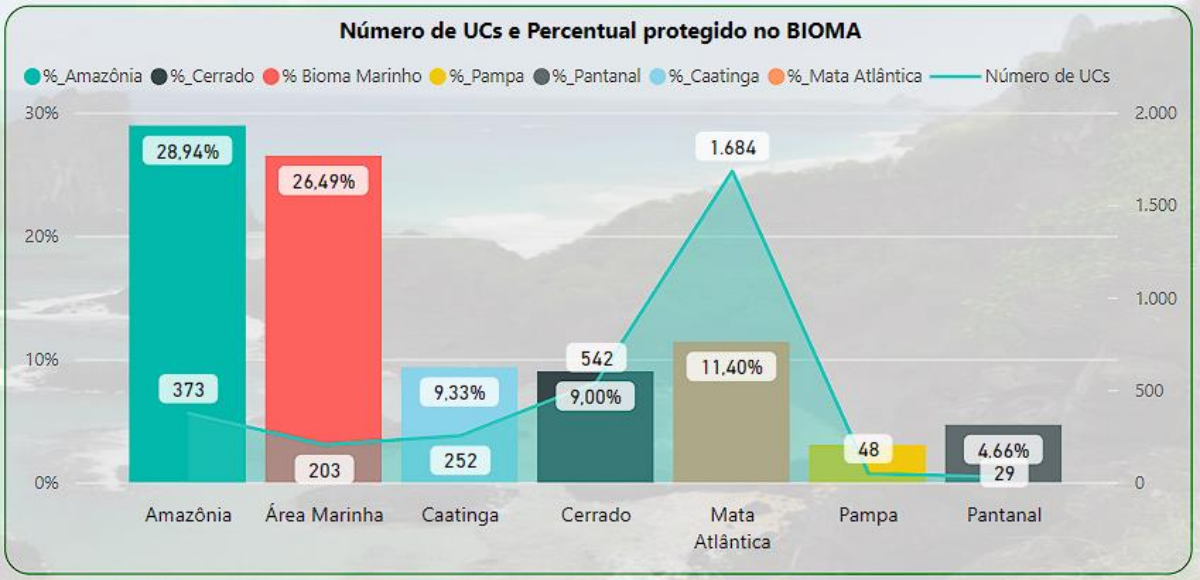

Fonte: Brasil (2022)

Figura 1 - Número de UCs e Percentual protegido no Bioma. Enfatizando a região Amazônica que possui a maior quantidade de áreas protegidas

A realização do trabalho permite a compreensão das principais estratégias conhecidas para auxiliar na diminuição de práticas devastadoras que agridem a região Amazônica, no qual, os artigos evidenciados conforme o quadro 1 a seguir são essenciais para essa temática e estão dentro de uma linha do tempo entre os anos de 2005 a 2022.

Quadro 1 - Principais estratégias encontradas para a conservação das Áreas Protegidas na região Amazônica

Autores	Título	Ano	Periódico	Principais estratégias
Vieira, I. C. G., Silva, J. M. C. & Toledo, P. M.	Estratégias para evitar a perda de biodiversidade na Amazônia	2005	Estudos avançados	Criação de programas que visem induzir a produção do conhecimento, conservação e geração de riquezas na região
Brienza-Júnior, S., Pereira, J. F. & Yared, J. A. G. et al.	Recuperação de áreas degradadas com base em sistema de produção Florestal energético-madeireiro: indicadores de custos, produtividade e renda	2008	Amazônia: Ciência & Desenvolvimento	Apresentam um modelo de produção florestal que combina as espécies castanha-do-pará (*Bertholletia excelsa* H.B.K.); andiroba (*Carapa guianensis* Aubl.), paricá (*Schizolobium amazonicum* Huber) e taxi-branco (*Sclerolobium paniculatum* Vogel).
Bortolon, B., & Mendes, M. S. S.	A importância da Educação Ambiental para o alcance da Sustentabilidade	2014	Revista Eletrônica de Iniciação Científica	Educação ambiental nas escolas
Soares-Filho, B. S.	O papel das áreas protegidas da Amazônia, em especial as com apoio do ARPA, na redução do desmatamento	2016	Funbio	Áreas protegidas são uma estratégia para conter o desmatamento
Fraxe, T. D. J. P., Rezende, M. G. G., Macedo, C. D. A., & Witkoski, A. C.	Áreas protegidas no amazonas: conservação e sustentabilidade como preceitos ambientais indissociáveis	2017	Revista Amazônica de Ensino de Ciências	Práticas de restauração ecossistêmica e preservação ambiental
Gazoni; J. L., & Brasileiro, I. L. G.	O turismo como um instrumento de proteção florestal na Amazônia: uma análise multivariada	2018	Revista Brasileira de Pesquisa em Turismo	Aumento na intensidade e frequência da fiscalização ambiental, implantação de novas legislações e o desenvolvimento do turismo
Fearnside, Philip M.	Destruição e Conservação da Floresta Amazônica	2022	Editora do INPA	Fatores limitantes, desenvolvimento e análise de lacunas de conservação da vegetação da Amazônia

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Após o início do século XXI, torna-se cada vez mais evidente que a Amazônia necessita de um modelo de desenvolvimento regional capaz de conciliar o progresso econômico, o bem-estar social e a preservação dos recursos naturais. Por meio destes, surgem os desafios e limitações das estratégias de conservação e uso sustentável de áreas protegidas na região amazônica (Veríssimo et al., 2011).

Exemplos desses desafios e limitações podem ser o desmatamento, as estradas, a mineração, a exploração madeireira, que são impactos diretos sobre as Áreas Protegidas, podemos citar ainda, outros fatores, como a caça, a grilagem, a agropecuária, a fragmentação que são impactos indiretos gerados por projetos de infraestrutura (Veríssimo et al., 2011).

Ainda que a adoção deste modelo apresente um enorme desafio, dois fatores oferecem significativas oportunidades para que isso ocorra na próxima década. O primeiro está relacionado com à importância estratégica dos recursos naturais da região, tanto para o Brasil quanto para o mundo, em termos de regulação climática e diversidade biológica. Em segundo lugar, a região possui riquezas excepcionais, incluindo produtos oriundos da floresta e da biodiversidade, o potencial hidrelétrico de seus rios e os abundantes depósitos minerais presentes (Veríssimo et al., 2011).

A reflexão sobre as fragilidades e potencialidades relativas a cada elemento de avaliação deve servir de base para o planejamento de estratégias que visem à melhoria de sua efetividade de gestão (Brasil, 2017). Existindo assim, diretrizes, metas e estratégias voltadas para a sustentabilidade do uso dos recursos naturais, no interior e/ou no entorno da UC.

Mesmo com tantas alternativas para alcançar os objetivos de promoção de atividades sustentáveis é necessário aprimoramentos para que sejam realizadas de maneira eficaz. É importante que as práticas de restauração ecossistêmica e preservação ambiental sejam implementadas de maneira concreta, em vez de apenas depender de ideais utópicas de conservação no futuro. Para que isso ocorra de forma efetiva, o apoio do Estado é fundamental, já que é responsável por estabelecer políticas públicas e fornecer recursos para que as ações de conservação possam ser realizadas (Fraxe et al., 2017).

Atualmente, a fiscalização ambiental está focando na responsabilização dos infratores, uma vez que os crimes já foram cometidos. Contudo, para combater o desmatamento e proteger a biodiversidade da Amazônia, é preciso atuar de forma preventiva, assim, a prevenção demandará recursos para que os órgãos possam evitar a ocorrência de crimes, principalmente pela vigilância sistemática das áreas protegidas (Barreto e Mesquita, 2009).

Dessa maneira, observa-se que a criação de Unidades de Conservação (UCs), como única medida de conservação ambiental, tem mostrado-se insuficiente. Nos últimos anos, tem havido uma crescente preocupação em manter essas áreas protegidas, bem como o estabelecimento de estratégias que visam promover a conservação desses ambientes. Por isso, esse tema tem sido objeto de intensas discussões (Torres e Oliveira, 2008).

As áreas protegidas, em geral, estão se tornando mais eficazes em reduzir o desmatamento, mesmo sob uma trajetória descendente do desmatamento em toda a Amazônia (Soares-Filho, 2016) e isso pode ser um fator pela criação do Programa Áreas Protegidas da Amazônia (ARPA) e outros meios que auxiliem nesse processo, como o Plano de Ação para Prevenção e Controle do Desmatamento da Amazônia Legal (PPCDAm) melhorando questões de fiscalização e combate ao crime ambiental, além do Instituto do Homem e Meio Ambiente da Amazônia (Imazon).

A vulnerabilidade das Unidades de Conservação pode ser apontada por alguns fatores, como: a fácil acessibilidade, favorecendo as atividades ilegais; a grande demanda por recursos naturais vulneráveis; a dificuldade de contratação e manutenção de funcionários; a dificuldade de monitoramento das atividades ilegais; e o valor de mercado dos recursos naturais, todos esses aspectos influenciam na falta de proteção dessas áreas (Macedo, 2008).

No Amazonas, as Unidades de Conservação de Uso Sustentável são mais comuns do que as de Proteção Integral. Essa escolha foi feita para evitar conflitos que poderiam surgir caso houvesse restrições à habitação dentro dessas áreas, devido à grande população presente. No entanto, o Estado do Amazonas tem enfrentado pressões para permitir o manejo florestal em áreas de conservação, desde que essas atividades sejam baseadas em práticas tradicionais de uso da terra (Fraxe et al., 2017).

Dessa maneira, Miranda et al. (2020) reforçam que a maioria das UCs, tanto na esfera federal quanto na estadual, são da categoria de Uso Sustentável o que reforça a importância de se pensar estratégias que garantam o uso e gestão dos recursos naturais existentes nesses territórios pelos povos e comunidades tradicionais que ali habitam. Pois, o uso sustentável de recursos, além de preservar a cultura e trazer benefícios socioeconômicos às comunidades, é também um aliado à conservação ambiental (Prado et al., 2019).

Para contribuir com a melhoria das áreas degradadas podemos evidenciar as diferentes

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formas de utilização da madeira, esse modelo não apenas fomenta práticas econômicas sustentáveis, mas também contribui para a preservação das florestas. Dessa maneira, o trabalho de (Brienza-Júnior, Pereira, Yared et al., 2008) ressaltam o modelo de produção castanha-do-pará (Bertholetia excelsa) x andiroba (Carapa guianensis) x paricá (Schizolobium amazonicum) x taxi-branco (Sclerolo-bium paniculatum) que podem auxiliar nas diferentes formas de utilização da madeira, visando diminuir a pressão sobre as florestas nativas. Dessa maneira, o reflorestamento é uma das formas mais eficazes de ajudar a recuperar áreas degradadas.

Existe um enorme desafio para realizar plantações de árvores em áreas previamente alteradas, pois há uma série de obstáculos ambientais e logísticos. Em sua pesquisa, (Brienza-Júnior et al., 2008) resssaltam que o desafio colocado é plantar árvores para diferentes propósitos em áreas já alteradas. A recuperação da capacidade produtiva de áreas degradadas deve levar em consideração aspectos ambientais, econômicos e sociais. Em muitos casos, a degradação do solo resulta na perda de nutrientes essenciais, tornando-o inadequado para o crescimento saudável das plantas. Além disso, a presença de resíduos tóxicos ou poluentes no solo pode comprometer a sobrevivência das mudas.

Na Amazônia, há três cenários principais de degradação ambiental, como: pastagens em grandes propriedades, florestas exploradas em áreas sob Plano de Manejo Florestal Sustentável - PMFS e áreas rurais de agricultores familiares (Almeida, Sabogal e Brienza-Júnior, 2006).

Iniciando a análise das preocupações ambientais na Amazônia, é crucial direcionar a atenção para o fenômeno conhecido como o Arco do Desmatamento. O Arco do Desmatamento é uma região da Amazônia com alto índice de desmatamento, decorrente de atividades ilegais, como a extração de madeira e a expansão de áreas para a agropecuária e mineração, várias UC sofrem com essas atividades (Brasil, 2018). Na região do município de Lábrea, somente 5% da destruição da floresta ocorre dentro das áreas de conservação, que incluem a Floresta Nacional Iquini, a Reserva Extrativista Purus, o Parque Nacional Mapinguari e a Reserva Extrativista Ituxi. Essas áreas protegidas são importantes para a preservação ambiental e ajudam a impedir o avanço do desmatamento no sul do estado do Amazonas (Fraxe et al., 2017).

Podemos destacar o Parque Nacional Mapinguari sendo uma importante unidade de conservação da biodiversidade localizada na Amazônia, que tem como objetivo preservar ecossistemas naturais e na manutenção das características biológicas e interações ecológicas com destaque para importantes enclaves de savana do Interflúvio Purus-Madeira que ocorre nessa região (Brasil, 2018).

Assim, as complexas questões de conservação e uso sustentável na Amazônia, mostram que é essencial explorar as diversas propostas e estratégias que têm sido implementadas para enfrentar os desafios ambientais na região. Entre elas, estão: a criação de áreas protegidas de diversos tipos, o aumento na intensidade e frequência da fiscalização ambiental, implantação de novas legislações e o desenvolvimento do turismo (Gazoni e Brasileiro, 2018). Podendo ser citadas também as práticas silviculturais na Amazônia, praticadas tanto em escala industrial como em escala comunitária e familiar e o monitorando a cobertura original.

As estratégias para desacelerar o desmatamento incluem a repressão através de procedimentos de licenciamento, monitoramento e multas. O rigor das penalidades deve ser suficiente para impedir os desmatamentos ilegais, mas não tão grande que as impeçam de ser executadas. Uma reforma política também é necessária para discutir as causas primordiais do desmatamento, incluindo o papel do desmatamento no estabelecimento da posse da terra (Fearnside, 2022).

Cada região demanda estratégias específicas, adaptadas às suas particularidades e desafios. Dessa maneira, esses meios que estão sendo criados têm como objetivo principal proteger os recursos naturais e a biodiversidade de uma determinada região, garantindo sua preservação para as gerações futuras, além disso, a categorização e classificação das Unidades de Conservação são elaboradas a partir das características de cada área, promovendo a conservação do ecossistema e restringindo atividades que possam prejudicar o funcionamento do ambiente (Fraxe et al., 2017). A diversidade biológica ou biodiversidade tem sido cada vez mais reconhecida como um dos elementos centrais para o desenvolvimento e bem estar da humanidade e é o grande responsável pelo equilíbrio ambiental global (Ferro, 2006).

Diversas outras estratégias estão relacionadas com o desenvolvimento de novas abordagens e técnicas ambientalmente eficientes, que possam fortalecer a política ambiental em âmbito estadual, nacional e global. Para se pensar em uma epistemologia ambiental no estado do Amazonas e embasar a construção de políticas ambientais inovadoras, é necessário dar o primeiro passo, que consiste em romper com o paradigma dualista do ambiente (Fraxe et al., 2017).

Além disso, a criação de programas que visem induzir a produção do conhecimento, conservação

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e geração de riquezas na região é uma alternativa eficaz, pois, com um bom planejamento que garanta que ocorra essa informação e conhecimento, pode receber destaque para subsidiar linhas de ação de manutenção dos principais processos biológicos, da promoção de uso dos recursos naturais, da conservação da biodiversidade e da gestão integrada do território (Vieira, Silva e Toledo, 2005).

De acordo com Barreto e Mesquita (2009) algumas alternativas podem ser destacadas como: priorizar o processamento dos casos em áreas protegidas; reforçar medidas recentes de comando e controle e aumentar a transparência sobre a responsabilização. Se todas essas medidas fossem realizadas, seria mais fácil combater o desmatamento ilegal na Amazônia e auxliaria na sua manutenção.

Torna-se relevante aferir se as estratégias já existentes são factíveis em um primeiro momento, para, posteriormente, aferir se as mesmas geram os resultados esperados em termos de melhoria do estado de conservação de alvo ou em termos de qualificação dos usos relacionados à UC (Brasil, 2017).

Algumas das estratégias mais utilizadas para a conservação são: educação ambiental, zoneamento e manejo de áreas protegidas, monitoramento e fiscalização, participação das comunidades locais, turismo sustentável, desenvolvimento e criação de UC de Uso Sustentável, ações para compatibilizar, integrar e otimizar a relação com a população do entorno ou do interior das unidades de conservação, disseminar uma consciência entre os proprietários de terras de que o desmatamento é um mau negócio, além do zoneamento ecológico e econômico; alternativas tecnológicas no uso do desmatamento e queimadas.

Para que realmente ocorra a maioria dessas estratégias, é preciso que esse conhecimento se inicie nas escolas, favorecendo à adoção de um comportamento conservacionista e consequentemente contribuindo para uma conservação mais efetiva da natureza. Desde cedo, é possível que as crianças desenvolvam uma consciência focada em valorizar a vida, por meio da adoção de novos hábitos e de um estilo de vida distante do consumismo excessivo e do desperdício dos recursos naturais (Bortolon e Mendes, 2014).

Existem diversas dificuldades na Educação Básica e no Ensino Fundamental das escolas públicas quando se trata de educação ambiental. Para que a conscientização sobre a preservação do meio ambiente ocorra de forma efetiva, é necessário que haja incentivo do poder público. É importante que sejam disponibilizados materiais para a realização de atividades e gincanas, além do apoio dos governantes e órgãos responsáveis pela preservação do meio ambiente. Dessa forma, será possível enfatizar a importância da sustentabilidade e criar projetos que visem a uma forma de viver mais consciente e responsável com o planeta (Bortolon e Mendes, 2014).

Além disso, quanto aos aspectos de preservação e conservação de espécies florestais, (Matos e Lima, 2023) citam que os estudos sobre recuperação ambiental envolvendo plantas precisam ser desenvolvidas em maior escala para o conhecimento dessas áreas e assim, acontecer a recuperação desses habitats, o manejo e de fato a conservação das espécies vegetais.

Uma outra opção é a divulgação dos resultados de pesquisa de modo que os mesmos cheguem ao agricultor de forma clara. Divulgação conjunta dos órgãos de pesquisa, criação de bancos de dados referentes ao tema, a fim de centralizar fonte das tecnologias e facilitar o repasse desses conhecimentos (Drumond et al., 2000). Dessa maneira, poderíamos incentivar cada vez mais as campanhas de reflorestamento; incentivar a criação de viveiros florestais dirigidos por comunidades e/ou associações de classes. Promover encontros de cursos e treinamentos sobre combate aos meios não sustentáveis. Poderíamos introduzir conceitos e noções básicas de proteção ambiental e sobre os recursos naturais, tanto no ensino básico quanto nos cursos de graduação.

Por meio desses cursos, as pessoas seriam capacitadas para identificarem espécies nativas da região amazônica com potencial forrageiro, melífero, madeireiro, frutífero, medicinal, aromático, ornamental e outros usos e desenvolvimento de métodos para seu cultivo sistemático.

Sendo que o governo e as organizações locais precisam investir mais esforços para alavancar e aumentar o protagonismo das comunidades na economia florestal e na gestão e uso dos recursos florestais madeireiros nesses territórios (Miranda et al., 2020).

Outra estratégia viável é fomentar o desenvolvimento de atividades econômicas sustentáveis nas florestas, fortalecendo as comunidades locais e aproveitando os recursos disponíveis nas regiões. Isso pode ser alcançado por meio do apoio a projetos de manejo florestal comunitário e empresarial, gestão de pesca, iniciativas de capacitação, programas educacionais e conscientização ambiental, além de políticas públicas e ações de comunicação (WWF, 2023).

É fundamental compreender a importância de programas inovadores como o de Manejo Florestal Comunitário e Familiar (MFCF) pois ele emerge não apenas como uma estratégia ambientalmente

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consciente, mas como um catalisador para o desenvolvimento sustentávelque e vem ganhando relevância como alternativa para a conservação e geração de emprego e renda no cenário amazônico (Miranda et al., 2020) sendo que o objetivo deste programa é coordenar as ações de gestão e fomento ao manejo florestal sustentável direcionadas para os povos, comunidades tradicionais e agricultores familiares que tiram sua subsistência das florestas brasileiras (Brasil, 2023).

As áreas protegidas podem fornecer uma extensa série de bens e serviços para as populações que vivem nela e no seu entorno, e para a sociedade como um todo (Scherl et al., 2006), como mostra os dados referente a evolução da implementação do Plano de Ação para Prevenção e Controle do Desmatamento na Amazônia Legal (PPCDAm) que trouxe resultados significativos para a contenção do desmatamento na Amazônia e Amazônia Legal (Mello & Artaxo, 2017).

Atualmente, as atividades pecuárias, a produção de soja, os investimentos em infraestrutura, a indústria madeireira e a grilagem de terras permanecem como os principais impulsionadores diretos do desmatamento na região da Amazônica (Mello & Artaxo, 2017).

As atividades de visitação pública também são estratégias para a conservação da biodiversidade, pois, é necessário conhecer para conservar e essa expansão de atividades ecoturísticas são uma alternativa para o desenvolvimento sustentável, tanto para a conservação de áreas públicas quanto de privadas (Wagner, Campos e Lima, 2023).

CONCLUSÕES

Como recomendações para aprimorar as estratégias de conservação e uso sustentável de áreas protegidas na região amazônica, podemos sugerir que ocorra o fortalecimento da participação das comunidades locais e povos indígenas na gestão das áreas protegidas, promovendo a capacitação e empoderamento desses grupos.

Investir em pesquisas científicas para compreender melhor a biodiversidade da região e os impactos das atividades humanas, com o objetivo de subsidiar a tomada de decisão e aprimorar as estratégias de conservação, além de desenvolver e implementar planos de manejo efetivos para as áreas protegidas, com metas claras e monitoramento constante dos resultados.

A combinação da conscientização com a tecnologia resulta na preservação, e é por isso que realizar campanhas de conscientização e educação ambiental com as comunidades locais e a sociedade em geral podem promover o diálogo e a cooperação entre os diversos setores envolvidos na gestão das áreas protegidas. É crucial acatar esses fatores, pois caso contrário, podem ocorrer alterações drásticas na vida na Terra.

Observa-se que o grande desafio na atualidade é investir na implementação e fiscalização das Áreas Protegidas. E essas áreas no Amazonas tem sido instrumentos importantes de conservação e preservação da biodiversidade, além de auxiliar na criação, expansão e no fortalecimento de unidades de conservação.

Portanto, se faz necessário adotar práticas sustentáveis em sua vida diária, como reduzir o consumo de plástico, economizar água e energia, usar transportes mais eficientes e evitar o desperdício de alimentos, esses pequenos detalhes auxiliam a melhorar as condições de subsistência dos recursos disponíveis e a diminuir a pressão sobre as áreas protegidas e todos esses problemas podem ser evitados ao serem inseridos no dia a dia, pois são decorrentes da falta de conhecimento da população em geral. Dessa maneira, é possível alcançar um equilíbrio entre o desenvolvimento econômico e a conservação ambiental, desde que sejam adotadas medidas efetivas e responsáveis.

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Lignocellulosic supplementation of the culture medium for mycelial development and production of phenoloxidase enzymes from Amazonian strains of gasteroid fungi (Basidiomycota)

Marcos Diones Ferreira Santana [a*], Sheyla Regina Marques Couceiro [a], Ceci Sales-Campos [b]

[a] Universidade Federal do Oeste do Pará, Brasil
[b] Instituto de Nacional de Pesquisas da Amazônia, Brasil
[*] Autor correspondente (santana.mdf@gmail.com)

INFO

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enzymatic production

ABSTRACT

Strains of gasteroid fungi are capable of oxidizing phenolic components and producing enzymes of great commercial value. However, the difficulty in producing biomass and, consequently, the low levels of expression of enzymes produced by these fungi hinder the advancement of biotechnological studies. A possible alternative may be supplementing the culture medium with lignocellulosic substrates, as observed in the cultivation of edible mushrooms, for example. Thus, the objective of this study was to analyze the effect of supplementing a potato-based culture medium with different concentrations of wheat and rice bran on mycelial development and the production of phenoloxidase enzymes of Amazonian strains of *Cyathus* and *Geastrum*. The basidiomas were collected in the Amazon of Pará, and the strains were obtained from mycelial isolation on Potato Dextrose Agar (PDA) medium. The strains were inoculated in semi-solid and liquid potato-based medium containing 10% and 20% rice bran and 10% and 20% wheat bran. For a period of 20 days, mycelial growth was observed in solid form, and for 40 days, the development of the strains in liquid medium was monitored, all incubated at 25°C in the dark. Growth and production of fresh and dry biomass were measured, as well as the production of phenoloxidase, arising from growth in liquid medium. Supplementation using 10% wheat bran was the most promising for the radial growth of four of the six species tested (*C. limbatus*, *G. hirsutum*, *G. echinulatum*, and *G. schweinitzii*), in addition to being the best treatment for the production of biomass and phenoloxidase enzymes for all species. Considering that wheat bran is easily accessible, this study stimulates basic science, especially in the Amazon, where Funga is still underestimated in terms of diversity and biotechnological potential.

Palavras-chaves
Culturas miceliais
farelos lignocelulósicos
fungos estrela-da-terra
fungos ninho-de-pássaros
produção enzimática

RESUMO

Suplementação lignocelulósica do meio de cultura para desenvolvimento micelial e produção de enzimas fenoloxidase de cepas amazônicas de fungos gasteroides (Basidiomycota)

Cepas de fungos gasteroides são capazes de oxidar componentes fenólicos e produzir enzimas de grande valor comercial. Porém, a dificuldade na produção de biomassa e consequentemente os baixos níveis de expressão das enzimas produzidas por esses fungos, impedem o avanço dos estudos biotecnológicos. Uma auternativa possível pode ser o uso da suplementação do meio de cultura com substratos lignocelulógicos, como como observado no cultivo de cogumelos cultiváveis, por exemplo. Assim, o objetivo desse estudo foi analisar o efeito da suplementação do meio de cultura a base de batata com diferentes concentrações de farelo de trigo e arroz no desenvolvimento micelial e na produção de enzimas fenoloxidases de cepas amazônicas de *Cyathus* e *Geastrum*. Os basidiomas foram coletados na Amazônia paraense e as cepas obtidas a partir do isolamento micelial em meio Batata Dextrose Ágar (BDA). As cepas foram inoculadas em meio semisólido e líquido a base de batata contendo 10 e 20% de farelo de arroz e 10 e 20% de farelo de trigo. Por um período de 20 dias observou-se o crescimento micelial emisólido e por 40 dias o desenvolvimento das cepas em meio líquido, todos incubados 25°C no escuro. O crescimento e a produção de biomassa fresca e seca foram mensuradas, assim como a produção de fenoloxidase, oriunda do crescimento em meio líquido. A suplementação usando farelo de trigo a 10% foi a mais promissora para o crescimento radial de quatro, das seis espécies testadas (*C. limbatus*, *G. hirsutum*, *G. echinulatum* e *G. schweinitzii*), além de ter sido o melhor tratamento para a produção de biomassa e enzimas fenoloxidases para todas as espécies. Considerando que o farelo de trigo é de fácil acesso, este estudo estimula a ciência de base, principalmente na Amazônica, onde a Funga ainda é subestimada em diversidade e potencial biotecnológico.

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INTRODUCTION

Given the increasing global demand for bioproducts associated with sustainable production, studies on fungi and its enzymes, particularly those that have potential for biotechnological application, have increased (Troiano et al., 2020). In this respect, gasteroid fungi (Basidiomycota) are recognized as important (Wang et al., 2014; Badshah et al., 2015; Srisurichan et al., 2017) for being able to produce several substances such as phenoloxidaseenzymes (PO) (Santana et al., 2016). PO have wide application in pollutantdegradation, effluent discoloration, wood pulp bleaching, removal of phenolics from wines, dyes oxidation, enzymatic conversion of chemical intermediates, biofuels production, xenobiotics degradation and bioremediation (Valmaseda et al., 1990; Banerjee and Vohra, 1991; D'Souza et al., 1999).

For some groups of mushrooms, the detection of PO can be done by direct reaction (Parra, 2008) or by grinding the basidiome. However, for gasteroid fungi, e.g. *Geastrum* Pers., the former technique is not efficient (Zamora et al., 2013) and the latter requires large or numerous basidiomes, which is not always possible given the nature of these fungi, that occur in low abundance and limited by climatic seasonality like most Amazonian fungi (Braga-Neto et al., 2008). Hence, the cultivation of strains may present advantages over other methods, mainly due to the availability of material for enzyme extraction over time.

Despite the recognition of the significant biotechnological potential of gasteroid fungi (Liu and Zhang, 2004), studies involving this group are predominantly taxonomic in nature. One of the contributing factors to this trend may be the challenges associated with obtaining mycelial cultures of these fungi, especially in the case of *Geastrum* species. *Geastrum* is a promising genus for the production of intriguing substances (Dore et al., 2007; Kuhar et al., 2016); however, its mycelial growth in conventional culture media is slow, posing difficulties in maintaining in vitro cultures and precluding enzyme production tests due to the limited amount of biomass generated (Sunhede, 1989; Stoytchev et al., 2001; Zamora et al., 2014).

Santana et al. (2016) reported that Amazonian strains of *Geastrum* have the capacity to produce phenoloxidase (PO). Although they have made recent advancements in obtaining mycelial cultures, testing different media, and studying improved cultivation conditions to facilitate investigations within this group (Santana et al., 2020), there remains a need to enhance the yield of in vitro cultures. In contrast, for *Cyathus* Haller, another gasteroid genus, there are more studies focusing on the extraction of substances of biotechnological interest, even though they are concentrated in a few species and specific regions of the world (Vasdev et al., 1995; Dhawan and Kuhad, 2002; Mishra and Bisaria, 2006). Obtaining and growing mycelial cultures for these fungi does not appear to be a significant challenge when compared to *Geastrum* cultures (Dhawan and Kuhad, 2003; Sharvit et al., 2021). Nevertheless, there is room for improvement, especially when considering cultivation systems.

Consequently, it is expected that mycelial cultures of gasteroid fungi can be enhanced through the supplementation of the culture medium, as observed in other fungal groups (Rossi et al., 2001; Benassi et al., 2012; Pérez-Pacheco et al., 2014; Olguin-Maciel et al., 2017; Szambelan et al., 2018). Utilizing rice and wheat bran can be particularly advantageous for achieving greater biomass or increasing enzyme production, as evidenced in the cultivation of edible mushrooms for similar purposes (Donini et al., 2006; Minotto et al., 2008). In the Brazilian Amazon regions, where the biodiversity of these genera continues to expand each year, it becomes imperative to propose protocols that open up new avenues for scientific research, including basic science, involving these species (Santana et al., 2020).

In this context, the aim of this study was to examine the impact of supplementing a potato-based culture medium, widely regarded as one of the most crucial culture media for fungi today, primarily due to its accessibility and the favorable findings reported by Santana et al. (2020). We investigated the influence of varying concentrations of rice bran and wheat bran on the mycelial development and phenoloxidase (PO) enzyme production in Amazonian strains representing different species of *Cyathus* and *Geastrum*.

MATERIAL AND METHODS

Sampling and identification of gasteroid fungi

Fresh and mature specimens of *Cyathus* and *Geastrum* were collected manually (Lodge et al. 2004) in a rainforest fragment (Amazon dense rainforest) near the Sílvio Braga Hydroelectric Power Plant (02°48'44.45"S, 54°17'56.23"W), Santarém, western Pará, Brazil. The specimens were packed separately in labeled plastic boxes. Species identification was performed based on morphological characters according to descriptions in the literature (Sunhede, 1989; Calonge et al., 2005; Sousa et al., 2014; Gois et al., 2021). Part of the samples were destined to obtain strains and

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another part was assembled in voucher specimens and incorporated into the collection of fungi of the HSTM Herbarium of the Federal University of Western Pará (Table 1).

Table 1 - Species used to obtain Amazonian strains of gasteroid fungi

Family	Species	Voucher number
Agaricaceae	*Cyathus albinus* Accioly, R. Cruz & Baseia	14790
	C. limbatus Tul. & C. Tul.	14883
	C. setosus H.J. Brodie	14957
Geastraceae	*Geastrum echinulatum* T.S. Cabral, B.D.B. Silva & Baseia	14901
	G. hirsutum Baseia & Calonge	14865
	G. schweinitzii (Berk. & M.A. Curtis) Zeller	14896

Mycelial culture obtaining

The *Geastrum* strains were obtained from sections removed from the pseudoparenchymatous layer of fresh basidiome exoperidium, set in PDA (Difco®). The *Cyathus* strains were obtained from sections of the inoculated peridioles in PDA. For all strains, the presence of a clamp connection in the mycelium, after satisfactory growth, was used as confirmation of a dicariotic mycelium (Taylor et al., 2000; Taylor et al., 2006). From the confirmation, mycelium fragments (3 × 3 mm) from the obtained strains were removed and inoculated in the center of Petri dishes (ø 90 mm) containing 15 mL of PDA and incubated at 25°C in the dark (Santana et al., 2020) to obtain pure cultures.

Effect of culture medium supplementation on mycelial growth

For the evaluation of mycelial growth, pure culture fragments (3 ×3 mm) were inoculated in the center of Petri dishes (ø 90 mm) containing 15 mL of PDA supplemented with 0 (control), 10 or 20% wheat bran or rice bran, locally sourced, previously sterilized at 121°C for 15 minutes. Petri dishes were then incubated for 20 days at 25°C in the dark (Santana et al., 2020). After this period, the average radial growth of the cultures was measured with a millimeter ruler set in orthogonal directions.

After the growth measurements, the Petri dishes were taken to the microwave oven (800 W for 20 seconds) to fuse the culture medium and then separate the fungal mycelium by filtration (Vargas-Isla and Ishikawa, 2008). After filtered, the mycelium was transferred to aluminum foil crucibles (of known mass) to measure their fresh mass. Finally, the crucibles were dehydrated in an oven at 60°C for 24 hours, and the temperature increased to 105°C until the mycelium mass reached a constant dry weight.

Effect of supplementation on biomass production in submerged culture

Supplemented mycelial growth was also analyzed in submerged culture of Potato Dextrose (PD). For this, strain fragments (3 × 3 mm) were set in 250 mL Erlenmeyer bottles containing 50 mL of PD + supplementation of 10 and 20% wheat bran or rice bran, previously sterilized at 121°C for 15 minutes, for 40 days. After the incubation period, mycelium was separated from the culture medium by filtration, and the fresh and dry mycelial mass was determined as described in the previous experiment.

Phenoloxidase production

The filtrates resulting from the previous experiment were used to evaluatethe supplementation by wheat bran and rice bran in the production of phenoloxidaseenzymes. Petri dishes (ø 90 mm) containing 15 mL of tannic acid agar culture medium (5%) sterile, three cup plates of 9 mm diameter were dug, filled with 0.1 mL of the filtrate. After 24 hours of incubation, the distance between the edge of each cup plate and the edge of the brownish oxidation halo was measured in orthogonal directions, with a millimeter ruler.

Data analysis

The experiments were carried out in a completely randomized design. For the experiments in solid culture medium, the experimental unit consisted of a Petri dish with five replicates per treatment; for experiments in liquid culture medium, the experimental unit was an Erlenmeyer bottle, also with five replicates per treatment. To evaluate the production of phenoloxidaseenzymes, the average of the three cup plates per Petri dish was considered, and each Petri dish was considered a sampling unit, with five

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replicates.

In the end, the measurements of growth and mycelial biomass of the strains and the size of the PO oxidation halo of the five repetitions for each species were submitted to two-way analysis of variance (ANOVA) between the five treatments (no supplementation, 10 and 20% wheat bran, 10 and 20% rice bran), followed by Tukey test if necessary. For the analyses, a significance level of 0.05 was set and software BioEstat 5.0 was used.

RESULTS

Effect of supplementation on mycelial growth

The radial mycelial growth of the strains was significantly influenced by the species of gasteroid fungi and by the supplementation (wheat bran or rice bran at 10 or 20%). Table 2 shows the final mycelial growth, where it can be observed that the strains of *Cyathus* species showed higher growth in relation to the strains of *Geastrum*. Overall, the best results of mycelial growth were observed in the treatment with 10% wheat bran supplementation. Rice bran supplementation in both concentrations conferred the lowest results in mycelial growth for all tested strains. Different results were only observed for *C. limbatus*, with higher growth in not supplemented culture medium (control); and for *C. albinus* strains, in which the control treatment did not differ from the treatments supplemented with wheat.

Table 2 - Mycelial growth (mm) of Amazonian strains of gasteroid fungi grown in Potato Dextrose Agar (PDA) medium supplemented with different concentrations of wheat bran and rice bran after 20 days of incubation at 25°C in the dark

Species	Treatments				
	PDA	PDA + Wheat		PDA + Rice	
		10%	20%	10%	20%
Cyathus limbatus	80.2 ± 1.1^{Ba}	61.4 ± 0.9^{Cb}	48.6 ± 1.1^{Cc}	37.6 ± 1.6^{Cd}	32.0 ± 3.4^{Bd}
C. albinus	90.0 ± 0.0^{Aa}	90.0 ± 0.0^{Aa}	90.0 ± 0.0^{Aa}	80.0 ± 0.0^{Ab}	52.6 ± 0.0^{Ac}
C. setosus	64.0 ± 1.9^{Cb}	74.0 ± 1.4^{Ba}	60.0 ± 0.9^{Bb}	45.0 ± 5.5^{Bc}	31.0 ± 0.7^{Bd}
Geastrum schweinitzii	16.2 ± 1.1^{Dc}	24.6 ± 0.5^{Da}	19.0 ± 0.7^{Db}	12.8 ± 1.3^{Dd}	6.4 ± 0.1^{Ce}
G. echinulatum	12.2 ± 1.6^{Eb}	15.6 ± 1.1^{Fa}	7.4 ± 0.5^{Fc}	11.6 ± 1.1^{Db}	4.3 ± 0.2^{Dd}
G. hirsutum	10.0 ± 4.7^{Fb}	18.5 ± 2.5^{Ea}	10.4 ± 5.3^{Ec}	1.3 ± 1.0^{Ed}	2.4 ± 2.2^{Ed}

Mean ± standard deviation of five replicates; different lettersindicate significantly different values (ANOVA, $p < 0.05$); lowercase letters refer to the comparison between treatments and uppercase ones refer to the comparison between strains.

In addition to the results presented in Figure 1, the variation in mycelial growth of fungal strains is visually shown. The strains cultivated without supplementation (control) presented irregular margins, fine and dense hyphae. The strains grown in medium supplemented with wheat bran presented denser hyphae, slightly irregular edges, larger in length and numerous branches and clamp connections. Supplementation also provided the emergence of basidiome initials in *C. albinus* strains. The strains grown in the presence of rice bran presented less dense mycelium, shorter hyphae and few branches that were closer to the surface of the culture medium

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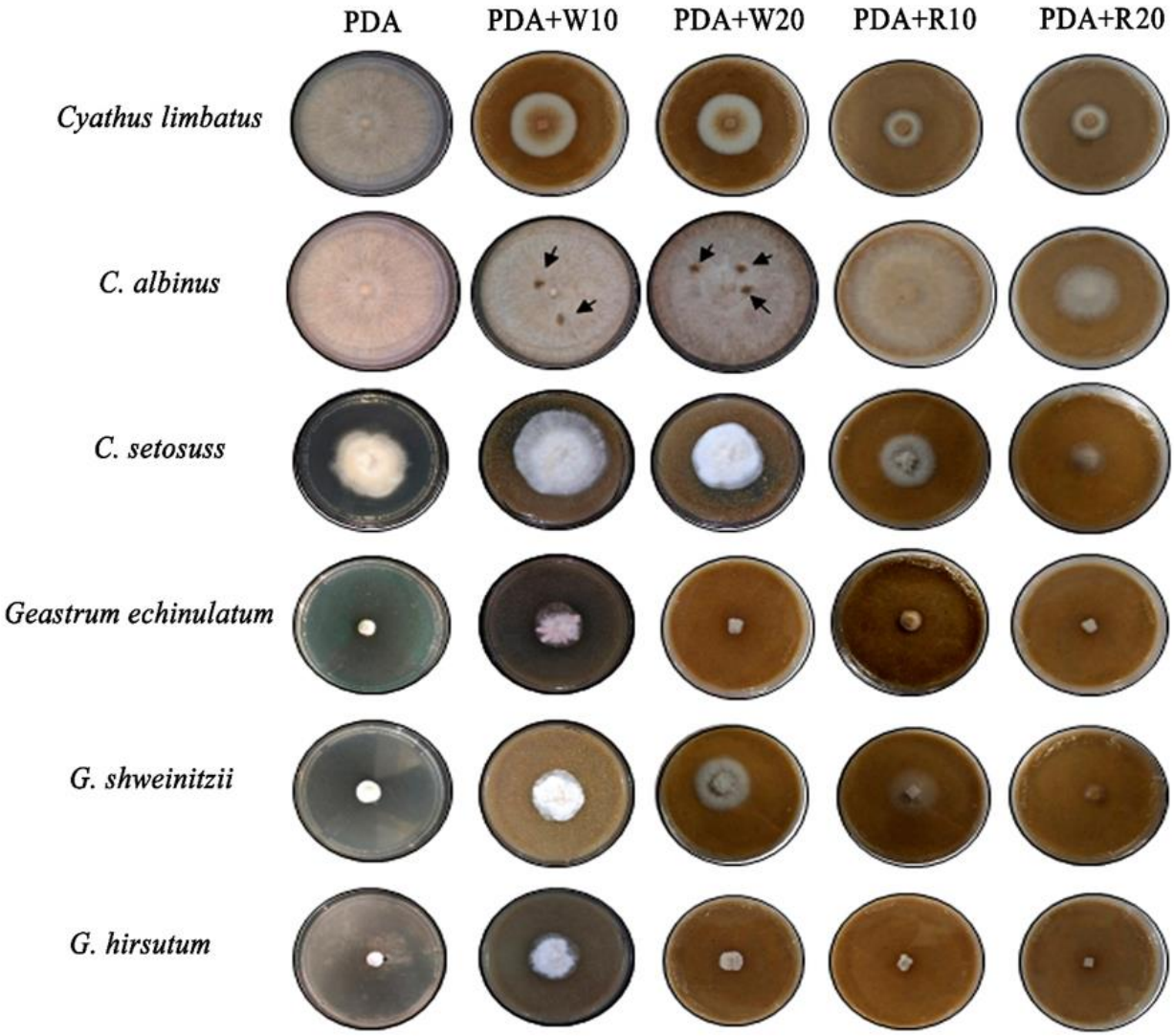

Figure 1 - Morphological aspects of Amazonian strains of gasteroid fungi grown in Potato Dextrose Agar (PDA) medium supplemented with wheat 10% (PDA + W10), wheat 20% (PDA + W20), rice 10% (PDA + R10) and rice 20% (PDA + R20) after 20 days in the dark. Arrows indicate the production of basidiome initials in culture medium

Similarly, biomass production was higher in the treatment supplemented with 10% wheat bran for both *Cyathus* and *Geastrum*; among the tested species, *Cyathus* strains were the most productive (Table3). Strains of *C. limbatus*, which had presented higher radial growth in non-supplemented medium, presented the highest biomass values in medium supplemented with 10% wheat bran, as well as the *C. albinus* strains, that reached the edge of the Petri dish in almost all treatments and showed the best results when supplemented with 10% wheat bran (Table 3). *G. echinulatum* produced the most biomass (compared to the control) among *Geastrum* strains, as well as the strains of *G. hirsutum*, which presented the lowest mycelial growth values, but obtained better biomass gains than the control (Table 3).

Effect of supplementation on biomass production in submerged culture

The biomass results in submerged culture medium reflected the same results as those observed in solid medium (Table 4). *Cyathus* strains presented the highest values of fresh and dry biomass production compared to *Geastrum* strains, especially when cultivated in the presence of 10% wheat bran, a treatment that conferred the best results in fresh and dry biomass production for both taxa. The treatment with 20% rice bran supplementation presented the lowest biomass gain (Table 4), and 10% rice bran had low values as well.

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Table 3 - Biomass produced by Amazonian strains of gasteroid fungi grown in Potato Dextrose Agar (PDA) medium supplemented with different concentrations of wheat bran and rice bran after 20 days of incubation at 25°C in the dark

Species	Treatments									
	Fresh mass (g)					Dry mass (g)				
	PD	PD + Wheat		PD + Rice		PD	PD + Wheat		PD + Rice	
		10%	20%	10%	20%		10%	20%	10%	20%
C. limbatus	5.7±0.4^{Ab}	11.3±0.6^{Aa}	4.0±1.1^{Ac}	3.0±0.4^{Bc}	1.4±0.4^{Ad}	0.2±0.1^{Bd}	0.9±0.1^{Aa}	0.4±0.2^{Bc}	0.6±0.1^{Ab}	0.4±01^{Ac}
C. albinus	4.7±0.6^{Bb}	7.3±2.4^{Ba}	2.4±0.8^{Bc}	4.0±0.8^{Ab}	1.3±0.2^{Ad}	0.3±0.1^{Ac}	0.8±0.0^{Aa}	0.6±0.1^{Ab}	0.6±0.1^{Ab}	0.3±0.1^{Bc}
C. setosus	3.9±0.3^{Cb}	6.3±0.5^{Ca}	2.7±0.7^{Bc}	1.2±0.2^{Cd}	0.7±0.3^{Bd}	0.2±0.1^{Bc}	0.8±0.1^{Ba}	0.4±0.1^{Bb}	0.2±0.1^{Bc}	0.1±0.0^{Cd}
G. schweinitzii	1.6±0.3^{Db}	2.9±0.2^{Da}	1.2±0.2^{Cc}	0.4±0.3^{Dd}	0.2±0.1^{Cd}	0.4±0.0B^{Cb}	0.7±0.1^{Ca}	0.1±0.0^{Cc}	0.01±0.0^{Cd}	0.01±0.0^{Cd}
G. echinulatum	0.8±0.1^{Eb}	2.0±0.2^{Ea}	1.0±0.3^{Cb}	0.7±0.1^{Dc}	0.3±0.1^{Cc}	0.3±0.1^{Db}	0.7±0.1^{Da}	0.1±0.0^{Dc}	0.02±0.0^{Cd}	0.01±0.0^{Cd}
G. hirsutum	0.5±0.1^{Fb}	0.8±0.1^{Fa}	0.3±0.1^{Dc}	0.2±0.1^{Ec}	0.2±0.1^{Cc}	0.02±0.0^{Ebc}	0.15±0.1^{Da}	0.05±0.0^{Db}	0.01±0.0^{Cc}	0.01±0.0^{Cc}

Mean ± standard deviation of five replicates; different letters indicate significantly different values (ANOVA, $p < 0.05$); lowercase letters refer to the comparison between treatments and uppercase ones refer to the comparison between strains.

Table 4 - Biomass produced by Amazonian strains of gasteroid fungi grown in of Potato Dextrose (BD) submerged medium supplemented with different concentrations of wheat bran and rice bran after 40 days of incubation at 25°C in the dark

Species	Treatments									
	Fresh mass (g)					Dry mass (g)				
	PD	PD + Wheat		PD + Rice		PD	PD + Wheat		PD + Rice	
		10%	20%	10%	20%		10%	20%	10%	20%
C. limbatus	12.2±0.3^{Bb}	15.4±0.4^{Ba}	8.5±0.6^{Bc}	4.3±0.4^{Cd}	2.8±0.4^{Ce}	0.4±0.0^{Cb}	1.5±0.1^{Ba}	0.3±0.1^{Cc}	0.2±0.0^{Cc}	0.1±0.0^{Bd}
C. albinus	17.2±0.3^{Ab}	19.4±0.4^{Aa}	8.5±0.5^{Bd}	13.4±0.4^{Ac}	7.9±0.9^{Bd}	0.7±0.0^{Bb}	2.3±0.1^{Aa}	0.5±0.1^{Ac}	0.8±0.2^{Ab}	0.3±0.1^{Ad}
C. setosus	11.1±0.5^{Bb}	14.2±0.3^{Ba}	11.9±0.9^{Ab}	11.1±0.3^{Bb}	9.4±0.3^{Ac}	0.9±0.1^{Ab}	1.3±0.0^{Ca}	0.5±0.0^{Bc}	0.3±0.0^{Bd}	0.1±0.0^{Be}
G. schweinitzii	2.5±0.3^{Db}	3.5±0.3^{Ea}	1.2±0.2^{Dc}	2.1±0.^{2Db}	1.4±0.3^{Dc}	0.3±0.1^{Db}	1.1±0.0^{Da}	0.1±0.0^{Dc}	0.3±0.0^{Bb}	0.01±0.0^{Bc}
G. echinulatum	2.9±0.1^{Db}	6.6±0.4^{Da}	2.1±0.1^{Cc}	1.3±0.2^{Ed}	0.8±0.1^{Ee}	0.1±0.0^{Eb}	0.9±0.1^{Da}	0.1±0.0^{Dcb}	0.1±0.0^{Cb}	0.01±0.0^{Bb}
G. hirsutum	3.7±0.1^{Cb}	9.1±0.2^{Ca}	1.1±0.1^{Dc}	1.2±0.1^{Ec}	0.3±0.1^{Ec}	0.8±0.0^{Ab}	1.1±0.0^{Ea}	0.04±0.0^{Ed}	0.2±0.0^{Cc}	0.02±0.0^{Cd}

Mean ± standard deviation of five replicates; different letters indicate significantly different values (ANOVA, $p < 0.05$); lowercase letters refer to the comparison between treatments and uppercase ones refer to the comparison between strains.

Phenoloxidase production

Only the strains of *Cyathus* species expressed the production of phenoloxidaseenzymes without supplementation. However, with supplementation, particularly wheat bran, there was an intensification in the production of enzymes for *Cyathus* strains. Also, the production of the enzyme by *Geastrum* strains was then visible (Figure 2; Table 5).

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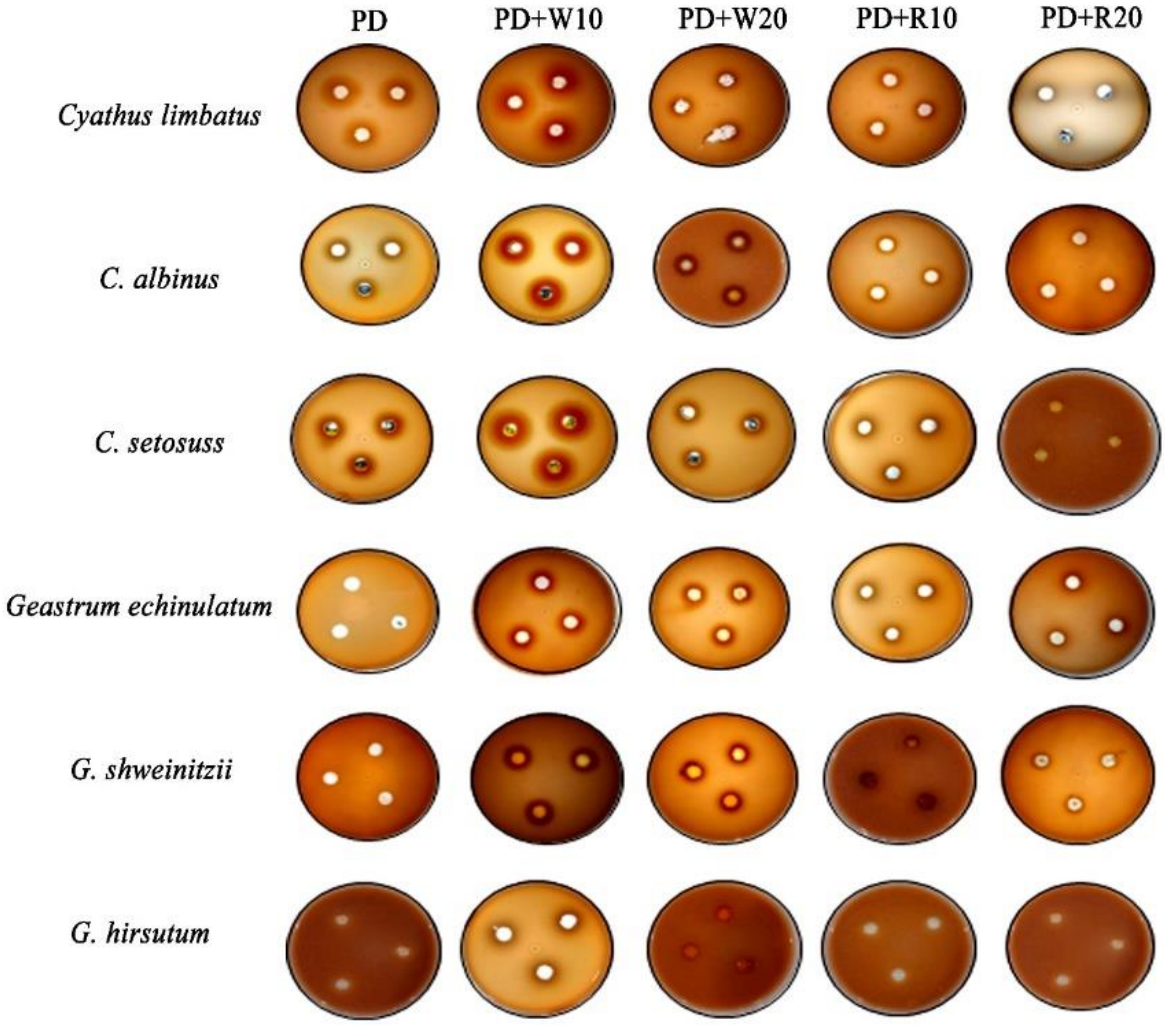

Figure 2 - Production of PO by Amazonian strains of gasteroid fungi grown in Potato Dextrose (PD) liquid medium supplemented with wheat 10% (PD + W10), wheat 20% (PD + W20), rice 10% (PD + R10) and rice 20% (PD + R20) after 20 days of cultivation in the dark

Supplementation with 10% wheat bran intensified the production of PO with larger oxidation halos for all strains of the gasteroid fungi tested, although the best result was measured for *Cyathus* species. Supplementation with rice bran contributed to the smallest halos observed (Table 5).

Table 5 - Oxidation halo (mm) indicated by the production of phenoloxidaseenzymes by Amazonian strains of gasteroid fungi grown in different concentrations of wheat and rice bran

Species	Treatments				
	PD	PD + Wheat		PD + Rice	
		10%	20%	10%	20%
Cyathus limbatus	13.8 ± 1.3^{Ab}	27.6 ± 1.1^{Aa}	11.6 ± 0.9^{Bc}	0.5 ± 0.1^{Bd}	0.2 ± 0.0^{Ae}
C. albinus	14.0 ± 1.0^{Ab}	25.8 ± 0.8^{Ba}	11.6 ± 1.1^{Bc}	0.5 ± 0.5^{Bd}	0.2 ± 0.1^{Ae}
C. setosus	13.8 ± 1.1^{Ab}	27.6 ± 1.1^{Aa}	14.0 ± 1.0^{Ab}	0.7 ± 0.4^{Ac}	0.2 ± 0.0^{Ad}
Geastrum schweinitzii	0 ± 0^{Bd}	11.6 ± 0.9^{Ca}	4.2 ± 0.8^{Cb}	0.3 ± 0.0^{Cc}	0.2 ± 0.1^{Ac}
G. echinulatum	0 ± 0^{Bd}	19.4 ± 1.1^{Ba}	3.8 ± 0.8^{Cb}	0.2 ± 0.1^{Cc}	0.2 ± 1^{Ac}
G. hirsutum	0 ± 0^{Bc}	1.3 ± 0.1^{Da}	0.3 ± 0.0^{Db}	0 ± 0^{Dc}	0 ± 0^{Bc}

Mean ± standard deviation of five replicates; different letters indicate significantly different values (ANOVA, $p < 0.05$); lowercase letters refer to the comparison between treatments and uppercase ones refer to the comparison between strains.

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DISCUSSION

The difference in development between the strains of the two genera may be attributed to the ecophysiological differences among species. These differences, coupled with substrate preferences, can influence mycelial growth and the production of biotechnologically significant enzymes (Wicklow et al., 1984). It is essential to understand that, under laboratory conditions, the growth and maintenance capacity of mycelial cultures depend on the adequate supply of nutrients provided by the culture medium (Prescott et al., 2002). Supplementation of the culture medium with lignocellulosic bran can be a valuable and advantageous technique for accelerating mycelial growth and biomass production in fungal strains (Donini et al., 2006; Minotto et al., 2008).

The *Geastrum* strains in this study were isolated from basidiomas that grew on leaf litter, where the need for enzyme production to degrade lignin is reduced (Wesenberg et al., 2003). This reduction may explain the absence of phenoloxidase enzyme production in a potato-based medium, for example. On the other hand, *Cyathus* strains were obtained from basidiomas that decompose trunks and branches, which stimulates a higher production of lignocellulosic enzymes (Sen et al., 2016). This difference in ecological niches likely contributed to the observed variation. When the culture medium was supplemented with 10% wheat bran, enzyme production was observed in *Geastrum* strains and increased in *Cyathus* strains.

For certain edible mushroom species, such as *Pleurotus ostreatus* (Jacq.) P. Kumm., wheat bran serves as a favorable substrate for mycelial development (Wang et al., 2001; Locci et al., 2008), as it did for the species in this study. Additionally, wheat-based substrates have been reported as effective inducers of enzyme activity (Mikiashvili et al., 2004; Papinutti and Lechner, 2008; Zhang et al., 2019; Cai et al., 2021). However, this enhancement comes at the expense of nutritional and cultural conditions, particularly oxygen levels and the availability of nitrogen (Silva, 2004). This relationship may help explain the differences in results observed with each type of bran used in the formulation of the culture media in this study. The low nitrogen content in rice bran may have had a similar inhibitory effect on gasteroid fungal strains, akin to what Rossi et al. (2003) observed in the cultivation of *Lentinula edodes* (Berk.) Pegler when they utilized the same type of bran.

These characteristics are likely associated with the ability to trigger the physiological mechanisms necessary for nutrient utilization from the culture medium (Mata et al., 2001). For instance, nitrogen, which is present in larger quantities in wheat bran, can be used more effectively, and its addition can also stimulate mycelial enzyme activity (Donini et al., 2006). However, it's worth noting that at high concentrations, nutrients can inhibit the development of certain fungal strains (Naraian et al., 2009). This observation might help explain the results obtained in this study, particularly in the case of the treatment with 20% wheat bran.

While the correlation between mycelial growth and phenoloxidase (PO) enzyme production hasn't been tested, the association between supplementation and enzyme production is widely documented for white rot fungi (Mikiashvili et al., 2005; Souza et al., 2006; Songulashvili et al., 2006, 2007; Osma et al., 2007), and this study may provide further corroboration. In the case of gasteroid fungi strains, the observed differences in mycelial growth, biomass production, and PO enzyme production, particularly in *Cyathus*, were also noted by Bernardi et al. (2008) in strains of *Agaricus brasiliensis* Fr. These findings can serve as motivation for advancing basic science with new research, highlighting the importance of obtaining fungal enzymes with significant biotechnological potential, especially in regions like the Amazon that have been underestimated in the scientific community.

CONCLUSION

This study deals with the first report, which presents promising results, regarding mycelial development and the production of enzymes of biotechnological interest using lignocellulosic products as a supplement to the culture medium for strains of gasteroid fungi in the Brazilian Amazon. Supplementing the potato-based culture medium with 10% wheat bran is recommended to enhance the mycelial development of the strains targeted in this study. It also serves as a significant inducer in the production of phenoloxidase enzymes, primarily by stimulating their synthesis in *Geastrum* strains and amplifying it in *Cyathus* strains. Furthermore, considering that culture media based on potatoes and wheat bran are readily available, this study broadens research horizons in regions with limited financial and human resources and little focus on basic science, particularly in the Amazon, where the diversity and biotechnological potential of fungi are still underestimated.

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Price volatility and demand of vegetables in three Brazilian Southeast supply centers between 2017 and 2021

Cindy Lee Cajachagua Pucuhuaranga[a], Risely Ferraz-Almeida[*b]

[a] Universidade de São Paulo, Brasil
[*] Autor correspondente (rizely@gmail.com)

INFO

Keywords
Lactuca sativa
Solanum lycopersicum
Solanum tuberosum
Allium cepa
Daucus carota

ABSTRACT

Vegetable consumption has increased in recent years due to the predisposition of people to have a healthy diet, which includes the ingestion of fibers, vitamins, and minerals present in fruits and vegetables. In Brazil, lettuce, tomato, carrot, onion, and potato are the five vegetables with the highest consumption. In this study, the price volatility and the price elasticity measure (price elasticity of supply, price elasticity of demand, or price inelasticity) of lettuce, tomato, carrot, onion, and potato were evaluated in three supply centers (*CEAGESSP, CEASAMINAS e CEASA/RJ*) between 2017 to 2021. The results indicated that all the evaluated products had similar price volatility behavior between 2019 to 2021 in the three supply centers. Moreover, the years with higher instabilities of prices were 2020 for potatoes and carrots, 2019 for lettuce, and 2018 for onion. The price elasticity or inelasticity had independent behavior per supply center. However, there are some periods of inelasticity where the price does not influence the quantity demanded or supplied of the evaluated products. In conclusion, there is high variability of price and volume for tomato, potato, onion, lettuce, and carrot during the year. There was a negative in the price and volume of vegetables which indicates that the reduction of volume increases the price of vegetables. The prices of commercialized vegetables are strongly influenced by the forces of supply and demand, which configures a seasonal characteristic.

Palavras-chaves
Lactuca sativa
Solanum lycopersicum
Solanum tuberosum
Allium cepa
Daucus carota

RESUMO

Volatilidade de preços e demanda de hortaliças na CEAGES/SP, CEASA/MG e CEASA/RJ durante 2017 e 2021
O consumo de hortaliças tem aumentado nos últimos anos devido à predisposição das pessoas para uma alimentação saudável, que inclui a ingestão de fibras, vitaminas e minerais presentes nas frutas e hortaliças. No Brasil, as cinco hortaliças com maior consumo são alface, tomate, cenoura, cebola e batata. Neste estudo calculou a volatilidade de preços e as medidas de elasticidades de preços (elasticidade preço da oferta, elasticidade preço da demanda ou inelasticidade preço) de cinco hortaliças em três centros de oferta (CEAGESSP, CEASAMINAS e CEASA/RJ) entre 2017 e 2021. Os resultados indicam que todos os produtos avaliados tiveram comportamento ou padrão semelhante de volatilidade de preços de 2019 a 2021 nos três centros de abastecimento. Além disso, os anos com maiores instabilidades de preços foram 2020 para batata e cenoura, 2019 para alface e 2018 para cebola. Além disso, o resultado mostra que a elasticidade ou inelasticidade de preço teve comportamento independente por centro de oferta, porém nesta pesquisa identificou alguns períodos de inelasticidade onde o preço não influencia na quantidade demandada ou ofertada do produto avaliado. Conclue que existe uma grande variabilidade de preço e volume de tomate, batata, cebola, alface e cenoura durante o ano. Existe uma correlação negativa do preço e volume das hortaliças o que indica que a redução do volume aumenta o preço das hortaliças no centro de abastecimento. Os preços das hortaliças comercializadas são fortemente influenciados pelas forças de oferta e demanda, o que configura uma característica sazonal.

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INTRODUCTION

Brazil is the greater producer of vegetables in the world with high production of garlic (*Allium sativum*), tomato (*Solanum lycopersicum*) potato (*Solanum tuberosum*), onion (*Allium cepa*), and carrot (*Daucus carota*) (EMBRAPA, 2023). The ingestion of fruits and vegetables is part of healthy eating patterns which is why in the last decade, one of the priorities around the world is to promote their consumption (Moreno and Ferraz-Almeida, 2023; Oliveira et al., 2022). The food demand depends on preferences or lack of access for socioeconomic or logistical reasons, being the last two the most recurrent in developing countries (FAO, 2021).

The price volatility is the variation of price changes around their mean value. At present, it is an ongoing concern because it may have a negative impact at the economic level on growth and poverty as reported by some economists. Thus, it is important to know the evolution of price volatility to develop different instruments and design appropriate policies to transfer risk or at least to lessen the extent of world market price volatility (Brander et al. 2023).

Additionally, it is important to understand the demand and supply of vegetables to help in the decisions regarding market performance and market activities. The elasticity is one of those analyses, and is an economic instrument that measures the rate at which quantities of a product respond to price changes; the percentage at which a one percent change in prices will cause a certain percentage change in quantities (Rosales and Mercado, 2020).

There are four different elasticity measurements: price elasticity of demand, income elasticity of demand, price elasticity of supply, and cross-price elasticity (Mankiw, 2001). The size of the price elasticities is important from a policy perspective because if the price elasticity is greater than one, any increase in the price will lead to a reduction in the quantity exported, so the governments have to stabilize the income of farmers with subsidies.

This study has two hypotheses: (i) due to the price volatilities in the fruits sector during the year, there is a pattern for each product, and (ii) the price elasticities are highly variable depending on the product within the vegetable sector. The aim is to analyze the price volatility, demand, and elasticity of vegetables in Brazil during 2017 and 2021.Sweet oranges also contain natural pigments known as carotenoids, displaying colors ranging from yellow to red. Classified as tetraterpenes (C40), these pigments are composed of isoprene units (C5). Although generally insoluble in water, carotenoids can dissolve in organic solvents like ethanol (Kultys and Kurck, 2022; Honda et al., 2019; Tiwari et al., 2019). Carotenoids fall into two categories: (a) carotenes, identified by a linear hydrocarbon chain with one or two cyclic structures at the ends, and (b) xanthophylls, oxygenated compounds derived from carotenes. Among the most prevalent in citrus peels are α-carotene, β-carotene, lutein, zeaxanthin, and β-cryptoxanthin (Figure 2) (Maoka, 2020; Meléndez-Martínez et al., 2019)

MATERIAL AND METHODS

Study characterization

The study was developed between 2017 and 2021. Lettuce, tomato, potato, onion, and carrot were the vegetables selected for the study due to the high demand and production in Brazil. The distribution centers are (i) *Companhia de Entrepostos e Armazéns Gerais de São Paulo (CEAGES/SP*; DC 1), located in Vila Leopoldina – São Paulo – São Paulo; (ii) *Companhia de Entrepostos e Armazéns Gerais de Minas Gerais (CEASA*/MG), located Rodovia BR-040 km 688, Kennedy, Contagem, Minas Gerais; (iii) *Centro de Abastecimento do Rio de Janeiro, (CEASA*/RJ), located in Irajá, Rio de Janeiro, Rio de Janeiro.

Data collecting and analysis

A data set was created using data from the *Boletim Hortigranjeiro*, available by the National Supply Company (CONAB, 2022) with information on vegetable prices from years 2017, 2018, 2019, 2020, and 2021. For all the calculus, the units for these prices were reais per kilograms (R$/Kg) to Brazilian reality and the prices were corrected by the index IPCA (*Índice de Preços ao Consumidor Amplo*), which measures the inflation of a set of products sold in retail, using the online calculator, available by IBGE (2022).

To analyze the price elasticities, we utilized the previous data and complemented it with information on the quantity sold. The quantity sold over the years was estimated by the bar graphs from the *Boletim Hortigranjeiro* (CONAB, 2022). The calculus of quantities used the ton unit in all the cases.

The price volatility was defined as price variability around a central value. The tendency of individual prices to vary from their mean value. Volatility is often defined as high deviations from a global tendency. In this study, we calculated the historical volatility, based on past prices of the last five years, using the coefficient of variation (CV) (Eq. 1), which is described in the investigations of Traore and Diop (2021). This measure was calculated per year from 2017 to 2021 and for each of the ten products selected previously.

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The price elasticity of demand or the elasticity of demand measures the responsiveness of consumers to a change in price. Sometimes price elasticities of demand are reported as negative numbers. This is because the percentage change in quantity will always have the opposite sign as the percentage change in price.

In the present study, we used the absolute value for dropping the minus sign and reported the results as positive numbers when we were comparing the price elasticities of demand of a specific product, but we maintained the negative sign to differentiate the price elasticities of demand from the price elasticities of supply. The price elasticity of demand is mathematically defined as the percentage at which a one percent change in prices will cause a certain percentage change in quantities (Mankiw, 2001). The price elasticity of demand was calculated according to Eq. 1.

Eq. 1

$$e_d = \left|\frac{\%\Delta Q_d}{\%\Delta P}\right|$$

where $\boldsymbol{e_d}$ is the price elasticity of demand or coefficient of demand, $\%\Delta Q_d$ is the percentage change in quantity demanded and $\%\Delta P$ is the percentage change in price.

To facilitate the calculation of the price elasticity of demand we used the midpoint method, described in Eq. 2, and based on studies of Mankiw (2001).

Eq. 2

$$e_d = \left|\frac{\frac{Q2-Q1}{\left(\frac{Q1+Q2}{2}\right)}}{\frac{P2-P1}{\left(\frac{P1+P2}{2}\right)}}\right|$$

where $\boldsymbol{e_d}$ is the price elasticity of demand, Q_1 is the quantity demanded at time 1, Q_2 is the quantity demanded at time 2, P_1 is the price at time1, and P_2 is the price at time 2.

We can interpret the $\boldsymbol{e_d}$ as follows: if the $\boldsymbol{e_d}$ is greater than one the demand is elastic, so the quantity demanded changes by a larger percentage than does price; if $\boldsymbol{e_d}$ is equal to 1, the demand is unitary elastic, so the percentage increase in quantity demanded is equal to percentage decrease in price; and if the $\boldsymbol{e_d}$ is less than 1, the demand is inelastic which means that quantity demanded is relatively insensitive to price (Mankiw, 2001).

The price elasticity of supply measures how much the quantity supplied responds to changes in the price. It is because sometimes producers of a good offer to sell more of it when the price of the good rises (Mankiw, 2001). Thus, economists compute the price elasticity of supply as the percentage change in the quantity supplied divided by the percentage change in the price (Eq. 3). In addition, the price elasticity of supply is never negative since price and quantity supplied are directly related.

Eq. 3

$$e_S = \left|\frac{\%\Delta Q_s}{\%\Delta P}\right|$$

where $\boldsymbol{e_s}$ is the price elasticity of supply or coefficient of supply, $\%\Delta Q_s$ is the percentage change in quantity supplied and $\%\Delta P$ is the percentage change in price. In the same way that the price elasticity of demand, the price elasticity of supply can be calculated by the midpoint method (Eq. 4) (Mankiw, 2001).

Eq. 4

$$e_S = \left|\frac{\frac{Q2-Q1}{\left(\frac{Q1+Q2}{2}\right)}}{\frac{P2-P1}{\left(\frac{P1+P2}{2}\right)}}\right|$$

where $\boldsymbol{e_s}$ is the price elasticity of supply, Q_1 is the quantity supplied at time 1, Q_2 is the quantity supplied at time 2, P_1 is the price at time 1, and P_2 is the price at time 2. The degree of price elasticity or inelasticity of supply is measured by the $\boldsymbol{e_s}$. If the $\boldsymbol{e_s}$ is greater than one the supply is elastic, which means that producers are relatively responsive to price changes. If the $\boldsymbol{e_s}$ is equal to 1 the supply is unit elastic, which indicates that the quantity produced changes in the same percentage as the price. On the other hand, if the $\boldsymbol{e_s}$ is less than 1, the supply is inelastic, so the producers are relatively insensitive to price changes (Mankiw, 2001).

The results of price volatilities and price elasticities are analyzed per vegetable. We showed the results in tables containing four statistical measures: standard deviation (SD), mean, maximum value, and minimum value to have a context in the data set (price volatilities and price elasticities calculated), per supply center (CEAGESSP, CEASAMINAS or CEASA/RJ) and from 2017 to 2021. Correlations between price and volumes in the supply center (CEAGESSP, CEASAMINAS, or CEASA/RJ) were tested by the Pearson correlation using a p of 0.05.

RESULTS AND DISCUSSION

Prices and volumes of vegetables in CEASA/GEAGES

There was a variation in price between the CEASA and GEAGES of lettuce, tomato, potato, onion, and carrot during 2017 and 2021 (Figure 1). The prices of vegetables are higher in DC 1 and DC 2, while DC 3 presented the lower price exception to the price of lettuce that was higher in DC 3 (Fig-

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ure 1). This result was expected because DC 1 attends all the demands of the great city in Brazil, as São Paulo capital and great-São Paulo.

In addition, DC 1 is considered the greater vegetable and fruit markets and the largest intermediary, playing the role of distribution center for other wholesale markets (Mayorga et al., 2017). The fruits and vegetables traded in DC 1 are produced in different regions of Brazil, mainly the vegetables produced in the nearby region. In the region of São Paulo, the area of incidence of the Cinturão + Verde in the Cabeceiras sub-basin, in the Alto Tietê, which comprises 10 municipalities, mainly Salesópolis, Biritiba-Mirim, Mogi das Cruzes, Suzano, Poá, Itaquaquecetuba, Ferraz de Vasconcelos, Arujá, Guarulhos, and São Paulo (FGV, 2022).

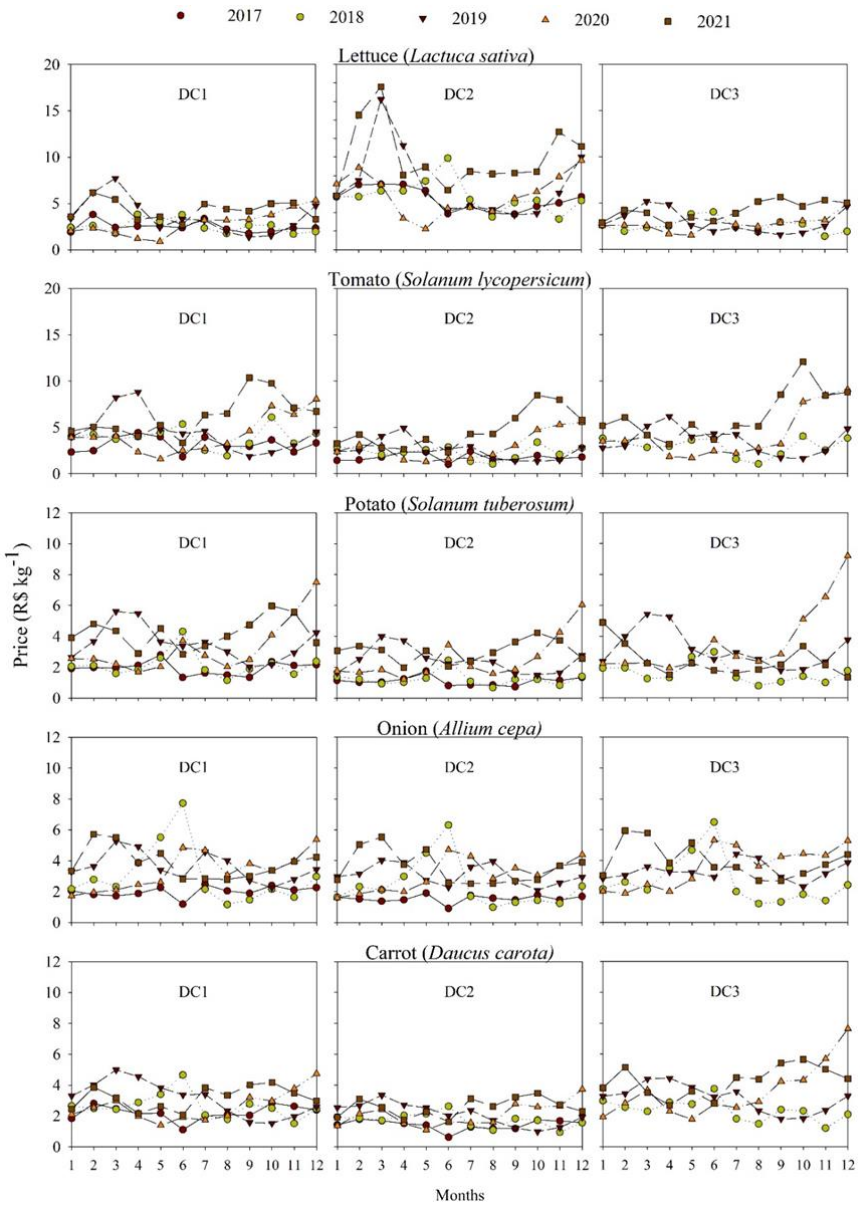

Figure 1 - Monthly price of lettuce, tomato, potato, onion and carrot in the CEAGES/SP (DC1), CEASA/MG (DC 2) and CEASA/RJ (DC3) between 2017 and 2021

Between supply centers, the lettuce presented a higher price in DC 2 (R$ 6.79 kg^{-1}), followed by DC 1 (R$ 3.14 kg^{-1}) and DC 3 (R$ 3.12 kg^{-1}). Tomato presented a higher price in DC 1 (R$ 4.40 kg^{-1}), DC 3 (R$ 4.24 kg^{-1}), and DC 2 (R$ 2.83 kg^{-1}), as well there was a higher price of potato in DC 1 (R$ 3.01 kg^{-1}), DC 3 (R$ 2.69 kg^{-1}) and DC 1 (R$ 2.04 kg^{-1}), Figure 1.

In DC 3, there was a higher price of onion with an average of R$ 3.38 kg^{-1} followed by DC 1 (R$ 3.15 kg^{-1}) and DC 1 (R$ 2.71 kg^{-1}), as well there was a higher price of carrot in DC 3 (R$ 3.32 kg^{-1}), DC 1 (R$ 2.75 kg^{-1}) and DC 2 (R$ 1.97 kg^{-1}), Figure

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1. In DC 1, there was a higher traded volume of lettuce (3,974 tons month^{-1}), tomato (21,159 tons month^{-1}), potato (21,298 tons month^{-1}), onion (8,930 tons month^{-1}), and carrot (814 tons month^{-1}), Figure 2.

During the months, there was a constant demand for tomatoes, potatoes, onions, and carrots (Figure 2). The second demand for vegetables was in DC 3, where there was a demand for lettuce (272 tons month^{-1}), tomato (7836 tons month^{-1}), potato (18,555 tons month^{-1}), and onion (7574 tons month^{-1}). While DC 2 presented the second higher volume of carrots with an average of 814 tons month^{-1} (Figure 2).

We can observe an alteration in lettuce demand with lower demand in the winter and higher demand in the summer. The years with higher instabilities of prices were 2020 for tomato, potato, and carrot, 2019 for lettuce, and 2018 for onion. Camargo Filho and Mazzei (2000) described that the quantity demanded of salad vegetables is higher in spring and summer, while vegetables, roots, and tubers have an increase in demand in autumn and winter. In our study, there was no alteration in demand for tomatoes, potatoes, onions, and carrots which are vegetables presented in diary food preparation. A similar result was presented by Furquim et al. (2023) with tomatoes in Goiás in constant demand during the year.

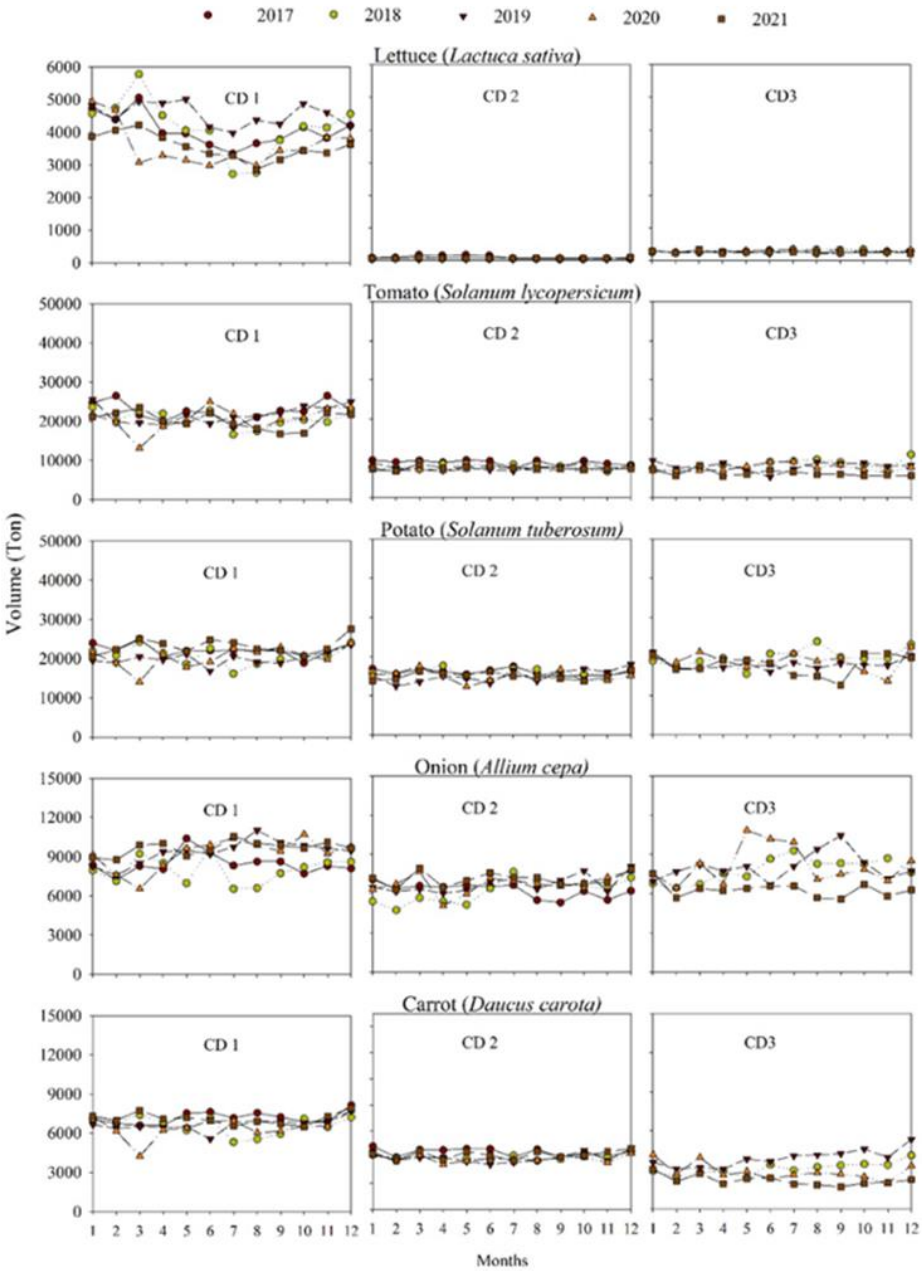

Figure 2 - Monthly volume of lettuce, tomato, potato, onion and carrot in the CEAGES/SP (DC1), CEASA/MG (DC 2) and CEASA/RJ (DC3) between 2017 and 2021

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In all supply centers, there was a significant interaction between the volume and price of vegetables with a negative r of -0.13 ($p<0.05$). This result indicates that the reduction of volume increases the price of vegetables in the all-supply center (Figure 3). The prices of commercialized vegetables are strongly influenced by the forces of supply and demand, which configures a seasonal characteristic (Pantoja et al. 2023).

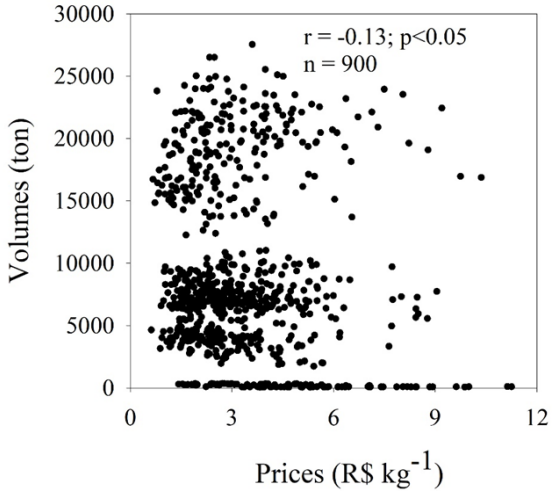

Figure 3 - Correlation between price and in the CEAGES/SP (DC1), CEASA/MG (DC 2) and CEASA/RJ (DC3) between 2017 and 2021. n: is the number of observations

There were significant influences between the supply center with significant correlations higher than 0.45 ($p<0.05$), Figure 4. For the prices, the higher influence was between CD 1 and CD 3 ($r = 0.82$; $p < 0.05$) with the price of vegetables concentrated and similar between them. While there CD 3 and CD 2 ($r = 0.45$; $p < 0.05$) presented a lower correlation (Figure 4).

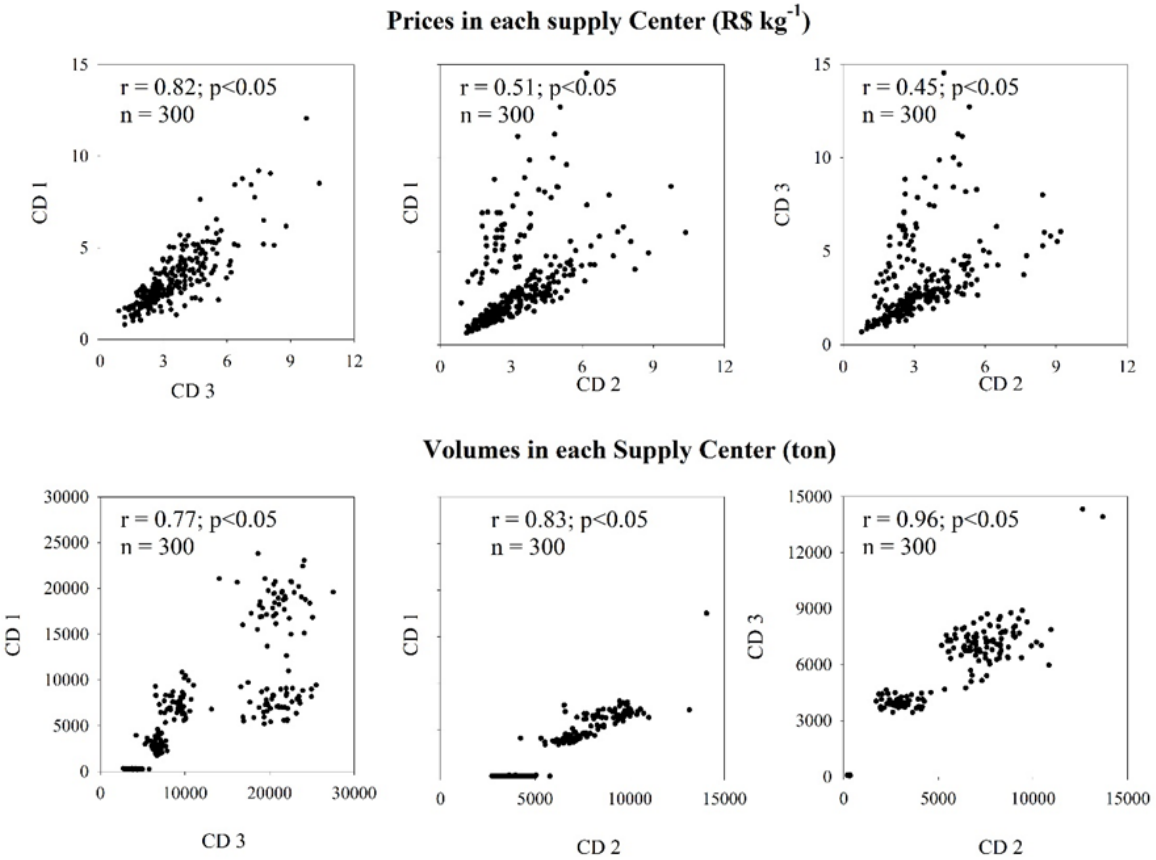

Figure 4 - Correlation between price and volumes in the CEAGES/SP (DC1), CEASA/MG (DC 2) and CEASA/RJ (DC3) between 2017 and 2021. n: is the number of observations

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Interest, for volume the higher correlation was noticed between CD 3 and CD 2 ($r = 0.96$; $p < 0.05$) followed by CD 1 and CD 2 ($r = 0.83$; $p < 0.05$), Figure 4. The influence on volumes and prices between the the supply center is associated with the demand and influence of each market (Pantoja et al. 2023). Mayorga et al. (2017) demonstrated that the price variation of melon in CD 1 influenced also the price variation in Açu/Mossoró/RN and Baixo Jaguaribe/CE represent the national biggest melon production areas impacting the Centers of Natal and Fortaleza

.

Elasticity measurements

There was variation between the elasticity price of vegetables during the years. The lettuce presents the alteration in the second semester and the initial of the first semester. While, there is a predominance of variation of price in the initial of the first semester for tomato, potato, onion, and carrot (Figure 5).

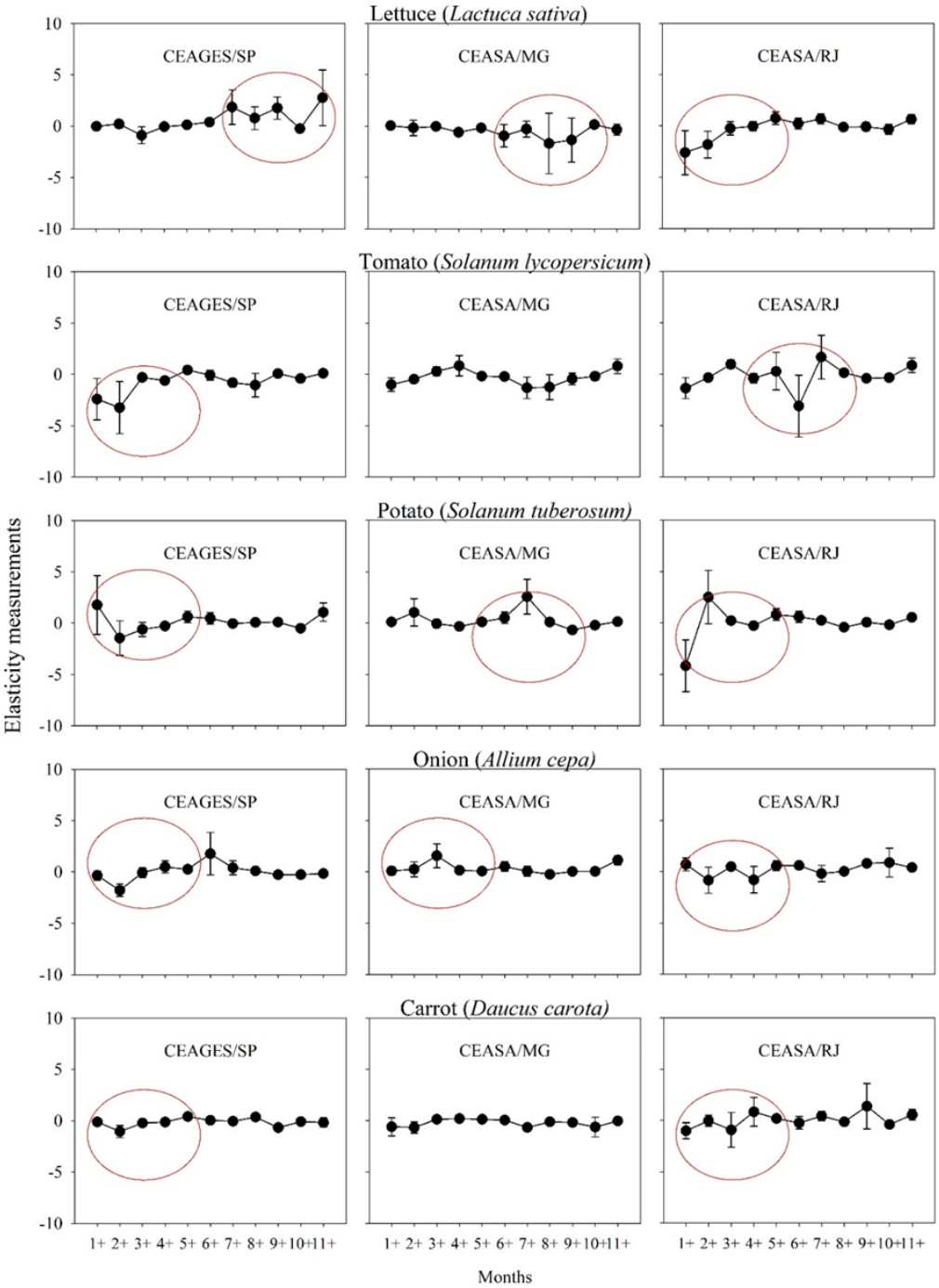

Figure 5 - Monthly elasticity price of lettuce, tomato, potato, onion and carrot in the CEAGES/SP (DC1), CEASA/MG (DC 2) and CEASA/RJ (DC3) between 2017 and 2021

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This result occurs because the price of vegetables has a direct effect on the quantity supplied in the market, which is influenced by the climate. In the field, the occurrence of greater or lesser rainfall and the temperature variation (heat or cold) determine the cost of production and yield at the station. Also, there was a larger volume of vegetables and fruits which are lost in the market impacting the price to consumers (Ferraz-Almeida and Martins, 2022)

Uçak et al. (2022) demonstrated that in Turkey there is a significant volatility spillover from the energy price index to the vegetable price index, whereas there is no statistically significant volatility spillover to the fruit price index. In Greece, Rezitis and Pachis (2020) showed that in the tomato and cucumber markets, which are regulated by the Common Market Organization of fruits and vegetables, producers are less vulnerable to volatility shocks transmitted from consumers. In contrast, in the non-regulated potato market, producers are affected by spillover effects from consumers.

CONCLUSIONS

The prices of vegetables are higher in DC 1 and DC 2, while DC 3 presented the lower price exception to the price of lettuce that was higher in DC 3. During the five evaluated years, four of the five vegetables (tomato, potato, onion, and carrot) were cheaper in DC 2. About the lettuce, the supply center where the prices were lower was DC 3. On the other hand, tomatoes and potatoes were more expensive in DC 1. Moreover, onions and carrots were more expensive in DC 3, and lettuce was more expensive in DC 2. About the price volatility, all the evaluated products had similar behavior from 2019 to 2021 in the three supply centers. The years with higher instabilities of prices were 2020 for tomato, potato, and carrot, 2019 for lettuce, and 2018 for onion. Based on the results concluded that there is high variability of price and volume for tomato, potato, onion, lettuce, and carrot during the year. There was a negative in the price and volume of vegetables which indicates that the reduction of volume increases the price of vegetables in the all-supply center. The prices of commercialized vegetables are strongly influenced by the forces of supply and demand, which configures a seasonal characteristic.

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Chemotaxonomic classification of closed-related sweet oranges (*Citrus sinensis* L.) using principal component and cluster analysis of color and bioactive compounds in the orange peels

Isabelli Bottene Casagrande[a], Gabriel Antonio Gianjope Casarotti[a], Claudio Lima[b*]

[a] Universidade de São Paulo, Brasil
[b] GS4/Science Consulting and Solutions
[*] Autor correspondente (claudio.gs4science@gmail.com)

INFO

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secondary metabolism
chemometrics

ABSTRACT

Chemotaxonomy is a systematic approach to plant classification based on discernible variations and similarities in their biochemical compositions, with a particular emphasis on secondary metabolites that often exhibit specificity within taxonomically related plant species. In the context of our investigation, we conducted a comprehensive chemotaxonomic analysis of sweet oranges utilized in concentrated juice production. This involved a thorough examination of antioxidant activities, characterization of flavonoid and phenolic profiles, UV-visible spectra scanning, and the implementation of color analysis. The results from cluster analysis demonstrated the superior efficacy of color analysis in discerning subtle distinctions among orange varieties. Further refinement in the analytical process requires the application of supplementary techniques after an exhaustive and precise extraction facilitated by organic solvents. Notably, all employed methodologies successfully discriminated between distinct orange types. While the compilation of extensive datasets presents inherent challenges, the incorporation of Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) enhances the interpretability of the obtained results. Considering that existing methodologies for assessing fresh fruit quality rely on subjective human visual inspection or orange juice analysis by ratio, which may lack precision in quantifying chemical constituents, there exists a growing demand for expedited techniques that can reliably ensure the quality of orange juice within the industry.

Palavras-chaves
laranja
classificação
composição química
metabolismo secundário
quimiometria

RESUMO

Classificação quimiotaxonômica de laranjas doces relacionadas (Citrus sinensis L.) utilizando análise de componentes principais e análise de agrupamento de cor e compostos bioativos nas cascas das laranjas
A quimiotaxonomia é uma abordagem sistemática para a classificação de plantas com base em variações e semelhanças discerníveis em suas composições bioquímicas, com ênfase especial em metabólitos secundários que frequentemente apresentam especificidade dentro de espécies de plantas taxonomicamente relacionadas. No contexto de nossa pesquisa, conduzimos uma análise quimiotaxonômica abrangente de laranjas doces utilizadas na produção de suco concentrado. Isso envolveu uma examinação minuciosa das atividades antioxidantes, caracterização dos perfis de flavonoides e fenólicos, varredura dos espectros UV-visíveis e a implementação da análise de cor. Os resultados da análise de agrupamento demonstraram a eficácia superior da análise de cor em discernir distinções sutis entre as variedades de laranja. Um refinamento adicional no processo analítico requer a aplicação de técnicas suplementares após uma extração exaustiva e precisa facilitada por solventes orgânicos. Notavelmente, todas as metodologias empregadas discriminaram com sucesso entre diferentes tipos de laranja. Embora a compilação de conjuntos de dados extensos apresente desafios inerentes, a incorporação da Análise de Componentes Principais (PCA) e Análise de Agrupamento Hierárquico (HCA) aprimora a interpretabilidade dos resultados obtidos. Considerando que as metodologias existentes para avaliar a qualidade de frutas frescas dependem de inspeção visual subjetiva humana ou análise de suco de laranja por meio de proporção, o que pode carecer de precisão na quantificação de constituintes químicos, existe uma demanda crescente por técnicas rápidas que possam garantir de maneira confiável a qualidade do suco de laranja na indústria.

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INTRODUCTION

Brazilian orange juice holds a prominent position as the largest industry globally (Blauer, 2023). Due to the extensive scale and cost-effectiveness of the final product, there is a pressing need to reduce production costs, particularly those linked to sorting fruits based on type and quality. In response to emerging market concepts, the orange juice industry has been fostering collaborations with academia, facilitating opportunities for open innovation (Granato et al., 2018; Saguy and Sirotinskaya, 2016). To comprehend the impact of chemical composition on color changes, it is essential to first understand orange trees (*Citrus sinensis* (L.) Osbeck), members of the Rutaceae family and prolific producers of the world's most cultivated fruits (Bayer et al., 2009; Pfeil and Crisp, 2008). While numerous orange varieties exist, sweet oranges dominate in Brazilian citrus belts, particularly for juice extraction (Rodrigues et al., 2019). The fruit comprises juice vesicles containing sugars, organic acids, vitamins, minerals, pectins, and pigments, shielded by a peel with a wax film responsible for the fruit's color and aroma compounds (Matheyambath et al., 2016). The peel is notably rich in phenolic compounds, flavonoids, carotenoids, and terpenes (Saini et al., 2022), This will significantly contribute to the theme of the present study.

Phenolic compounds are secondary metabolisms produced by plants, usually to protect them from environmental adversities or even as an attraction for pollinators, and which have in their structure the phenol group, an aromatic ring with a hydroxyl group (Chen et al., 2020a; Malta and Liu, 2014). These compounds are classified according to their main skeleton, having as a basic structure a benzene ring (C6). The main classes are phenolic acids (C6-C1), flavonoids (C6-C3-C6), coumarins (C6-C3), tannins (C6-C3-C6n), lignins (C6-C3n), among others (Vuolo et al., 2019; Tsimogiannis and Oreopoulou, 2019). The polyphenol content is higher in the citrus peel than in the pulp, such as caffeic, ferulic, synaptic, and p-cumaric acids (Bouhafsoun et al., 2018). Flavonoids are the largest class of plant phenolics, whose basic structure contains two aromatic rings connected by a bridge with three carbons. They can be divided into six different groups: flavones, flavanones, flavonols, isoflavones, anthocyanidins, and catechins. Some studies suggest that flavonoids represent about 2–3% (w/v) in citrus peels (Denaro et al., 2021; Nieto et al., 2021). Naringin, neoeriocitrin, and neohesperidine are the main flavanones found in the bergamot (400–1,000 mg/100 g), lemon (400–600 mg/100 g) and sour orange (380–1,100 mg/100 g) peels (Wang et al., 2021). Hesperidin and narirutin are most abundant in sweet oranges (270–350 mg/100 g of dry peel) (Sawalha et al., 2009). Flavonoids have aromatic chromophores indicating absorption in the UV region (250 nm), which can undergo π, π* excitation and react from excited states π, π*. They may also contain carbonyl chromophores and absorb UV in the region of 300 nm (Krishnan et al., 2021). This characteristic allows us to analyze the flavonoids by scanning in the UV region from 200 to 360 nm, and this diversity in composition and its chemical structures will contribute to the establishment of rapid analysis protocols for distinguishing between types of sweet oranges.

Some chemical structures of the main citrus peel flavonoids are shown in Figure 1.

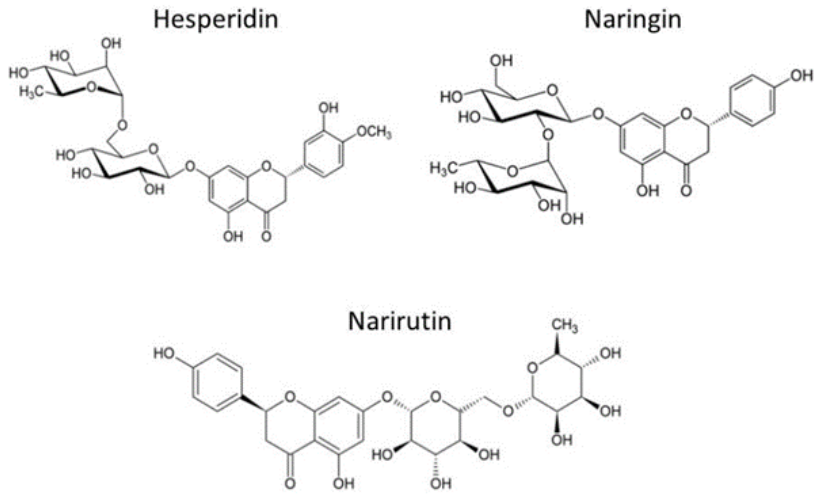

Figure 1 - Chemical structure of the main citrus peel flavonoids: hesperidin, naringin, and narirutin

Sweet oranges also contain natural pigments known as carotenoids, displaying colors ranging from yellow to red. Classified as tetraterpenes (C40), these pigments are composed of isoprene units (C5). Although generally insoluble in water, carotenoids can dissolve in organic solvents like

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ethanol (Kultys and Kurck, 2022; Honda et al., 2019; Tiwari et al., 2019). Carotenoids fall into two categories: (a) carotenes, identified by a linear hydrocarbon chain with one or two cyclic structures at the ends, and (b) xanthophylls, oxygenated compounds derived from carotenes. Among the most prevalent in citrus peels are α-carotene, β-carotene, lutein, zeaxanthin, and β-cryptoxanthin (Figure 2) (Maoka, 2020; Meléndez-Martínez et al., 2019)

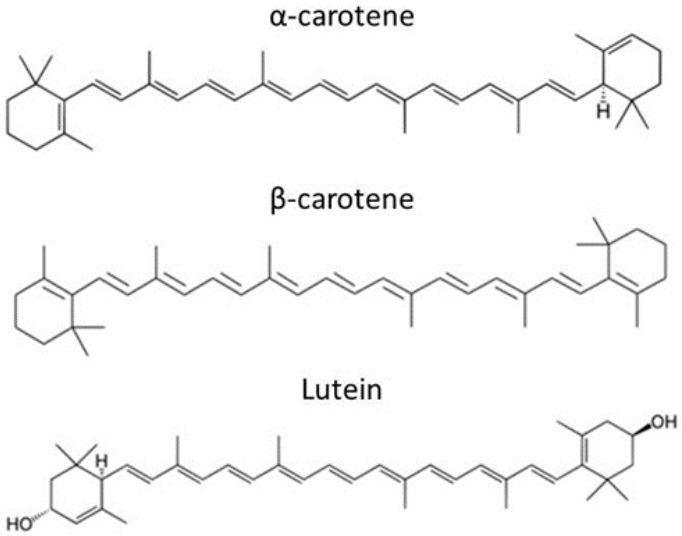

Figure 2 - Chemical structures of carotenoids present in orange peels

Utilizing instrumental measurements to assess the color of orange juice through its carotenoids offers a valuable tool for quality control. Carotenoids (along with chlorophylls) primarily absorb light in the visible region, and their *in vivo* measurement is feasible when exposed to UV light (Pérez-Gálvez and Fontecha, 2020; Virtanen et al., 2020). As indicated by Flores-Hidalgo et al. (2017), electronic properties can be examined through energy representation in boundary orbitals, as demonstrated in UV-visible absorption spectroscopy. Certainly, light is undeniably a primary environmental factor influencing the color of fruits, significantly impacting the absorption spectrum by their carotenoid composition (Lado et al., 2019). The color holds undeniable importance as a pivotal parameter for quality control, especially in the context of fresh fruits (Pigni et al., 2020; Murador et al., 2019).

The significance of phenolic compounds in orange peel is apparent, yet there is no consensus on their quantification and identification methods; while many authors recommend liquid phase chromatographic methods (with or without coupling with mass spectrometers) for pigment quantification and identification, the extraction protocols for these compounds are widely debated due to high costs or lengthy sample preparation times (Saini and Keum, 2018). The pursuit of fast analytical and exploratory data methods that facilitate recognizing similarities between samples is crucial for the fruit and its derivatives industries (Baqueta et al., 2020; Quelal-Vásconez et al., 2020). Granato et al. (2018) propose that principal component analysis (PCA) reduces dimensionality, visualizing intricate connections between bioactive compounds and functional properties, while hierarchical cluster analysis (HCA) categorizes similar items, revealing patterns; their comprehensive multivariate approach concurrently examines these components, unveiling subtle connections often overlooked in univariate analyses, with a primary focus on bioactive compounds, exploring their correlation with sensory, nutritional, and processing attributes, and conducted with a discerning perspective, critically evaluating the limitations, challenges, and implications of using PCA and HCA, providing a nuanced exploration of intricate relationships in foods, like fruits. Additionally, Granato et al. (2017) underscored the pivotal role of experimental design and statistical data analysis in understanding interconnections among nutrition, biology, pharmacology, functional properties, and chemical components in foods and their extracts; in the evaluation of diverse food extracts and bioactivities, the application of chemometric tools and statistical methodologies gains heightened relevance, as discussed by Granato et al. (2014). Spagolla et al. (2009) also reported an intercorrelation among phenolic compounds in *Vaccinium ashei*, extracted in hydroalcoholic solutions, and their effects on in vitro biological activity; the same classes of compounds that have been associated with the coloring of *V. ashei* fruits.

The implementation of intelligent systems

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capable of automatically sorting based on predetermined standards (Pérez-Gálvez et al., 2020) or utilizing various measurable variables reflecting similarities in the physical and chemical properties holds significant promise for the typification of orange types in the industry (Basri et al., 2019). Given the intricacies involved in human visual perception for orange quality control or distinguishing between varieties, this study assesses the discriminative capacity among three commonly found sweet orange varieties in juice industries. It proposes a straightforward method for extracting and analyzing the chemical composition of orange peel, coupled with chemometric analysis.

MATERIAL AND METHODS

Harvesting of orange samples in the industry

Sweet oranges (*Citrus sinensis* L.) were carefully harvested and sourced from Citrosuco Agroindustry S/A, Brazil. The harvesting of the Hamlin, Pêra-Rio, and Valencia cultivars occurred in June, July-August, and September 2019, respectively, at a farm situated in the São Paulo state within the Bebedouro orange belt, Brazil. The harvest timing was meticulously determined based on fruit ripeness, guided by the fruit ratio, a measure encompassing the ratio between total soluble solids and acidity. For this study, a specific ratio of 14 was rigorously established during the selection of fruits, ensuring a precise and standardized approach for the present research.

Preparation of methanolic extracts from orange peel

Peels from three varieties of oranges (Hamlin, Pêra-Rio, and Valencia) were finely grated and combined with analytical-grade methanol at a ratio of 1:5 (w/v). The mixture underwent ultrasonication in a water bath (Unique model T28220; frequency of 25 kHz and power of 120 W) at 35 °C for 1 hour. Subsequently, the liquid phase was separated by filtration using a qualitative filter (0.8 mm diameter pore) to completely isolate the fibrous components. The methanolic extracts underwent distillation to partially remove the solvent, and the concentrated extracts were stored at 4 °C until compositional analyses were conducted. The samples were designated as Hamlin orange peel, Pêra-Rio orange peel, and Valencia orange peel methanolic extracts.

Colorimetric analysis of total phenolic compounds

The quantitative analysis of total phenolic compounds in methanolic extracts of orange peel was conducted utilizing the Folin-Ciocalteu spectrophotometric method, following the procedure outlined by Mazen and Nashat (2019). Tannic acid served as a known standard for constructing the calibration curve. The ethanolic extracts were appropriately diluted, and a 1 mL aliquot of each sample was transferred to a test tube containing 0.5 mL of Folin-Ciocalteu reagent (diluted in water at a 1:10 ratio, v/v). After shaking, the test tubes were allowed to rest for 40 minutes. Subsequently, 2.5 mL of 20% sodium carbonate (Na_2CO_3) was added to the mixture. Absorbance readings were taken at 725 nm using a UV-Mini 1240 spectrophotometer (Shimadzu, Japan). A blank sample using ultrapure water was prepared under the same conditions. The results were expressed in tannic acid equivalents as the mean±standard deviation (in mg tannic acid equivalent per g of sample).

Colorimetric analysis of total flavonoids

The total flavonoid analysis of methanolic extracts from orange peel was carried out following the spectrophotometric method proposed by Sartori et al. (2013), with rutin serving as a known standard for constructing the calibration curve. In test tubes, 0.5 mL of methanolic extracts were combined with 4.3 mL of 70% ethanol, 0.1 mL of 2% aluminum chloride, and 0.1 mL of sodium acetate at 1 mol L^{-1}. After shaking, the test tubes were left undisturbed for 40 min, and absorbance readings were taken at 415 nm using a UV-Mini 1240 spectrophotometer (Shimadzu, Japan). A blank sample using ultrapure water was prepared under identical conditions. The results were expressed in rutin equivalents as the mean±standard deviation (in mg rutin equivalent per g of sample).

High-performance liquid chromatography (HPLC) profiles of orange peel methanolic extracts

Flavonoid analysis of methanolic extracts from orange peel was conducted following the method outlined by Aguiar et al. (2012), employing a high-performance liquid chromatography model LC-20AT with a photodiode array detector (model SPD-M20A; Shimadzu, Japan). The elution of compounds occurred on a C18 column model Shim-pack CLC ODS(M) using a mobile phase consisting of solvent A (0.2% formic acid in ultrapure water) and solvent B (acetonitrile), with an elution flow of 1.0 mL min^{-1} and the column maintained at 40 °C. The gradient system was as

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follows: 0 to 8 min: 10 to 13% B; 8 to 25 min: 13 to 20% B; 25 to 40 min: 20 to 40% B; 40 to 45 min: 40 to 60% B; 45 to 50 min: 60 to 10% B; 50 to 65 min: 10% B. A 10 µL injection volume was used, and the concentration of naringin and hesperidin was determined against a calibration curve utilizing authentic flavonoid standards (HPLC grade) at 270 nm.

Color analysis directly on orange peels by CIELab system

The color of the orange peel was assessed using the CIELab system, employing the Minolta colorimeter (model CR-300, Japan), with guidance provided by Dr. A. P. Jacomino from the University of São Paulo. Three readings were captured at equidistant points in the equatorial region of 15 whole fruits for each variety. The results were presented in terms of luminosity (L*), a* coordinate, b* coordinate, hue angle (*h*), and chromaticity (c).

Spectral scanning of orange peel methanolic extracts

The determination of the UV-visible spectral absorption maximum was conducted by combining 10 µL of each methanolic extract from the orange peel with 5 mL of analytical-grade methanol. The spectra were swiftly acquired using a UV-Mini 1240 spectrophotometer (Shimadzu Co., Japan) across the range of 200 to 600 nm.

***In vitro* antioxidant activity by DPPH radical scavenging**

According to Mensor et al. (2001), each methanolic extract from orange peels (0.5 mL) was placed in test tubes containing 3 mL of 95% ethanol (v/v) and 0.3 mL of DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical at a concentration of 0.3 mmol mL^{-1} in methanol. The solutions were thoroughly mixed and left undisturbed for 40 min at room temperature in a dark container. The negative control was prepared by replacing the extracts with ethanol (used as a diluent). The color change was measured using a spectrophotometer at 517 nm, and the DPPH radical scavenging activity was determined as the antioxidant activity percentage (AA%):

$$AA\% = 100 - \frac{(Aa - Ab) \times 100}{Ac}$$

Where: Aa = absorbance of orange peels methanolic extracts; Ab = absorbance measured of methanol; Ac = absorbance of negative control.

Total antioxidant capacity by phosphomolybdenum method

Following the procedure outlined by Prieto et al. (1999), 0.1 mL of ethanolic extracts from orange peels was introduced into test tubes containing 1 mL of a reactive solution (comprising sulfuric acid at a final concentration of 3 mol L^{-1}, monobasic sodium phosphate at 0.1 mol L^{-1}, and ammonium molybdate at 30 mmol L^{-1}). The test tubes were maintained at 95 °C for 90 min. After cooling to room temperature, the absorbance of the samples was measured using a spectrophotometer at 625 nm. A blank control was prepared by substituting the extracts with ultrapure water. A calibration curve was established by diluting ascorbic acid (HPLC grade) at 200 µmol L^{-1} and adding quantitative ultrapure water to achieve concentrations ranging from 10 to 200 µmol L^{-1}. The antioxidant capacity of the methanolic extracts was expressed as ascorbic acid equivalent, specifically in micrograms of ascorbic acid per milliliter of orange peel methanolic extract.

Statistical data analysis

In this analysis, the study delved into the statistical differences in key components of orange peels, namely total phenolics, total flavonoids, antioxidant activities, and the content of narigin and hesperidin, determined using high-performance liquid chromatography (HPLC). To scrutinize these differences, a one-way analysis of variance (ANOVA) and Tukey's means test were employed, revealing insights into the variability among the samples. The results, crucial for understanding the overall trends, were presented as the mean value±standard deviation based on a sample size of n=9. For a more in-depth exploration, the study extended its analysis to include Pearson correlation, hierarchical clustering analysis (HCA), and principal component analysis (PCA) using the statistical package MetaboAnalyst v. 5.0 (Xia et al., 2009). These sophisticated statistical techniques provided a nuanced understanding of the interrelationships between the various parameters studied. Furthermore, the study incorporated data from UV-visible spectra of orange peel ethanolic extracts and direct color analysis on the orange peel surface using the CIELab system into the PCA and HCA analyses. These additional datasets were processed with metabolomics data analysis software, and the data were normalized by the sum, with scaling performed using Pareto, without undergoing any data transformations. This comprehensive statistical approach aimed to untangle and decipher the intricate relationships

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among the diverse parameters, offering a robust and nuanced comprehension of the bioactive compounds and functional properties present in orange peels.

RESULTS AND DISCUSSION

Chemical profiles and antioxidant activities from methanolic extracts of orange peels

The peel of citrus fruits is a reservoir of essential oils and volatile substances, contributing to the characteristic aroma of these fruits, which includes terpenes and other secondary metabolites serving as protective agents against abiotic factors. However, this study serves as a foundation for distinguishing between types of oranges based on the chemical composition of their peels. Monoterpene hydrocarbons, notably limonene (87.9%), myrcene (2.4%), and α-pinene (0.5%), along with monoterpenoid alcohols such as linalool (1.2%), and aliphatic aldehydes, including octanal (1.3%) and decanal (0.2%), have been identified as predominant in sweet oranges by Njoroge et al. (2009). These compounds exhibit antioxidant properties, as demonstrated by Singh et al. (2010), highlighting D-limonene's remarkable capacity to inhibit free radicals. Despite the availability of various analytical techniques for characterizing orange peel composition, the challenge arises on an industrial scale due to the massive volumes of oranges processed. Consequently, there is a demand for rapid, non-destructive methodologies that can effectively differentiate orange types based on their quality. Colorimetric techniques, widely used in the food industry, including orange juice, proved effective in discriminating between orange types through the assessment of total phenolic and flavonoid contents, along with in vitro antioxidant activities. Principal component analysis revealed significant variability, with PC1 explaining 99.1% of the differences between clusters. Unlike color analysis, PCA exhibited no overlap between Pêra-Rio and Valência oranges, indicating the potential for differentiation among orange types such as Hamlin, Pêra-Rio, and Valencia. The HeatMap diagram further illustrated substantial differences between orange types, highlighting Valencia's prominence across various evaluated parameters, except for antioxidant activity against free DPPH radicals.

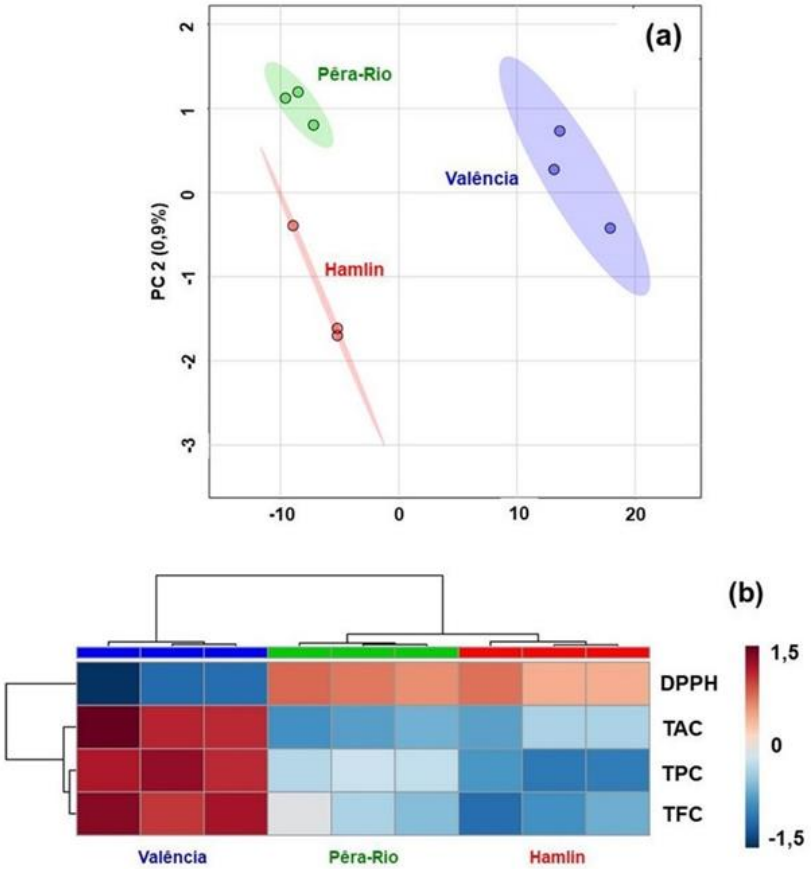

Figure 3 - (a) Principal Component Analysis (PCA) and (b) HeatMap of total phenolic (TPC) and total flavonoid (TFC) contents and *in vitro* antioxidant activities from methanolic peel extracts of Hamlin, Pêra-Rio and Valencia orange types. Where: DPPH, antioxidant activity by free DPPH radical scavenging; and TAC, total antioxidant capacity by phosphomolybdenum method

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While the Hamlin and Pêra-Rio stood out from the Valência type for phenolic compounds, total flavonoids, and total antioxidant capacity, both are similar one each other. Valencia had greater prominence in all data. Box Plot data analyses (Figure 4) allow us to observe what variables most influenced each orange type. The antioxidant activity by DPPH was more important to Pêra-Rio and Hamlin while antioxidant activity by phosphomolybdenum method was to Valência**.**

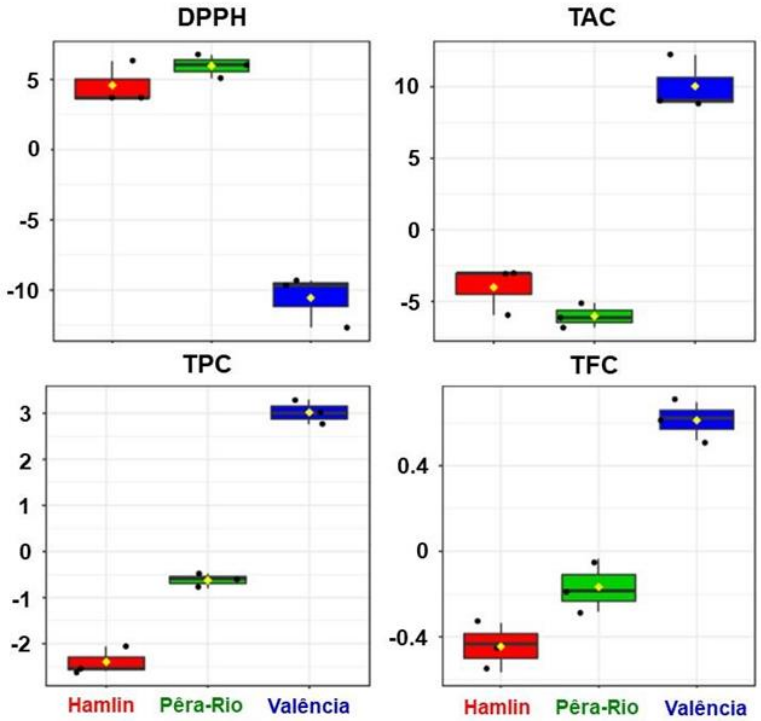

Figure 4 - Box Plot of total phenolic, total flavonoid contents, and *in vitro* antioxidant activities of methanolic extracts of peel from Hamlin (*red box*), Pêra-Rio (*green box*), and Valência (*blue box*) oranges. Where: DPPH, antioxidant activity by free DPPH radical scavenging; TAC, antioxidant activity by phosphomolybdenum method; TPC, total phenolic compounds and TFC, total flavonoid compounds

Liew et al. (2018) determined the total phenolic and total flavonoids in the peel of *Citrus sinensis* using methanol as solvent and found total phenolic contents of 36.1±2.9 mg TAE/gram dried peel and total flavonoids contents of 4.6±0.1 mg RE/gram dried peel. Melo et al. (2014) using ethanol as a solvent to extract the phenolic compounds from sweet orange bagasse found 8.1±0.5 mg TAE/gram of freeze-dried peel. For the antioxidant activity by DPPH free radical scavenging, Pereira et al. (2017) found similar values to this work with antioxidant activity above 60% for methanolic extracts of Hamlin, Pêra-Rio, and Valencia cultivars. Hegazy and Ibrahim (2012) analyzed the methanolic extract of sweet orange peel extracted by the Soxhlet technique and the antioxidant activity for DPPH radical scavenging of 73.4% was found. Sagar et al. (2020) and Seelam et al. (2016) reported that the activity for DPPH radical scavenging of 89% in *Malpighia emarginata* fruits.

Table 1 - Total phenolic compounds, total flavonoids, activity for DPPH radical scavenging, and total antioxidant capacity of methanolic extracts of orange peel from Hamlin, Pêra-Rio, and Valência cultivars (n = 15)

Orange types	Total phenolics (mg/g)[a]	Total flavonoids (mg/g)[b]	DPPH radical scavenging (%)	Total antioxidant capacity (µg/mL)[c]
Hamlin	5.92	0.23	78.16	34.07
Pêra-Rio	4.71	0.18	58.20	23.61
Valência	7.19	0.28	61.40	45.16

[a]mg TAE/g peel (mg of total phenolic compounds in tannic acid equivalent per gram of orange peel); [b]mg RE/g peel (mg of total flavonoid in rutin equivalent per gram of orange peel); and [c]µg AAE/mL extract (µg of ascorbic acid equivalent per mL of methanolic extract of orange peel)

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The antioxidant activity by DPPH radical scavenging showed that there is a moderate negative interaction between total flavonoids and weakly negative with total phenolic compounds. Thus, the higher concentration of phenolic compounds and total flavonoids do not have a direct relationship with the increase in antioxidant activity by DPPH radical scavenging (Figure 5). Aguiar et al. (2012), and Aguiar et al. (2009) have shown that soybean flavonoids are widely found as conjugate forms, and they present low antioxidant activity. Thus, the active site of flavonoids could not be able to scavenge the free radicals during the in vitro antioxidant tests. Besides, orange peels have other compounds that may have antioxidant activity, such as terpenes and carotenoids. Carotenoids are known as vitamin A precursors (such as retinol, retinal or retinoic acid) and they are associated with cellular protection against free radicals, due to their antioxidant activities, either through the reaction with singlet oxygen and peroxyl radical scavenging (Rufino et al., 2010). While, total antioxidant capacity by the phosphomolybdenum method, which has a broad spectrum of antioxidant activity evaluation or is a non-specific method, presented a moderate positive correlation with the total phenolic and total flavonoid contents. According to Kaska et al. (2019), the phosphomolybdenum method as total antioxidant capacity was used to ascertain the antioxidant properties in plant extracts. According to Prieto et al. (1999) and Nur-Alam et al. (2013), this methodology had been associated with the reductones presence once those compounds present reducing capacity on Mo+4 to Mo+5 and development of green phosphate/ Mo+5 complex.

Wan et al. (2011) analyzed total phenolic and total flavonoid contents no significant difference was noted for two different extraction temperatures (90 and 100 °C). The extracts obtained at a higher temperature exhibited a significant antioxidant activity by free DPPH radical scavenging compared with extraction at lower temperatures. As our group showed (Aguiar et al., 2012), under high heating (121°C/30 min) the malonyl isoflavones present in soybean flour were converted into glycosyl forms and increased in vitro antioxidant activity.

Between total phenolic compounds and total flavonoids, the correlation was strongly positive (Figure 3), since flavonoids belong to the chemical group of phenolic compounds. Many authors report that quercetin, hesperetin, and naringin and its conjugate forms, rutin, hesperidin, and naringenin, were widely found as flavonoids in citrus fruits (Singh et al., 2020; Bento et al., 2017).

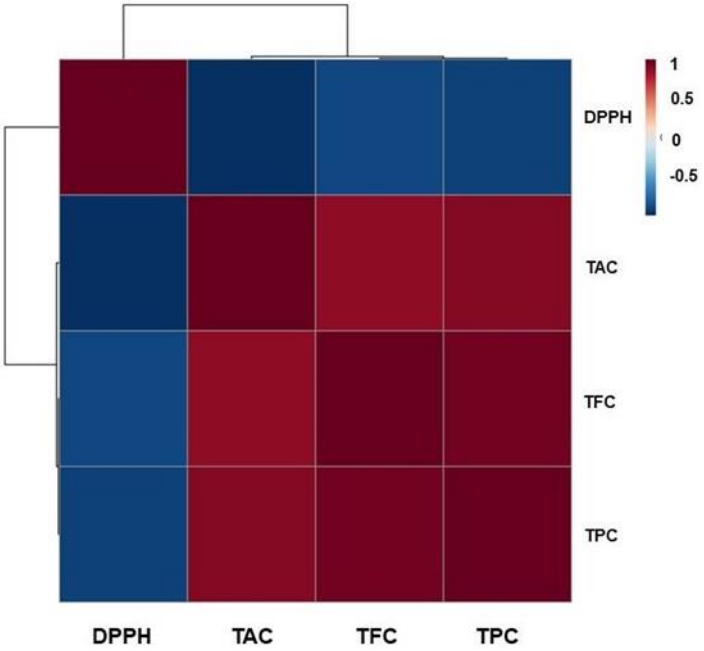

Figure 5 - Pearson correlation for total phenolics (TPC) and total flavonoids contents (TFC), antioxidant activity by DPPH free radical scavenging (DPPH) and total antioxidant capacity by phosphomolybdenum method (TAC)

High-efficiency liquid chromatography and co-chromatography analyses were carried out against flavonoid authentic patterns (HPLC grade) and made it possible to note the presence of major flavonoids (hesperidin and naringin) in methanolic extracts of orange peels. Hesperidin was present in all methanolic extracts of orange peel analyzed. Its concentrations vary between the orange types, with

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the highest content present in the Hamlin orange, followed by Valencia and Pêra-Rio, respectively. Naringin was not detected in the extracts from the Pêra-Rio and Valencia orange types. Whereas, the methanolic extract of Hamlin peel presents low concentration. Like Gómez-Mejía et al. (2019), Chen et al. (2020b), and Singh et al. (2020), low naringin concentrations were found when compared to hesperidin. Although the RP-HPLC analysis showed the highest concentrations of hesperidin and naringin were found in the Hamlin extracts, for the other analysis (total phenolic and total flavonoid) the results were more expressive for the methanolic extracts from Valência orange (Table 2 and Figure 6). Probably, other flavonoids were present in the analyzed orange peel samples, such as catechin and rutin (Singh et al., 2010), neoerocitrin, lutein, narirutin, apigenin, neohesperidin, or diosmin (Manthey and Grohmann, 2001).

Table 2 - Concentrations of hesperidin and naringin in the methanolic extracts of Hamlin, Pêra-Rio and Valencia orange peels

Orange types	t_R (min)	UV $\lambda_{máx}$ (nm)[a]	Compounds[b]	Concentration (µg/mL)[c]
Hamlin[d]	31.9	220 and 280	Naringin	382.7 ± 2.3
	33.6	224 and 276	Hesperidin	2,076.8 ± 2.7[A]
Pêra-Rio	33.4	224 and 276	Hesperidin	1,026.7 ± 5.8[C]
Valência	33.6	224 and 276	Hesperidin	1,562.9 ± 2.3[B]

[a]UV-visible spectra were carried out with the methanolic extracts from orange peels and the Figure 7 shows these spectra of corresponding peaks to hesperidin and naringin in the samples. [b]The authenticity of each flavonoid was checked by co-chromatography, retention time and UV-visible spectra from photodiode-array detector against authentic standards (HPLC grade). [c]Concentrations of each flavonoid were presented as mean±standard deviation and the same capital letter within a column (for hesperidin concentrations) are not significantly different according to the Tukey test at $p<0.01$.

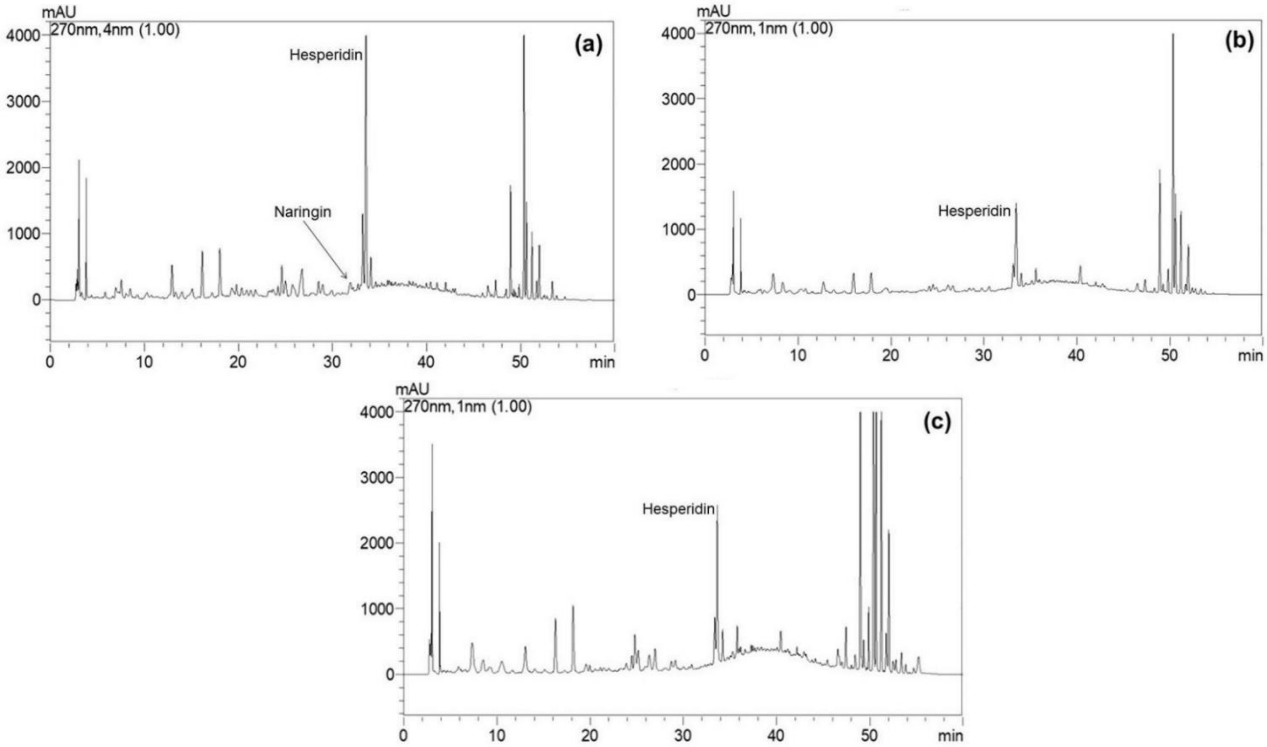

Figure 6 - RP-HPLC chromatograms of hesperidin and naringin from methanolic extracts from three orange peel types: (a) Hamlin; (b) Pêra-Rio; and (c) Valência

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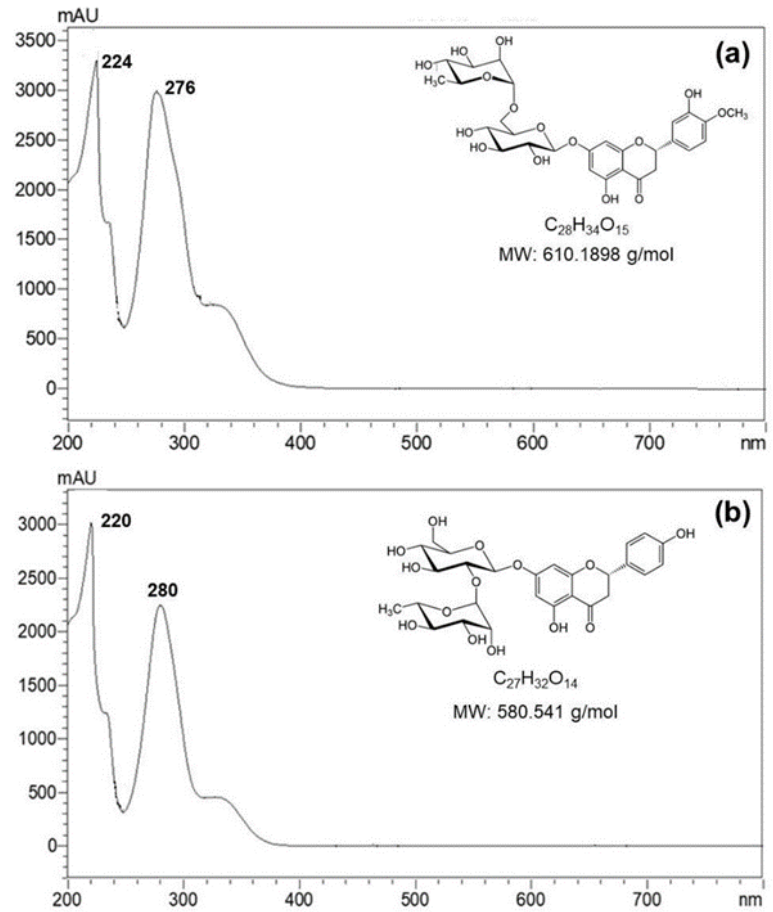

Figure 7 - UV-visible spectra of hesperidin and naringin from methanolic orange peel extracts

Color analysis from methanolic extracts of orange peels

By analyzing the samples by CIELab colorimetry it was possible to notice a statistical difference between the orange types studied. PC1 explained 96.9% (Figure 8a) of the data variability, and the Hamlin samples stood out from the other orange types since there was an overlap between the data of the Pêra-Rio and Valencia types. The same small overlap of some data was noticed in the HeatMap hierarchical analysis (Figure 8b).

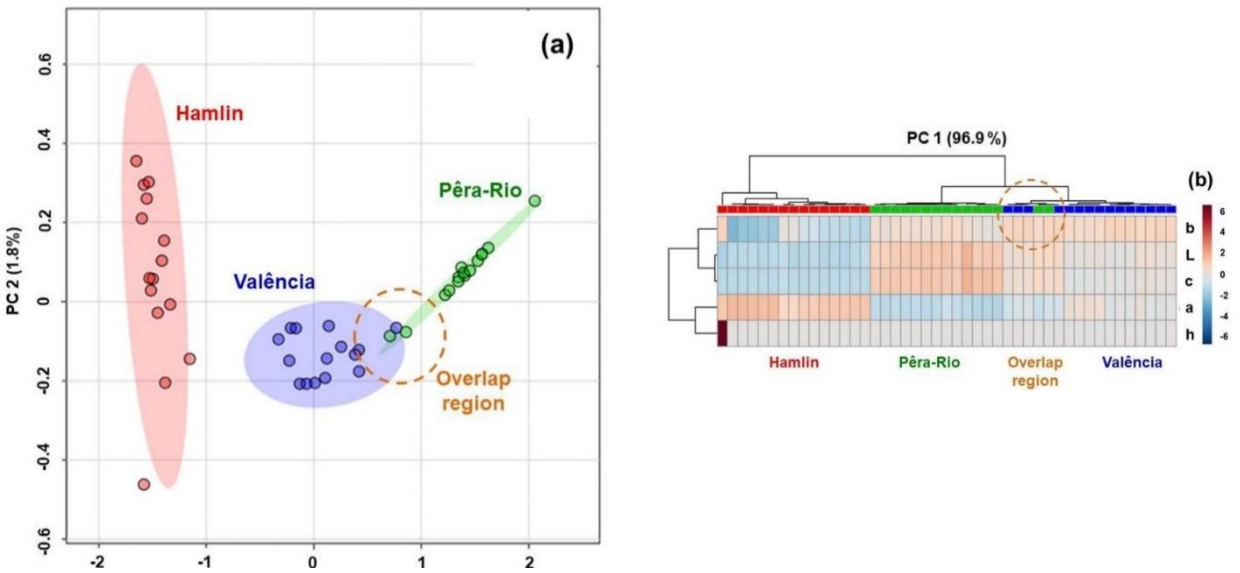

Figure 8 - (a) Principal Component and (b) HeatMap analysis for CIELab colorimetry for direct and fast analysis in peels' surface of Hamlin, Pêra-Rio, and Valência orange types

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It was noted by HeatMap analysis in Hamlin orange, that the a* coordinate was more significant than others, while for Pêra-Rio were L* and c*, in opposite to Valência that the b* coordinate was significant. These differences between parameters and orange types are important to discrimination by CIELab analysis, promoting the use of this technique in industrial processing scale. CIELab analysis is fast, cheap, and friendly to use. In addition, the dendrogram (results not shown) analysis confirmed the high difference between Hamlin and other orange types. The dissimilarity was upper than 30% between Hamlin and others, and between Pêra-Rio and Valência was 15%.

On the colorimetric analyses by Space Color from the CIELab system (Figure 9), the Hamlin was far from other orange types by plan distances. According to Pozzan and Triboni (2005), the Hamlin fruits are perfectly colored, intense orange and without spots, while Pêra-Rio presents fruits with a greenish-yellow color and Valencia accentuates intense yellow. Color nuances are a result of the thermal amplitude. In regions with mild temperatures, the peel color becomes more orange. Another factor is associated with the long periods of rain and sunshine that activate the chlorophyll synthesis and leave the fruits greenish.

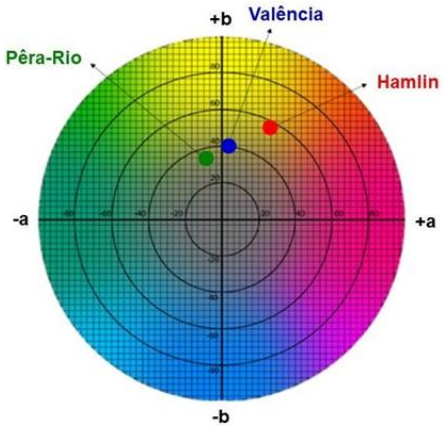

Figure 9 - Coordinates obtained by colorimetric analysis from Space Color of the CIELab system on Hamlin, Pêra-Rio and Valencia orange surfaces

UV-visible spectral scanning analysis from methanolic extracts of orange peels

UV-visible maximum absorption scanning from 200 to 600 nm was carried out with methanolic extracts from orange peels of Hamlin, Pêra-Rio, and Valência. The PCA did not show efficiency in distinguishing the orange types. Thus, we chose to analyze the UV-Vis data by PLS-DA (Figure 10a), which uses a multivariate curve, which was more successful in discriminating the orange types, although this differentiation was not entirely clear.

Component 1 (PLS-DA) explained 71.3% of the variability, in particular, between Valencia and the other orange types. However, Hamlin and Pêra-Rio did not show a great difference in the analysis of PLS-DA. It was verified that the analysis by scanning the UV-visible maximum spectrum did not prove to be a technique capable of differentiating the peel compositions from the orange types.

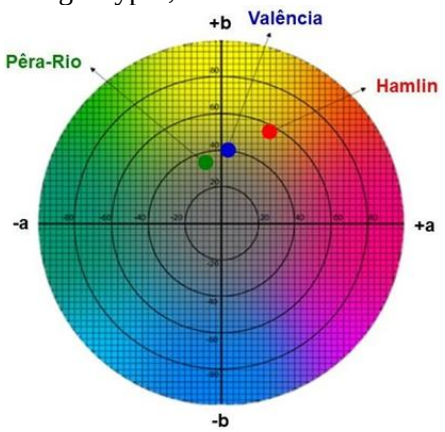

Figure 9 - Coordinates obtained by colorimetric analysis from Space Color of the CIELab system on Hamlin, Pêra-Rio and Valencia orange surfaces

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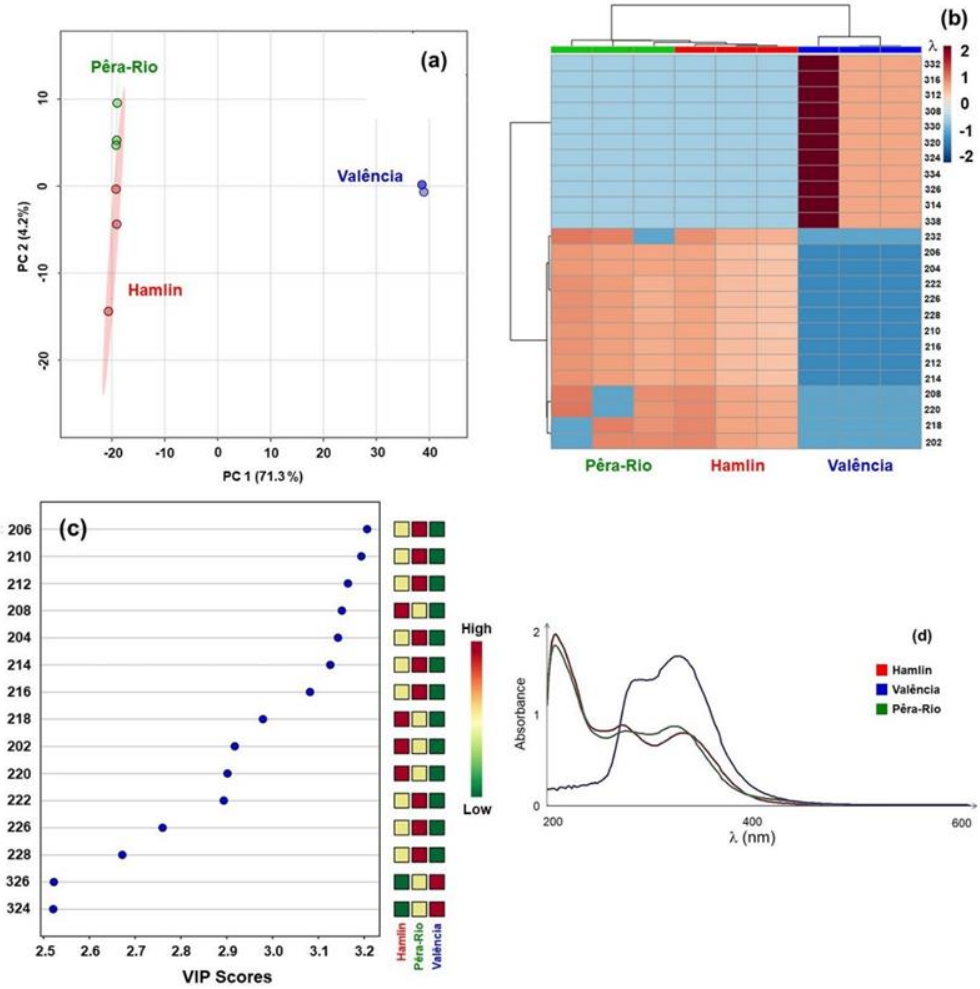

Figure 10 - (a) PLS-DA; (b) HeatMap; (c) VIP Score; and (d) overlapped UV-VIS spectra of methanolic extracts of peels from Hamlin (red); Valência (blue) and Pêra-Rio (green) orange types

However, a more detailed treatment of the data by HeatMap and VIP Score was effective in showing the difference between the characteristic wavelengths of each methanolic extract. It was noted that Valencia presented characteristic wavelengths in the range of 308 to 338 nm, with the wavelengths 324 and 326 nm being the most pronounced. According to Holser (2012), ferulic acid has a maximum absorbance at 215 nm with additional absorbances at 287 and 312 nm. In contrast, *p*-coumaric acid exhibits maximum absorption at 286 nm with additional absorbances at 209 and 220 nm. Harnly et al. (2007) reported that flavonoids can be identified and quantified based on UV-Vis absorption between 200 and 600 nm. The extracts from Hamlin and Pêra-Rio presented characteristic wavelengths in the range of 202 to 232. However, the wavelengths 204, 206, 210, 212, 214, 216, 222, 226, and 228 nm were more pronounced for Pêra-Rio, and 202, 208, 218, and 220 nm were more pronounced for Hamlin. Borello and Domenici (2019) reported that between

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390 and 560 nm, carotenoid and chlorophyll derivatives contribute to the spectrum, while the region between 630 and 700 nm can be safely attributed to the contribution of chlorophyll-exclusive derivatives. According to Makarska-Bialokoz and Kaczor (2014), chlorophyll forms have the maximum absorption at different wavelengths. Many organisms capable of conducting photosynthesis have chlorophyll which absorbs red light at approximately 662 nm and under violet light at around 430 nm. Chlorophyll *b* strongly absorbs light at 642 nm and blue light at 453 nm. Plants with more complex chlorophyll systems absorb light in the greatest range. During the photosynthesis process, the light is absorbed by chlorophylls below 480 nm and from 550 to 700 nm. Light between 480 and 550 nm (i.e., green) is not absorbed by chlorophylls, but they are reflected.

CONCLUSIONS

The cluster analysis showed that the color analysis was more effective, as its analysis was performed directly on the surface of the orange peels. The other techniques must be performed after extensive and careful extraction by organic solvents. However, all the applied techniques are capable of distinguishing the types of orange analyzed in this work. In addition, large datasets must be prepared, often difficult to interpret, but the use of PCA and HCA increases interpretability during orange differentiation. Optical methods coupled with chemometrics would greatly benefit from accessible, up-to-date tools by automatization process of orange identification.

AUTHOR CONTRIBUTIONS

Conceptualization: C.L. and I.B.C.; funding acquisition: C.L.; investigation: I.B.C., G.A.G.C., and C.L.; methodology: I.B.C., G.A.G.C., and C.L.; supervision: C.L.; writing (original draft preparation): I.B.C., G.A.G.C.; writing (preparation, review, and editing): C.L. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest regarding this article.

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Analysis of seed dormancy breakage and seedling growth in sweet sorghum (*Sorghum bicolor* L.) through the electrical stimulation method: a scientific perspective aimed at promoting bioethanol production

Johann Pereira Sousa [a], Claudio Lima [b*]

[a] Universidade de São Paulo, Brasil
[b] GS4/Science Consulting and Solutions, Brasil
[*] Autor correspondente (claudio.gs4science@gmail.com)

INFO

Keywords
Saccharine sources
electro-culture
seedlings
biomass
biofuel

ABSTRACT

This pioneering study meticulously focused on the underexplored realm of sweet sorghum (*Sorghum bicolor* L.), utilizing commercial seeds to mitigate potential biases. The research intricately interwove the intricate facets of electrical stimulation, delineating its profound implications on the estimative bioethanol production of sweet sorghum, with potential extrapolation to future investigations involving sugarcane. Executed within the controlled confines of a greenhouse, after seed treatment procured from a local market, the commercial seeds underwent an electrifying metamorphosis. In the pursuit of enhancing the fresh biomass output of sweet sorghum, a nuanced interplay with electrical currents transpired. Noteworthy findings surfaced, elucidating that the attainment of optimal outcomes necessitated meticulous control over the applied electrical current. The identified optimal parameter advocated maintaining a conservative threshold of 50 mA during a seed treatment duration spanning approximately 15 min. However, as the experimental cadence intensified, the narrative evolved with a bold revelation, indicating that exploration of higher electrical currents, peaking at 150 mA, could yield favorable results under the condition of a truncated seed treatment time, notably as brief as 5 min. The delicate equilibrium achieved in this electrical choreography presented a compelling prospect for redefining the production paradigm of sweet sorghum. After analyzing the germination results, the estimative production projections derived from treatment #3 paint a vivid picture of the sorghum biomass production potential. A visionary forecast materialized, envisioning an impressive 25 t/ha of sweet sorghum, coupled with a remarkable total conversion rate into bioethanol reaching 1.5 million cubic meters. This transcendence transcends the boundaries of a conventional study; it signifies a leap into the future, pushing the envelope of conventional wisdom in the relentless pursuit of innovation in sustainable energy.

Palavras-chaves
Fontes sacarinas
eletrocultura
mudas
biomassa
biocombustível

RESUMO

Análise da quebra de dormência de sementes e do crescimento das plântulas de sorgo sacarino (Sorghum bicolor L.), por meio do método de estímulo elétrico: uma visão científica voltada para o incentivo da produção de bioetanol
Este estudo pioneiro concentrou-se meticulosamente no domínio pouco explorado da produção de sorgo sacarino (*Sorghum bicolor* L.), utilizando sementes comerciais para mitigar possíveis viéses. A pesquisa entrelaçou de maneira elaborada as facetas complexas da estimulação elétrica, delineando suas profundas implicações na estimativa de produção de bioetanol do sorgo sacarino, com possíveis extrapolações para futuras investigações envolvendo a cana-de-açúcar. Executado dentro dos limites controlados de uma estufa, após o tratamento das sementes adquiridas em um mercado local, as sementes comerciais passaram por uma metamorfose eletrizante. Na busca por aprimorar a produção de biomassa fresca do sorgo sacarino, uma interação sutil com correntes elétricas transcorreu. Descobertas notáveis emergiram, esclarecendo que a obtenção de resultados ótimos demandava um controle meticuloso da corrente elétrica aplicada. O parâmetro ótimo identificado preconizava a manutenção de um limiar conservador de 50 mA durante um período de tratamento das sementes com duração de aproximadamente 15 min. Entretanto, à medida que a cadência experimental se intensificava, a narrativa evoluía com uma revelação audaciosa, indicando que a exploração de correntes elétricas mais elevadas, atingindo 150 mA, poderia gerar resultados favoráveis sob a condição de um tempo de tratamento de sementes truncado, notadamente tão breve quanto 5 min. O equilíbrio delicado alcançado nessa coreografia elétrica apresentava uma perspectiva convincente para redefinir o paradigma de produção do sorgo sacarino. Após análise dos resultados de germinação, as projeções estimativas de produção derivadas do tratamento #3 nos trazem um quadro vívido do potencial de produção de biomassa de sorgo. Uma previsão visionária se concretizou, projetando impressionantes 25 toneladas por hectare de sorgo sacarino, acompanhadas de uma notável taxa de conversão total em bioetanol atingindo 1,5 milhão de metros cúbicos. Esta transcendência vai além de um estudo convencional; ela representa um salto para o futuro, empurrando os limites da sabedoria convencional na incessante busca por inovação em energia sustentável.

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INTRODUCTION

Brazilian agriculture relies heavily on imported fertilizers, constituting approximately 85% of its fertilizer consumption, totaling 39.2 million t, and positioning the country as the fourth-largest global consumer, accounting for around 8% of the worldwide consumption (Ogasawara et al., 2013). In the 2021/22 crop year, Brazil witnessed a significant uptick in sweet sorghum production, reaching approximately 3 million metric tons, marking a substantial 43% increase compared to the preceding year (Ozbun, 2022). The Brazilian sorghum crop exhibits remarkable productivity, serving diverse roles as a food and feed source, and a key component in ethanol production (Jardim et al., 2020). Notably, Rezende and Richardson (2017) posit that sugarcane cultivation yields a risk premium benefit of around US$ 8.7 thousand per year in average annual net cash income. Their research suggests that integrating sweet sorghum into the crop mix, serving as a raw material for ethanol production in sugarcane mills, could reduce costs by spreading fixed expenses. Consequently, mitigating the cost of producing sweet sorghum seedlings emerges as a viable strategy for large-scale fuel ethanol production.

It is noteworthy in contemporary times the substantial investment in technological innovation, particularly in the field of energy transition. A vast number of calls are opened each year to foster scientific and technological research in the quest for sustainable technological solutions, both from an environmental and economic perspective, to promote sustainable energy generation from renewable sources. Among the great success stories, Brazil has been producing immeasurable volumes of biofuel from sugarcane since the 1970s (Gonçalves et al., 2023; Vega et al., 2024). Bioethanol is a globally recognized success for providing clean and liquid bioenergy for combustion engines (Eaglin, 2022). However, while the production costs of biofuels in Brazil remain among the lowest in the world, the expenses associated with acquiring synthetic fertilizers through the importation from a limited number of countries that possess extensive deposits and mines of key minerals (potassium and phosphate) have increasingly restricted access to the essential minerals for chemical fertilizers (Abay et al., 2023; Bair and Mahutga, 2023).

Currently, researchers have published papers detailing advancements in biofertilizer production or enhancements in nutrient assimilation efficiency by plants, particularly C4 plants, the foundation for bioenergy production worldwide (such as corn, sorghum, or sugarcane) (Altaf et al., 2023; Wasi et al., 2023; Alqahtani, 2023). Traditionally, the global economy is based on increasing crop production, especially food and feed, by the large-scale use of pesticides and fertilizers. Since the consumption profile has been more critical regarding the residual presence of these chemicals, which can negatively affect their health, public food safety agencies have sought alternatives to the use of fertilizers, for example. The availability of deposits of basic elements (nitrogen, phosphorous, or potassium) for the production of fertilizers in an area of armed conflict has also been another reason for the search for large-scale crop production using alternative techniques to stimulate plant growth. According to Amante (2019), in the Philippines, the agriculture fuels economy is significant to study innovations like electro culture to create more efficient ways to grow their crops or as reported by Christianto and Smarandache (2021) to reduce dependence on fertilizers.

Meanwhile, little has been spoken or published about a technology extensively studied years ago – electroculture. The stimulation of seed germination or plant propagation through electrical stimulation has not been in the headlines within scientific circles recently. The investigation into biopotentials, bioelectricity, or bioelectrogenesis was a vast domain of scientific exploration, with multiple facets yet to be fully elucidated (Vodeneev et al., 2015; Li et al., 2021; Mohan et al., 2016). In 1746, the Scottish scientist Dr. Von Maimbray conducted an experiment where he subjected two myrtles to electrification, observing a remarkably accelerated growth of branches, a phenomenon previously unreported (Manquiam et al., 2019). Capron (1883) documented that those trees exposed to the aurora borealis exhibited enhanced growth rates. Given the inherent potential difference across cellular membranes, characterized by a negative interior and positive exterior, it is hypothesized that this potential arises from the asymmetric distribution of ions, notably Na^+, K^+, Cl^-, and HPO_4^{2-}.

Living organisms, as part of their adaptive strategies, acquire environmental information and manifest it through physiological processes to optimize performance within the prevailing edaphoclimatic conditions, thereby maintaining homeostasis. Techniques involving electric current, magnetism, monochromatic light, and sound can influence the germination and growth of plants. Notably, electrical stimulation stands out as a technology utilized for various applications, including seed treatment and seedling growth (Acosta-Santoyo et al., 2018; Sánchez et al., 2018; Xu et al., 2022; Yuan et al., 2021).

Electrical stimulation manifests as either endogenous or exogenous phenomena. Endogenous occurrences are triggered by environmental stressors, such as fluctuations in temperature, which play a pivotal role in plant physiology (Lee and Oh, 2023;

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Kim and Oh, 2023). This involves the regulation of key processes like respiration, transpiration, stomata opening, protein and hormone synthesis, and the gene expression of enzymes and ribosomal proteins. Exogenous electrical stimulation influences cellular metabolism by interacting with the physical and chemical components of plant structures. This interaction can yield both positive and negative effects, impacting aspects such as germination, biomass yield, fruit quality, and resilience to extreme events like frost (Li et al., 2019; Chen et al., 2019a; Chen et al., 2019b).

In this groundbreaking investigation, we push the envelope by delving into the revolutionary realm of directly applying electric current to saline solutions housing ungerminated sweet sorghum seeds. Our objective is to scrutinize the profound influence of this novel approach on seed development, including germination time, biometric parameters encompassing root and shoot length, and the resultant biomass yield arising from the experimental treatments. This avant-garde exploration seeks to unravel the untapped potential and redefine the conventional boundaries in the domain of seed germination and plant growth modulation. Based on the obtained results, we have shed light on the biomass production potential of sweet sorghum, a prominent C4 plant utilized as a renewable source of bioenergy. This is achieved through an estimated biomass production calculation and, consequently, grounded in scientific agronomic data, the potential conversion into liquid energy, specifically biofuel in the form of bioethanol.

MATERIAL AND METHODS

The experiment was carried out in an indoor cultivation greenhouse installed at the Hugot Sugar Technology laboratories at the University of São Paulo, Brazil. Experiments were carried out controlling some variables such as lighting, water availability, and ambient temperature to optimize the culture performance, according to the appropriate agronomic characteristics of sweet sorghum. The cultivation was carried out in a plastic vase with 200 g of inert substrate, in sufficient numbers for the statistical design adopted. Two Shoppled LED tube lamps (20 W) were used for lighting, with 1480 Lumens, totaling 2960 Lumens (i.e., 40.0 µmol m^{-2} s^{-1}). Electric current was provided by a switched source, providing a different working current adapted to an e-Stimulator® current distribution donated by GS4|Science Consulting and Solutions (Figure 1). An Amanco irrigation system was used to supply potable water for irrigation. The system was controlled by a hydraulic interrupt coil driven and an analog Rohdina timer, set to irrigate 15 min every 8 h, resulting in a water depth of 150 mL per vase.

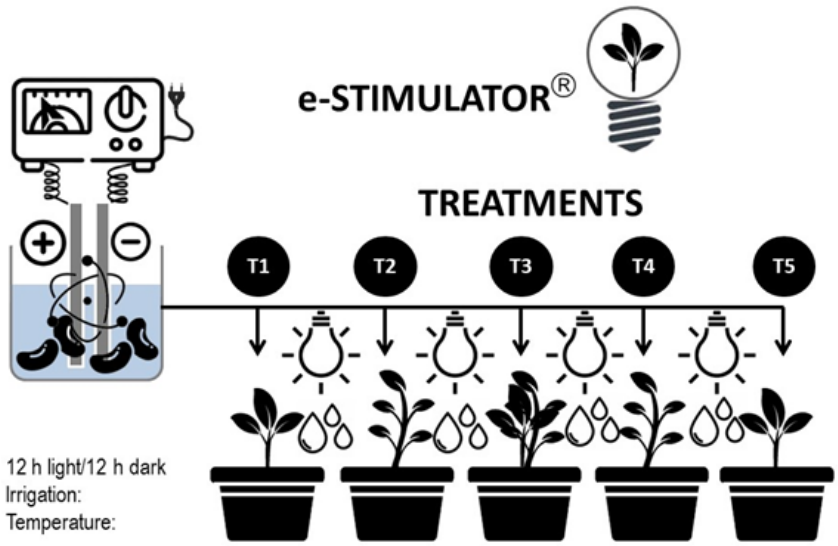

Figure 1 - Illustration of the experimental site for seedling electrical stimulation of sorghum seeds (*Sorghum bicolor* L.)

In each vase according to experimental design (Table 1), 30 g of healthy seeds of sweet sorghum (*Sorghum bicolor* L. Moench) was used per vase, totaling 8 treatments (7 tests and one control). The seeds were submitted to different current ranges (0; 50; 100; and 150 mA) for different times (0; 5; 10; and 15 min) into KCl-based solution at 1%wt in distilled water. The ratio of seeds to water was 1:10 (w/v).

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Table 1 - Experimental design and experimental data matrix for seedling electrical stimulation of sorghum seeds (*Sorghum bicolor* L.) and real values of shoot and root lengths and biomass weight at 21 days after planting (n = 7)

Runs	I (mA)	Time (min)	Shoot Length (mm)	Root Length (mm)	Biomass Weight (g)
#1	50	5	108.3 ± 30	43.4 ± 12	3.10 ± 0.3
#2	150	5	176.4 ± 34	87.5 ± 9	3.94 ± 0.2
#3	50	15	149.4 ± 39	95.4 ± 12	5.10 ± 0.2
#4	150	15	94.6 ± 23	16.8 ± 3	0.92 ± 0.1
#5	100	10	156.3 ± 39	33.9 ± 8	2.05 ± 0.3
#6	100	10	124.0 ± 14	30.8 ± 4	0.67 ± 0.1
#7	100	10	127.7± 22	30.4 ± 5	1.64 ± 0.2

Data were collected weekly (biometrics: root and shoot length) for 21 days after planting (DAP) and then used for further statistical analyses. All the tests performed were tabulated, containing the data referring to the analyses, allowing to evaluate and discuss through the Minitab Statistical software (free trial version), by surface analysis of response to the current and time variations, making it possible to verify the germination difference from one trial to another. The data also were analyzed by principal component (PCA) and hierarchical clustering (HCA) analyses to confirm the most important variables within each cluster using MetaboAnalyst online tools (Chong *et al.*, 2019).

RESULTS AND DISCUSSION

Effect of electrical stimulation on sweet sorghum growth

After each electrical stimulation treatment, the sorghum seeds were planted in vases and their development profiles were monitored weekly using a caliper to measure the plants' lengths. As described in the Material and Methods, ten plants from each vase were randomly collected so that shoot and root length measurements were taken. The results presented in Table 1 served as the foundation for the subsequent multivariate and cluster analyses. Given that it involves a 2^2 full factorial design with three repetitions at the central point, the outcomes enabled a comprehensive analysis of the interactions between the applied current (50, 100, or 150 mA) and the seed treatment time at these currents. The aim was to seek the optimal interaction between the current and its application time on sweet sorghum seeds, or at the very least, initially determine if there were statistically significant differences among the treatments. The response variables (biometric measurements of the seedlings) were utilized as reference indicators to ascertain whether there was a significant effect among the experimental trials. As evident, each trial yielded a distinct combination of responses following the seed treatments.

According to the original measures data and by the cluster analysis (Figure 2A) and hierarchical analysis (Figure 2B) it was possible to verify that the original repetitions data presented great values dispersion. Thus, for correct data analysis, a Discriminant Analysis was performed to identify the closest repetition values. These normalized values were used for statistical analysis and data discussion.

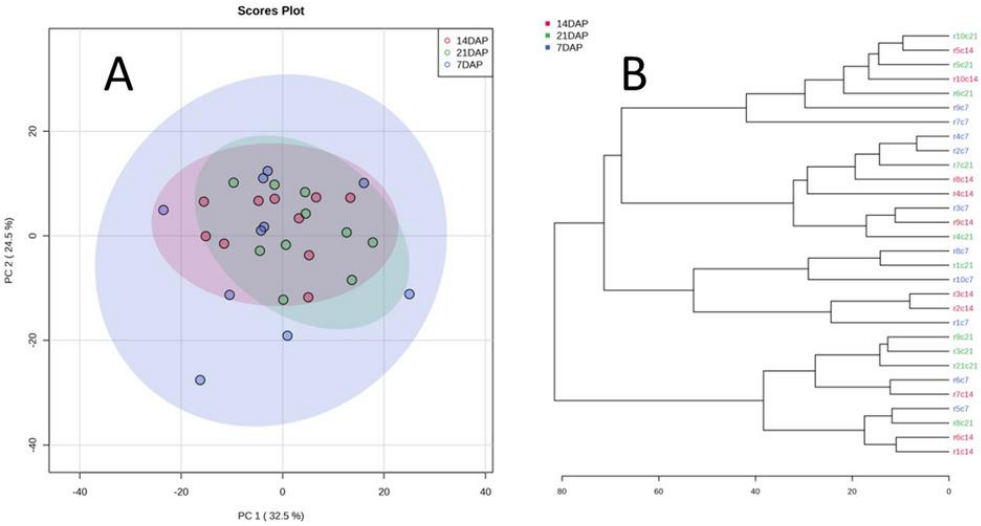

Figure 2 - Principal component analysis (**A**) and hierarchical analysis (**B**) of original data collected from shoot lengths of sorghum seedlings at 7, 14, and 21 days after planting (DAP)

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Figure 3 shows the profile of those seedlings. It is possible to note some subtle differences between the sorghum seeds' development. The difference was the result of the current applied and the time at which the seeds were exposed to the current, as shown in Table 1.

There is a great variability in the plant's lengths for the same treatment (Figure 3), i.e., the electrical stimulation effect has a generally negative impact on plant development.

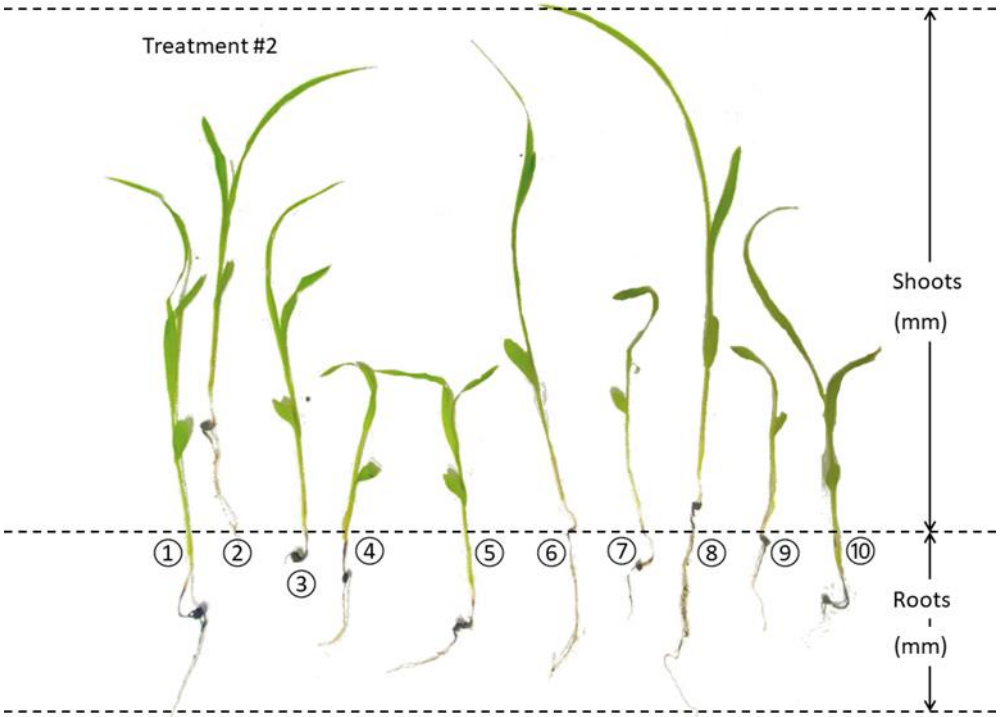

Figure 3 - Random growth profile of sweet sorghum after treatment by electrical stimulation of seeds

After previous statistical analysis of original data, we noted that the electrical stimulation by direct transfer of electrons from graphite electrodes into the soil, and the seedling of sweet sorghum is very different (Figures 4 and 5). There is a significant effect of current intensity and applied time on the germination capacity of the seeds under the same cultivation conditions (i.e., light radiation, nutrients, and water supply). It is even possible to notice the difference in plant development between the treatments.

During germination, we could notice that the current, which at the beginning was the factor with the greatest weight by the Pareto analysis (Figure 6), following the interaction between current and treatment time had the greatest impact on the vegetative development of the plants.

Figure 4 - Seed germination profile among sweet sorghum electrical stimulation treatments

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It is plausible that an increase in seed treatment time may impact the vegetative biochemistry of the seedlings, resulting in a lower rate of vegetative development in sorghum plants. Despite treatment #3, where the combination of factors was 150 mA and 15 min of seed treatment, proving to be one of the most favorable for the vegetative development of sorghum seedlings, it also exhibited statistical similarity to treatments #2 and #5 when considering 21 days after planting (DAP). In treatment #2, the results, in absolute terms, achieved the most significant stimuli in the development of sorghum seedlings. Statistically, the treatments were largely similar. However, absolute differences between the results and treatments were discernible, suggesting that electrical stimulation with a lower current and longer treatment time, as well as a higher current and shorter treatment time, could potentially be viable options for sorghum seed electrical stimulation (Figure 5).

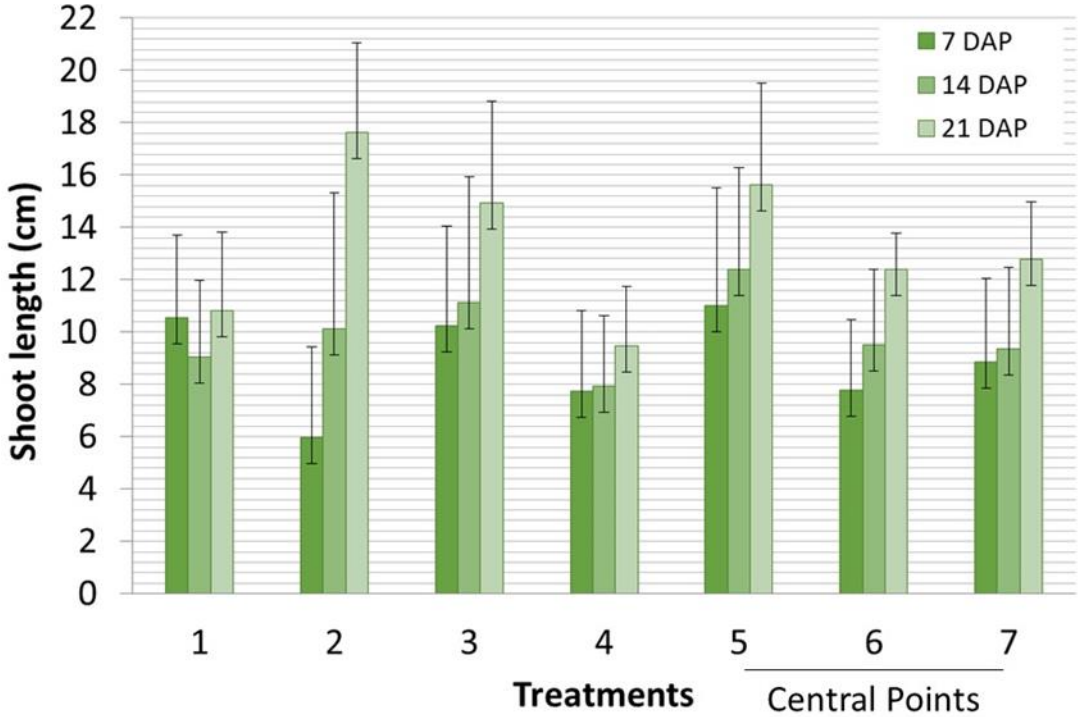

Figure 5 - Vegetative profile of sweet sorghum under electrical stimulation after 7, 14 and 21 days after planting (DAP)

Pareto charts illustrate that current, within the experimental conditions, was the most significant factor, but rather that the exposure time of sorghum seeds to those currents was important to promote seedling germination and also significant. Current (Factor A) is an important factor, since the A×B interactions (current versus treatment time) were also important, although seed treatment time (Factor B) was not statistically significant (**Figure 6**).

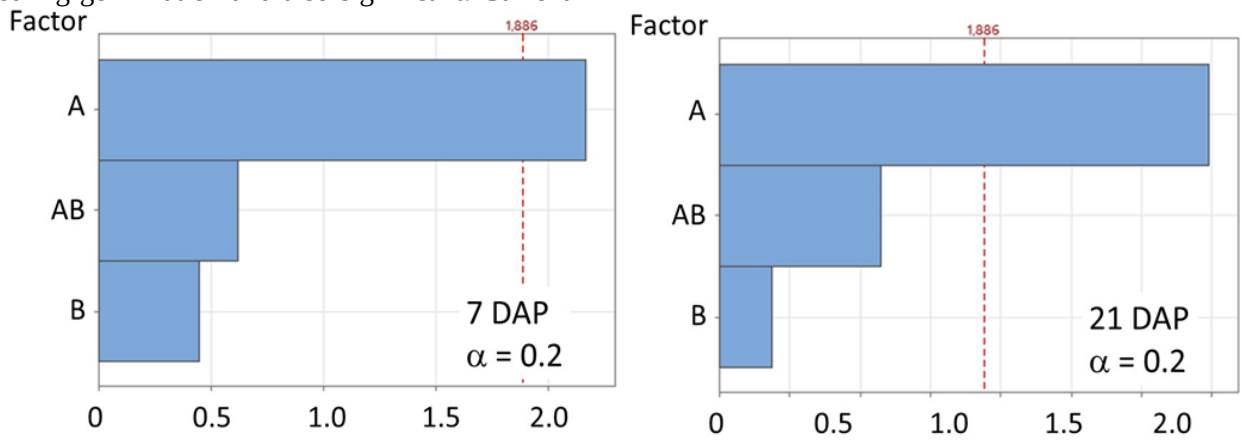

Figure 6 - Standardized Pareto chart of multivariate analyze of sweet sorghum vegetative development under electrical stimulation. The red line shows the F-value for shoot lengths. DAP: days after planting of seeds in the vases

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The combination of current and seed treatment time resulted in interesting results regarding sweet sorghum germination. By analyzing the main effects and interactions, it is possible to notice that 7-DAP, the behavior of the vegetative development is different from the plant development after 21 days. It is so interesting to observe that after 7 days, there is a rapid development of the plants in some treatments, while in others, especially in those where the current was higher (150 mA), it was not favorable. The vegetative development after 7 days had a better performance in those treatments where the current was lower (50 mA) and with a longer exposure time of the seeds to electrical treatment. If we analyze the results of 14-DAP, we still have that the treatment with lower current is favorable, although we begin to notice that the treatment time has no effect. The same can be observed by analyzing the factors' interactions, in which the current of 50 mA and the longer treatment time (15 min) were the conditions that most favored the initial sorghum plant developments. However, the longer the vegetative development time, the vegetative behavior of the plants demonstrates that for those treatments where the current was higher (150 mA), good results can also be obtained as long as the time of exposure to high currents is shorter (i.e., 5 min). Thus, the choice of current intensity will depend on the seed exposure time to that current, and it must be inversely proportional, that is, the higher the current, the shorter the exposure time and vice versa (Figure 7).

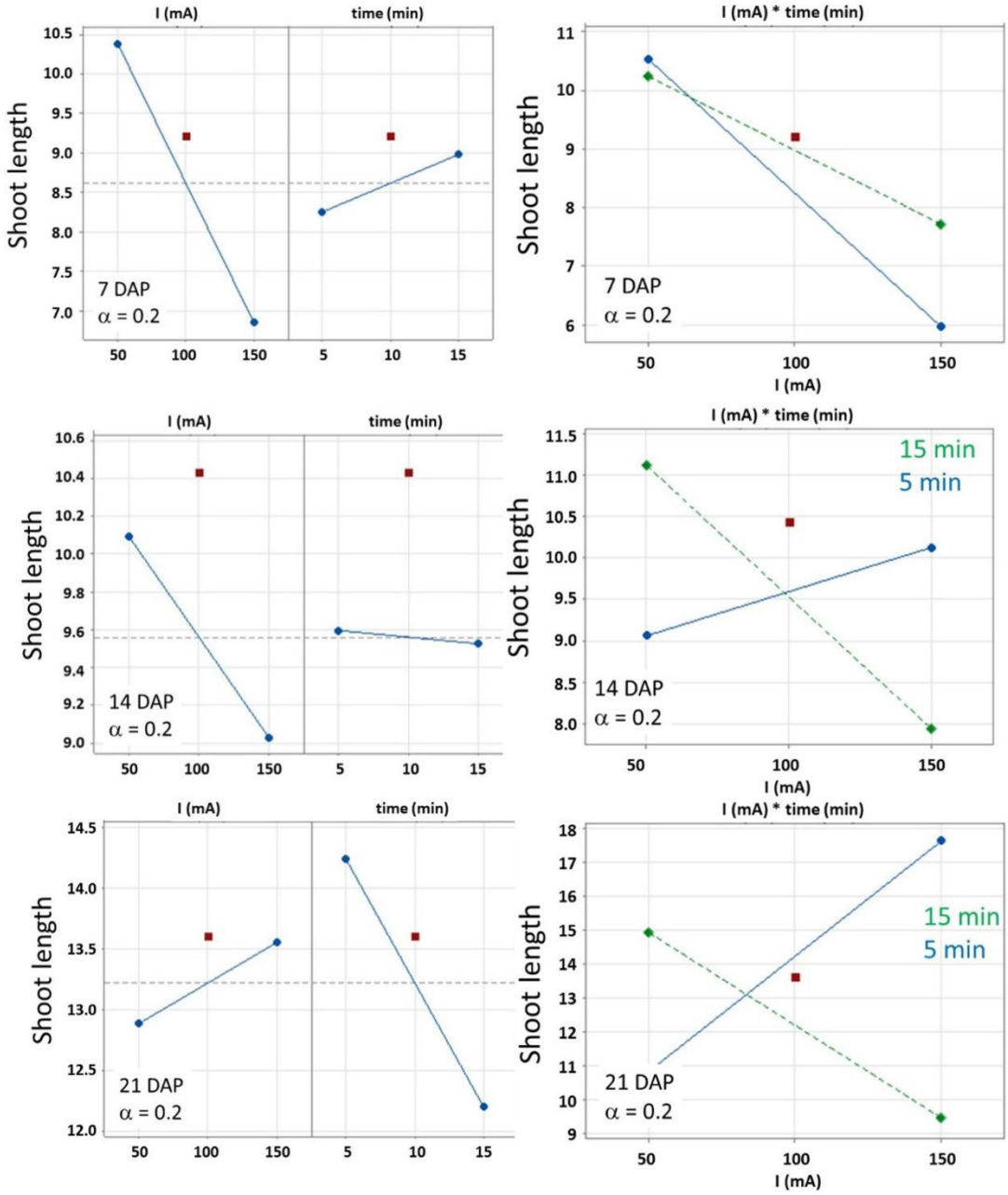

Figure 7 - Main and interaction effects of the two factors analyzed for shoot length of sweet sorghum

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Since the application of current in biological organisms in a saline solution directly impacts the biochemical reactions in the embryo and, therefore, in the organism's physiology, the application of high currents for prolonged times can negatively affect the plant's vegetative cycle. Black *et al.* (1971) reported that electrical stimulation of tomato seeds increased the assimilation of ions, in particular calcium, potassium, and phosphorus. In addition, Songnuan *et al.* (2015) reported that under high-energy fields, *Arabidopsis thaliana* seeds had higher levels of gene expression at nearly tenfold (PAD3 and PR1) compared with untreated seeds.

Hepler (2005) reported that Ca^{2+} is a crucial regulator, working as a second messenger in plant cell growth and development and controlling membrane structure and function. According to Lee and Oh (2021), kale submitted to a current of 50 mA resulted in an increase in fresh mass, with greater active transport of ions by the roots, which increased the calcium content by 72%, phenolic compounds by 57% and by 70% the antioxidant capacity of the plants. We also observe the effects of current and time on the development of sweet sorghum roots (Table 1). The current with less intensity favored the root development, in the same way in the time factor, where a shorter exposure period of seeds to the current also favored the root development. Taking into account the fact that the smaller the root system, the smaller the exposure area to nutrient absorption, such as calcium, which consequently alters receptor proteins in the plant cell membrane, impairing the assimilation of ions from the soil.

Thus, the electrical stimulation effect on sweet sorghum development is defined by a combination of factors, such as seed treatment time and the intensity of the applied current. Therefore, the best way to analyze the results is through multifactor analysis, such as response surface analysis.

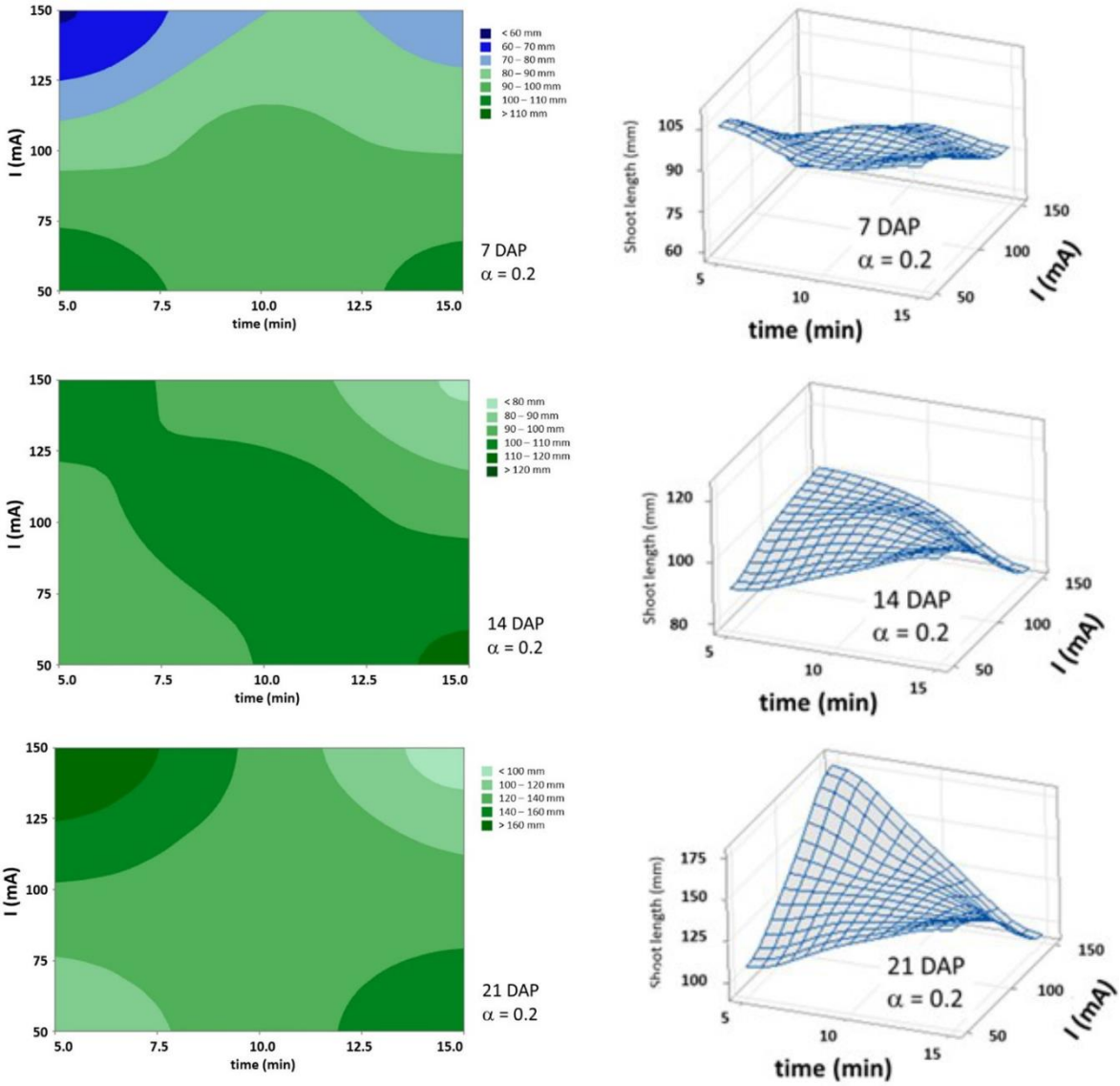

Figure 8 - Contour and surface plots of shoots development of sweet sorghum after electrical stimulation treatment

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Bioethanol estimative production by biomass weight

After 21 days of cultivation, all plants in each vase were collected and the fresh mass was measured on an analytical balance with an accuracy up to 0.01 g. The electrical stimulation treatments that most promoted the vegetative development, in biomass weight, were treatments #3 and #2, with 5.1±0.2 g and 3.94±0.2 g, respectively. Again, we have a dichotomous profile in the vegetative development of sweet sorghum under electrical stimulation, i.e., if high current was used, the shorter exposure time is favorable and vice versa. Treatment #3 was carried out with a current of 50 mA and 15 min of exposure to the electrical treatment, while treatment #2 was carried out at 150 mA and 5 min to the treatment (Figure 8 and Table 1).

According to Assis and Morais (2014), the potential for generating ethanol from sweet sorghum in Brazil is 3.2 m^3 ha^{-1} to a biomass productivity of up to 55 t ha^{-1}, which would result in an ethanol yield of 58.6 L t^{-1}. During our experimental trials, polypropylene vases with a 14 cm diameter were used for planting, which resulted in a planting area of approximately 22.0 cm^2 of planted area (= 2.2 × 10^{-7} ha).

Based on these area proportions and the values obtained, in grams, of fresh biomass, for Treatment #3, the estimated production of sweet sorghum would be 25 t/ha, with a total conversion rate into bioethanol of 1.5 × 10^9 L (= 1.5 million m^3 of bioethanol), as long as the proportions are maintained. For the calculations, harvest losses due to pests and diseases, among other factors, were not considered.

CONCLUSIONS

Undoubtedly, the preceding electrical stimulation applied to sweet sorghum seeds induces a shift in physiological behavior, significantly impacting fresh biomass production. To optimize the increase in fresh biomass, it is advisable to regulate the current at its minimum, approximately 50 mA, with a seed treatment duration of around 15 min. Intriguingly, noteworthy results can also be achieved at higher currents, such as 150 mA, provided the seed treatment time is minimized, for instance, to 5 minutes. Nonetheless, the current study unequivocally illustrates the discernible interference in the vegetative development of sweet sorghum, emphasizing the critical role of the duration of seed exposure to a specific electric current. Extended seed treatment may impact seedling biochemistry, lowering the sorghum plant's vegetative development rate. Some treatments had favorable outcomes with 150 mA and 15 min of seed treatment, while it was statistically resembled to other treatments at 50 mA (at 21 DAP). Results yielded the most significant stimuli in sorghum seedling development. While statistically similar, noticeable differences suggest that electrical stimulation with lower current and longer treatment or higher current and shorter treatment could be potential options for sorghum seed stimulation. Therefore, the electrical stimulation of sorghum seeds could shorten the germination and establishment time of the sorghum crop for biomass production and the potential production of bioenergy by approximately 1.5 million cubic meters of bioethanol.

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Potential of *Albizia zygia* and *Albizia lebbeck* leafy biomasses as organic soil amendments on the growth and yield of okra, *Abelmoschus esculentus* (L.) Moench

Adams Latif Mohammed [a*], Frank Addai [a], Yasser Arafat Tackie [b]

[a] Department of Agroforestry at the Kwame Nkrumah University of Science and Technology, Ghana
[b] Eberswalde University for Sustainable Development Schicklerstraße, Alemanha
[*] Autor correspondente (adamsinho224@gmail.com)

INFO

Keywords
Albizia spp
soil fertility
okra
agroforestry

ABSTRACT

In the array to assess the suitability of using amendments from nitrogen fixing trees in agroforestry, the response of okra to *Albizia lebbeck* and *Albizia zygia* leafy biomasses as soil amendments were investigated at the Faculty of Renewable Natural Resources Demonstration Farm in the Kwame Nkrumah University of Science and Technology, Kumasi-Ghana. The objective of this study was to assess the effect of different levels of *A. lebbeck* and *A. zygia* leafy biomasses on the growth and yield of okra in a randomized complete block design. Four treatments were used and allocated as T1 (Control, no biomass), T2 (0.2454 kg of *A. lebbeck* leafy biomass), T3 (0.2932 kg of *A. zygia* leafy biomass), T4 (0.2127 kg of *A. lebbeck* leafy biomass + 0.1466 kg of *A. zygia* leafy biomass). The treatments were replicated four times. Growth in height, stem diameter, fruit yield and fruit dry weight were the parameters investigated on. The application of *A. lebbeck* and *A. zygia* leafy biomasses significantly improve the growth and yield of okra compared to the control treatment during the 12-weeks period of the experiment ($p<0.05$). Therefore, *A. lebbeck* and *A. zygia* leafy biomass amendments are recommended for use by okra farmers, as it has great prospects for use in soil fertility improvement. Also, further investigations with other levels of *A. lebbeck and A. zygia* mulch can be carried out to determine optimum rate for optimum growth and yield of okra, *Abelmoschus esculentus* (L.) Moench.

Palavras-chaves
Albizia spp
fertilidade do solo
quiabo
agrofloresta

RESUMO

Potencial das biomassas foliares de Albizia zygia e Albizia lebbeck como emendas orgânicas do solo no crescimento e rendimento do quiabo, Abelmoschus esculentus (L.) Moench
No intuito de avaliar a adequação do uso de corretivos de árvores fixadoras de nitrogênio na agrossilvicultura, a resposta do quiabo às biomassas folhosas de Albizia lebbeck e Albizia zygia como corretivos do solo foi investigada na Fazenda de Demonstração da Faculdade de Recursos Naturais Renováveis da Universidade de Ciências Kwame Nkrumah. e Tecnologia, Kumasi-Gana. O objetivo deste estudo foi avaliar o efeito de diferentes níveis de biomassas foliares de *A. lebbeck* e *A. zygia* no crescimento e produção de quiabo em delineamento de blocos casualizados. Quatro tratamentos foram utilizados e alocados em T1 (Controle, sem biomassa), T2 (0,2454 kg de biomassa folhosa de *A. lebbeck*), T3 (0,2932 kg de biomassa folhosa de *A. zygia*), T4 (0,2127 kg de biomassa folhosa de *A. lebbeck* + 0,1466 kg de biomassa folhosa de *A. zygia*). Os tratamentos foram replicados quatro vezes. Crescimento em altura, diâmetro do caule, produtividade e massa seca dos frutos foram os parâmetros investigados. A aplicação de biomassas folhosas de *A. lebbeck* e *A. zygia* melhorou significativamente o crescimento e a produção de quiabo em comparação ao tratamento controle durante o período de 12 semanas do experimento ($p<0,05$). Portanto, os corretivos de biomassa foliar de *A. lebbeck* e *A. zygia* são recomendados para uso pelos produtores de quiabo, pois apresentam grandes perspectivas de uso na melhoria da fertilidade do solo. Além disso, investigações adicionais com outros níveis de cobertura morta de *A. lebbeck* e *A. zygia* podem ser realizadas para determinar a taxa ideal para crescimento e rendimento ideais de quiabo, *Abelmoschus esculentus* (L.) Moench.

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INTRODUCTION

Agricultural sustainability is bolstered by incorporating tree leafy biomass, which enhances soil fertility, provides organic matter, and promotes a balanced ecosystem. Utilizing tree leaves as biomass in agriculture contributes to long-term soil health and productivity while minimizing environmental impact. *Albizia zygia* (J.F) Macbr and *Albizia lebbeck* (L.) Benth, two species of the Albizia genus, are renowned for their significant contributions to agroforestry and sustainable land management (Ogunniyi et al., 2023). These *Albizia* species are local and exotic species, respectively, domiciled in Ghana (Akoto et al., 2022). They are leguminous trees valued for their ability to fix nitrogen, improve soil fertility, and provide valuable biomass in the form of leaves (Adelani, 2023; Akoto et al., 2022). According to Alamu et al. (2023), these attributes have garnered attention as they offer potential benefits to crop growth and yield enhancement in the context of agroecosystems.

In Ghana, vegetable production plays a vital role in the agricultural sector, contributing significantly to food security and economic livelihoods. The diverse agroecological zones in the country allow for the cultivation of a wide variety of vegetables, meeting both local and export demands. Farmers employ sustainable practices, and ongoing initiatives aim to enhance productivity, improve crop resilience, and promote the overall sustainability of vegetable farming in Ghana of which okra production cannot be left out. Okra, *Abelmoschus esculentus* (L.) Moench is a staple vegetable crop in many tropical and subtropical regions due to its nutritional content and culinary versatility (Amin et al., 2022; Elkhalifa et al., 2021). In addition, it is a widely cultivated vegetable crop with economic importance, serving as an ideal candidate for examining the potential influence of *A. zygia* and *A. lebbeck* leafy biomass on crop performance (Kaur et al., 2023; Kumae et al., 2021). However, like many crops, okra growth and yield are often constrained by factors such as inadequate soil fertility and nutrient deficiencies. Amponsah-Doku et al. (2022), Tadele (2019), and Talabi et al. (2022) reported that, suboptimal growth conditions can lead to reduced crop yield and quality, impacting the livelihoods of farmers and food availability in the tropics. The relationship between these nitrogen-fixing trees and okra has the potential to improve agricultural sustainability and food security, particularly in regions with resource limitations in sub-Saharan African countries (Dagar et al., 2020; Kebede, 2021; Roslan et al., 2020).

Furthermore, agricultural systems in the tropics face challenges such as soil degradation, unpredictable climate patterns, and the threat of pests and diseases. These issues hinder sustainable productivity and necessitate innovative solutions for resilient and thriving tropical agriculture. According to Reza & Sabau (2022), agricultural systems in many regions, including those in sub-Saharan Africa, face multifaceted challenges that hinder crop productivity and food security. Tesfaye et al. (2023) and Zanli et al. (2022) indicated that soil degradation, nutrient depletion, and limited access to external fertilizers are some of the primary issues plaguing these systems in the tropics. In addition, conventional monoculture practices often result in reduced soil fertility and increased susceptibility to pests and diseases decreasing crop yields in developing countries (Adedibu, 2023; Altieri, 2019; Armengot et al., 2020). To address these challenges and promote sustainable agricultural practices, the integration of leguminous trees like *A. zygia* and *A. lebbeck* into cropping systems has gained attention (Dev et al., 2022; Hasanuzzaman & Hossain, 2023). According to Adelani (2023), these trees have the potential to enhance soil fertility through biological nitrogen fixation and the provision of leafy biomass, which can serve as green manure or mulch. However, while the benefits of these trees are well-documented in terms of soil improvement, their specific impact on crop growth and yield, especially on crops like okra, remains an area of research that requires deeper exploration (EO et al., 2023).

Therefore, the need for a comprehensive understanding of the influence of *A. zygia* and *A. lebbeck* leafy biomass on okra growth and yield is underscored by the potential advantages this integration can offer to smallholder farmers. Such knowledge can inform evidence-based recommendations for sustainable agroforestry practices that enhance crop performance, reduce the reliance on external inputs, and contribute to food security in resource-constrained regions. The results of this research will help educate poor resource farmers on the potential of *A. zygia* an indigenous agroforestry tree species for soil improvement in crop production in the tropics more importantly Ghana. The objectives of the study were to determine the effect of *A. lebbeck* and *A. zygia* leafy biomasses on the morphological growth (height and stem diameter) of okra and to determine the effect of *A. lebbeck* and *A. zygia* leafy biomasses on the yield (number of fruits and dry weight of fruits) of okra in Ghana.

METHODOLOGY

Location of the study area

The experiment was conducted at the Agroforestry Department Faculty's demonstration farm,

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which is located on the Kwame Nkrumah University of Science and Technology (KNUST) campus in Kumasi, Ghana. The site is located within the Oforikrom municipality within the Ashanti region of Ghana. According to the 2021 National Population and Housing census, Oforikrom Municipal Assembly's population is 213,126 made up of 107,426 males and 105,700 females. This farm is positioned in the humid Semi-Deciduous Forest zone of Ghana, with geographical coordinates approximately at 6.40°N latitude and 1.37°W longitude and elevated 250 to 300 meters above sea level (Mohammed et al., 2023).

Rainfall

The area experiences a distinct rainfall pattern, manifesting in two primary cycles annually. Typically, the average precipitation ranges from 1250 to 1500 mm. The primary rainy season, termed the major wet season, spans from May through July, followed by a minor rainy season from September to November. Moreover, there are two dry spells, one extending from December to March, constituting the lengthier dry season, and the other occurring in August, representing the shorter dry period (Mohammed et al., 2023). The predominant climate in the region is tropical wet and dry or savanna climate.

Temperature and Humidity

The typical daily temperature at the location averages at 25.6°C. In the coldest period spanning from December to February, the mean temperature decreases to about 20°C, whereas in the warmest month of March, the average peak temperature reaches 33°C. On an annual average, the temperature at this site is approximately 26.61°C, and the relative humidity stands at 67.6% (Mohammed et al., 2023).

Soil type

The soil at the experimental site is characterized as Ferric Acrisol, displaying strong acidity and effective drainage. Additionally, the texture of the soil was identified as sandy-loam (Mohammed et al., 2023).

Soil properties at the study site

pH	N (%)	P (mg/kg)	K (mg/kg)	O.C (%)	O.M (%)	Moisture (%)	Bulk density (g/cm^3)	Temperature	C/N ratio
5.4	0.17	33.2	18.9	0.85	1.5	9.6	1.02	30.4	6.6

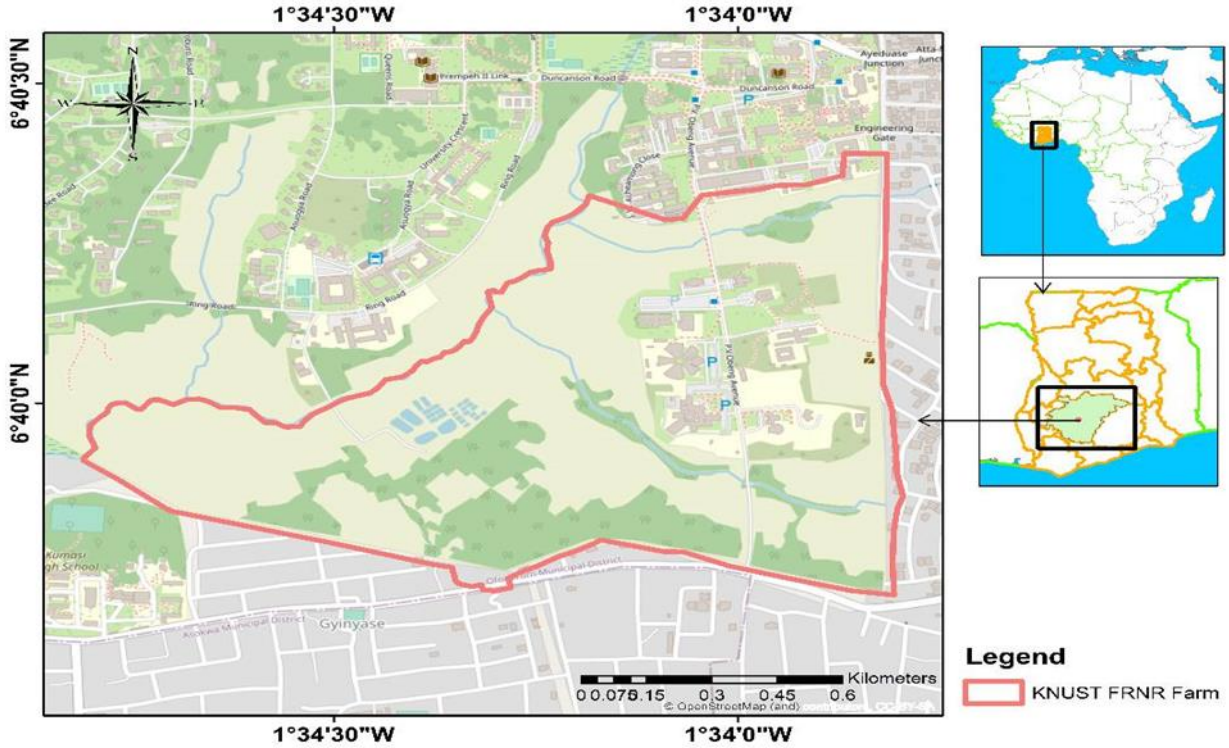

Figure 01 - Map of the study area showing the Faculty of Renewable Natural Resources Demonstration Farm, KNUST-Kumasi, Ghana

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Experimental Approach and Procedure

Sources of leafy biomasses

The *A. lebbeck* and *A. zygia* leafy biomasses were obtained at the department of Agroforestry research farm in the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi-Ghana. These components were applied separately or in combination at different levels of dosage.

Source of okra seeds and planting

The seeds of okra were obtained from the Crop Research Institute of Ghana, Kwadaso-Kumasi. Irrigation was carried out before and after sowing consistently throughout the dry season of the experiment. Each hill was planted with four seeds at a depth of 1.5 cm and spaced at 0.6–0.5 m a week after integrating the biomass into the soil. Okra seeds were sown at stake at a planting distance of 0.6 m x 0.5 m. One week after germination, the okra seedlings were selectively reduced to one seedling per hill through thinning. This thinning process aimed to promote consistent growth among the remaining plants in the field, reducing competition for nutrients and water (Manrique-Alba et al., 2020). Watering was carried out as required using a watering can.

Albizia lebbeck and *Albizia zygia* leafy biomasses application

Different quantities of *A. lebbeck* and *A. zygia* biomasses were weighed using an electronic balance and applied to each sub plots as a sole application and in combinations at a rate of 75kg N/ha by incorporation. Leafy biomasses of *A. lebbeck* and *A. zygia* was applied through incorporation into the soil two weeks before planting (WBP) in various treatments during the field experiment (0.4254, 0.2932, 0.21271 and 0.1466 kg per plot).

Weed control

Manual weed control was carried out using a hoe, cutlass, and by hand.

Pest and disease control

Cyber force was administered at a concentration of 0.5 liters per hectare (Mohammed et al., 2022) for caterpillar and beetle control. The application started two weeks after germination (2 WAG) and continued until the fruiting stage.

Harvesting

At maturity, the okra fruits were harvested by hand and using a knife.

Experimental Design and Treatments Allocation

The experiment was laid out in a Randomized Complete Block Design (RCRD) with four treatments randomly allocated and replicated four times. The size of the experimental site was 8.7 m x 7.5 m (65.25 m^2) with a total of 16 plots. Each unit plot size was 1.8 m × 1.5 m (2.7 m^2) with 16 hills per plot. An alley of 0.5 m between plots and blocks was maintained to ensure a true reflection of each treatment per plot.

Blocks allocation

BLOCK 1	BLOCK 2	BLOCK 3	BLOCK 4
T4	T1	T2	T3
T1	T3	T4	T2
T3	T2	T1	T4
T2	T4	T3	T1

Fertilizer treatments

Treatments	Interpretation
T1	No biomass (Control)
T2	0.4254 kg of *A. lebbeck* leafy biomass/plot (1575 kg/ha of *A. lebbeck* leafy biomass)
T3	0.2932 kg of *A. zygia* leafy biomass/plot (1086 kg/ha of *A. zygia* leafy biomass)
T4	0.21271 kg of *A. lebbeck* leafy biomass + 0.1466 kg of *A. zygia* leafy biomass/subplot (788 kg/ha of *A. lebbeck* + 542 kg/ha of *A. zygia* leafy biomasses)

Nitrogen demand of okra is 75 kgN/ha (Mohammed et al., 2022)

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Data collection and analysis

Weekly data on the growth and yield aspects of okra were gathered starting from the second week after planting (2 WAP) up to the twelfth week after planting (12 WAP). Growth parameters included plant height and stem diameter. Plant height was determined by measuring from the ground surface to the top of the tallest leaf using a meter ruler while stem diameter was measured at a point 5 cm above the ground using a digital caliper.

Data concerning yield parameters were gathered, specifically the quantity of fruits and their dry weight upon harvest. Counting the number of pods or fruits was done visually, while the dry weight of pods per fruit was determined by employing an electronic balance after subjecting them to oven drying at 105°C within a laboratory setting. Subsequently, statistical analysis utilizing the Analysis of Variance (ANOVA) method was performed using STATISTIX 10 software at a significance level of 5% (Analytical software, 2013). To compare the means that displayed significant differences, the Least Significant Difference (LSD) was utilized. The outcomes were then displayed through tables and graphs, generated using Microsoft Excel version 2021.

RESULTS

Effect of *Albizia lebbeck* and *Albizia zygia* leafy biomass on the morphological growth of okra

Height (cm) of okra from 2 to 12 weeks after planting (WAP)

The effect of various leafy biomass treatments of *A. lebbeck* and *A. zygia* on the height of okra during the 12-week growth period is summarized in Table 01. Weekly height measurements indicated significant differences ($p < 0.05$) from 2 to 12 weeks after planting (WAP) for each treatment. At 2 WAP, the tallest okra plants were observed in T3 (8.9 cm), followed by T2 (8.76 cm) and T4 (8.6 cm), while the shortest plants were in T1 (5.08 cm) with a percentage difference of 54.65%. Although there were no notable differences between T3, T2, and T4, they all exhibited significant variation from the control (T1). Similar trends persisted from 4 to 12 WAP with percentage difference of 32.65% and 18.84% respectively.

Table 01 - Effect of *Albizia lebbeck and Albizia zygia* leafy biomass on the Height (cm) of okra from 2 to 12 weeks after planting (WAP)

TREATMENT	2 WAP (±SeM)	4 WAP (±SeM)	6 WAP (±SeM)	8 WAP (±SeM)	10 WAP (±SeM)	12 WAP (±SeM)
T1	5.08±1.2^{b}	18.28±1.1^{b}	23.8±0.6^{b}	24.18±0.5^{b}	26.18±0.5^{b}	28.08±0.8^{b}
T2	8.76±0.4^{a}	25.28±0.6^{a}	29.34±0.4^{a}	29.82±0.3^{a}	31.82±0.6^{a}	33.72±0.4^{a}
T3	8.90±0.3^{a}	25.40±2.^{4a}	29.34±0.4^{a}	30.02±1.2^{a}	32.02±0.4^{a}	33.92±0.9^{a}
T4	8.60±0.8^{a}	25.40±1.5^{a}	31.04±0.8^{a}	31.34±0.3^{a}	33.34±1.2^{a}	35.21±0.7^{a}
P-VALUES	0.0087	0.0156	0.0000	0.0000	0.0003	0.0000
LSD	2.28	4.78	1.99	2.15	2.56	2.26
CV (%)	18.86	13.15	4.55	4.84	5.39	4.48

Means in the column accompanied by the same letter (s) are not significantly difference at ($P \leq 0.05\%$) using Least Significance Difference (LSD), where CV=Coefficient of Variation, WAP=Weeks After Planting, SeM=Standard error of Mean

Mean Height (cm)

There was a significant difference between the treatments means with respect to growth in height of okra over the experimental period, ($p < 0.05$) ($p= 0.0372$, $F= 2.94$, $DF= 3$, $CV=34.18$, $LSD=4.97$). The tallest plants were recorded in T4 (27.5 cm), followed by T3 (26.6 cm), T2 (26.5 cm) and finally, T1 had the shortest plants (20.9 cm). There were no significant differences between T4, T3 and T2, however, they were significantly different from T1 (control) (Figure 02). Therefore, the percentage difference between T4 (27.5 cm) and T1 (20.9 cm) is approximately 27.27%.

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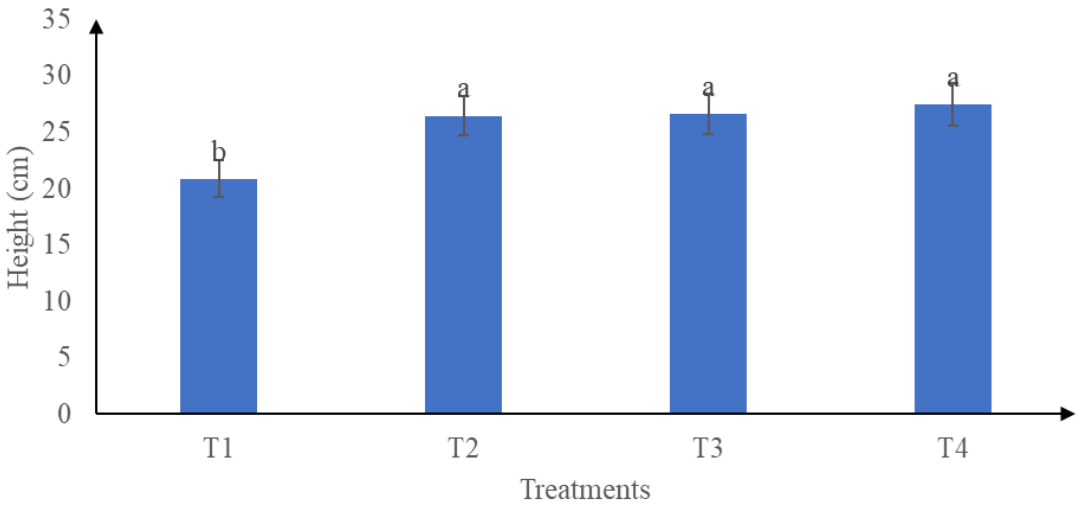

T1= Control; T2= 0.4254 kg of *A. lebbeck*; T3= 0.2932 kg of *A. zygia*; and T4= 0.2127 kg of *A. lebbeck* + 0.1466 kg of *A. zygia*

Figure 02 - Effect of *Albizia lebbeck* and *Albizia zygia* leafy biomass on the height (cm) of okra

Stem diameter (mm) of okra from 2 to 12 weeks after planting (WAP)

The effect of *A. lebbeck* and *A. zygia* leafy biomass on the stem diameter is summarized in Table 02. The growth pattern of okra stem diameter mirrored that of its height between 2 to 12 weeks after planting (WAP). Initially, at the second week, no significant variations were observed among the treatments, p>0.05 with a percentage difference between T4 (1.5 mm) and T1 (1.2 mm) being approximately 22.22%. However, starting from the 4th to the 12th WAP, Analysis of Variance revealed notable differences in the treatment means. In the fourth week, the most robust plant growth was observed in T3 (3.8 mm), statistically comparable to T4 (3.7 mm) and T2 (3.6 mm), but significantly distinct from the control treatment (T1) (1.5 mm) with a percentage difference of 86.79%. Similar trends were observed between the 6th and 12th WAP with the percentage difference being 106.25% and 100% respectively.

Table 02 - Effect of *Albizia lebbeck and Albizia zygia* leafy biomass on the Stem diameter (mm) of okra 2 to 12 WAP

TREATMENT	2 WAP (±SeM)	4 WAP (±SeM)	6 WAP (±SeM)	8 WAP (±SeM)	10 WAP (±SeM)	12 WAP (±SeM)
T1	1.2±0.3	1.5±0.1^{b}	1.5±0.2^{b}	1.5±0.2^{b}	1.6±0.4^{b}	1.6±0.4^{b}
T2	1.4±0.2	3.6±0.3^{a}	4.9±0.6^{a}	3.4±0.2^{a}	4.8±0.6^{a}	4.8±0.5^{a}
T3	1.5±0.2	3.8±0.5^{a}	4.8±0.4^{a}	3.8±0.3^{a}	4.8±0.2^{a}	4.8±0.1^{a}
T4	1.5±0.2	3.7±0.7^{a}	4.7±0.3^{a}	3.8±0.3^{a}	4.8±0.1^{a}	4.8±0.2^{a}
P-VALUES	0.7636	0.0100	0.0001	0.0001	0.0000	0.0001
LSD	0.66	1.41	1.14	0.78	1.07	1.11
CV (%)	31.04	29.2	18.86	15.94	17.34	17.94

Means in the column accompanied by the same letter (s) are not significantly difference at (P≤ 0.05%) using Least Significance Difference (LSD), where CV=Coefficient of Variation, WAP=Weeks After Planting

Mean Stem diameter (mm)

The mean stem diameter of okra plant as affected by the different treatments at the end of the experiment is shown in Figure 03. Stem diameter of okra followed the same pattern to plant height where there was a significant difference between the treatment means throughout the experimentation, (p < 0.05) (p= 0.0000, F=23.17, DF=3, CV=37.06, LSD=0.70). The biggest stem diameter was recoded in T3 (3.88 mm) which did not differ significantly from T4 (3.87 mm) and T2 (3.85 mm) but they were significantly different from T1 which recorded the smallest stem diameter (1.48 mm) with a percentage difference of 89.55%.

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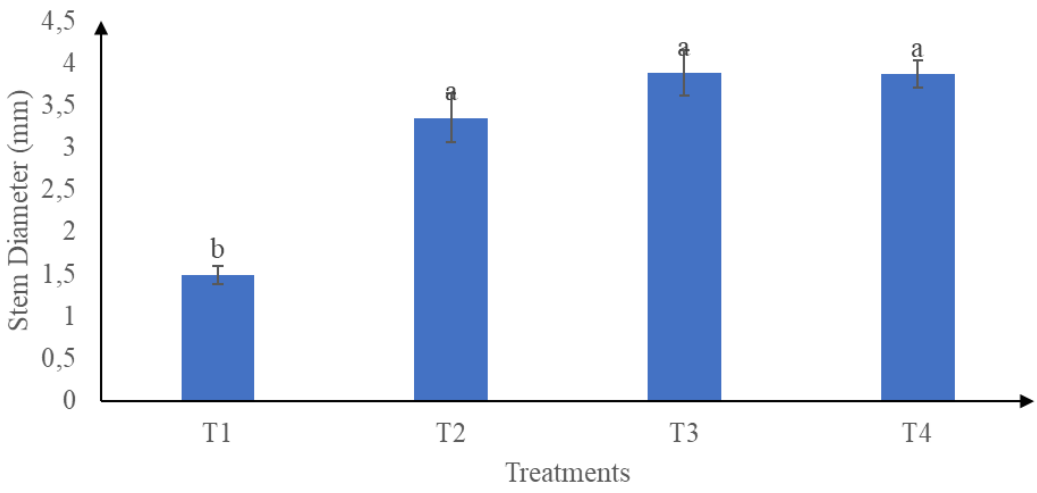

T1= Control / T2= 0.4254 kg of *A. lebbeck* / T3= 0.2932 kg of *A. zygia* / T4= 0.2127 kg of *A. lebbeck* + 0.1466 kg of *A. zygia*
Figure 03 - Effect of *Albizia lebbeck* and *Albizia zygia* leafy biomass on the Stem diameter (mm) of okra

Effect of *Albizia lebbeck* and *Albizia zygia* leafy biomass on the Yield of okra at harvest

Fruit yield/ha

The effect of different levels of *A. lebbeck* and *A. zygia* leafy biomass application regimes on the fruit yield/ha component of okra at harvest. There was a significant difference between treatment means, ($p < 0.05$) ($p= 0.0214$, $F=4.71$, $DF=3$, $CV=32.71$, $LSD=77143$). Mean values of okra fruit yield during the period of experimentation indicated that, T4 biomass application regime recorded the maximum number of fruits (184852) followed by T3 (179037), T2 (176667), and the minimum number of fruit yield/ha was recorded in fertilizer application regime of T1 (71742) with a percentage difference between T4 and T1 being 88.16%.There was no significant difference between T2, T3 and T4, but they differed significantly from T1 (Figure 04).

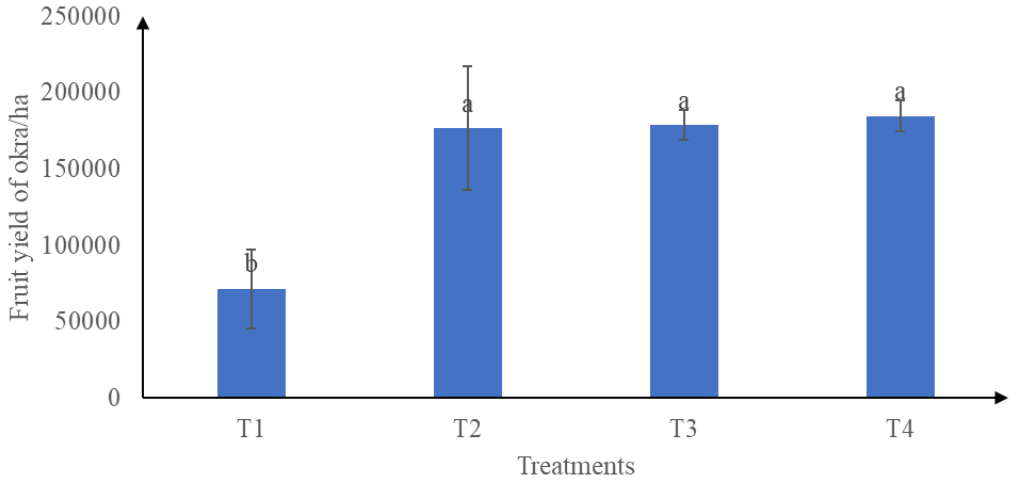

Figure 04 - Effect of *Albizia lebbeck* and *Albizia zygia* leafy biomass on the Yield/ha of okra. T1= Control; T2= 0.4254 kg of *A. lebbeck*; T3= 0.2932 kg of *A. zygia*; and T4= 0.2127 kg of *A. lebbeck* + 0.1466 kg of *A. zygia*.

Dry weight kg/ha

Figure 05 shows the effect of different biomass application regimes on the dry weight component of fruits at harvest. There was a significant difference between treatment means, with the maximum dry weight of fruits recorded in T4 (338.89 kg/ha) followed by T3 (338.41 kg/ha), T2 (334.26 kg/ha) and the minimum dry weight was recorded in T1 (169.11 kg/ha). There were significant differences between T2, T3 and T4, but they were significantly different from the control treatment (T1). The percentage difference between T4 and T1 is 66.73%.

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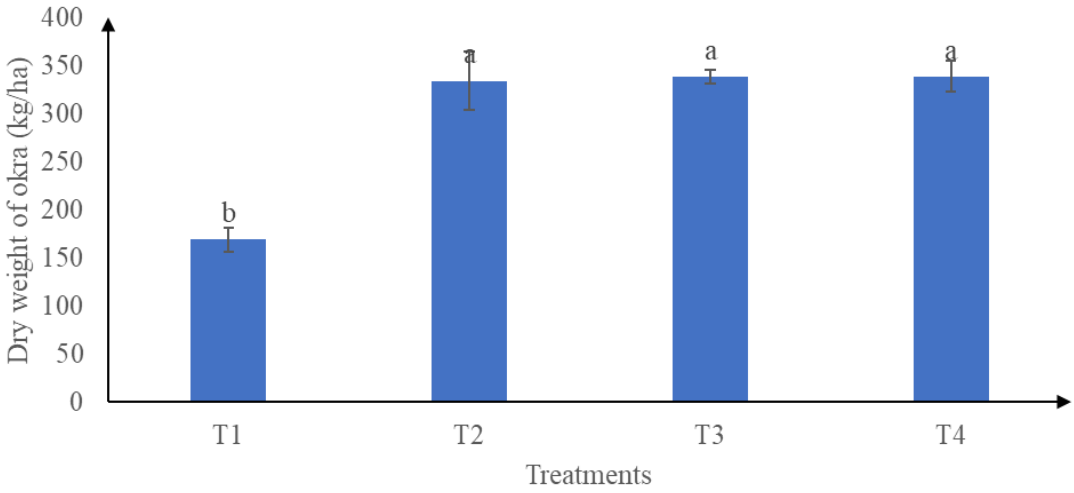

Figure 05 - Effect of *Albizia lebbeck* and *Albizia zygia* leafy biomass on the Dry weight kg/ha of okra T1= Control; T2= 0.4254 kg of *A. lebbeck*; T3= 0.2932 kg of *A. zygia*; and T4= 0.2127 kg of *A. lebbeck* + 0.1466 kg of *A. zygia*.

DISCUSSION

Effect of *Albizia lebbeck* and *Albizia zygia* leafy biomass on the morphological growth and yield

The utilization of *A. lebbeck* and *A. zygia* leafy biomass has demonstrated a positive impact on the morphological growth and yield of plants. These organic materials contribute to enhanced plant development and increased yield, showcasing their potential as beneficial amendments in agricultural practices. According to Muzamil et al. (2021) and Rao et al. 2022), enhancing soil fertility is bolstered through the incorporation of natural materials, enriching soil organic content. The practice of modifying soil aims to enhance its structure and physical attributes, like its ability to retain water, permeability, water penetration, drainage, aeration, all conducive to fostering optimal conditions for root development (Franzluebbers, 2023; Shakywal et al., 2023).

The results from the study have shown that okra demonstrated significantly greater growth when tree legume leafy residues were applied compared to the control (okra without manure). This observation highlights the improved growth performance of okra when organic residues are added to enhance soil nutrients. This result confirms the findings of Sun et al. (2020) and Zhao et al. (2020) whom reported that the utilization of organic matter can enhance soil nutrients, ultimately boosting crop productivity. Additionally, *A. zygia* and *A. lebbeck* leafy biomasses are rich in nutrients, including nitrogen, phosphorus, potassium, and other essential minerals. This could be that when incorporated into the soil as organic amendments, they release these nutrients during decomposition (Magingo, 2017). The findings support similar studies by Mukhopadhyay & Masto (2022) and Singh & Kumar (2022) whom reported that the use of *A. lebbeck* and other *Albizia* species could increase nutrient availability in the soil providing a favorable environment for enhanced plant growth, as evidenced in taller and bigger okra plants.

Furthermore, organic amendments play a vital role in enhancing soil fertility and promoting healthy crop yields. By adding organic matter such as compost or manure to the soil, essential nutrients are released slowly over time, improving soil structure, water retention, and microbial activity. This not only boosts nutrient availability for plants but also contributes to sustainable agricultural practices, reducing reliance on synthetic fertilizers and fostering long-term soil health. According to Essling (2020), Hombegowda et al. (2022) and Murugaragavan et al. (2022), organic matter from the leafy biomasses might have improved soil structure by enhancing aggregation, aeration, and water-holding capacity. Mitra et al. (2023), Sharma & Kumar (2023) and Zvinavashe et al. (2021) reported that better soil structure could facilitate root penetration, nutrient uptake, and water absorption, promoting healthy root development and overall plant growth. Vigorous height and stem diameter growth in the *Albizia* species amended plots compared to the unamended plots might be attributed to the presence of organic matter from *A. zygia* and *A. lebbeck* leafy biomasses which could have encouraged beneficial microbial activity in the soil. This agrees with Edwards & Arancon (2022), Soumare et al. (2023), Turp et al. (2023), and Younas et al. (2022) whom indicated that the presence of microorganisms in the soil could break down organic matter, thereby converting it into plant-available nutrients.

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These symbiotic relationships between plants and soil microbes can significantly boost plant growth and yield (Diagne et al., 2020; Khaliq et al., 2022).

The addition of *A. zygia* and *A. lebbeck* leafy biomasses like other organic amendments could have helped reduced soil erosion by improving the soil's ability to retain moisture and withstand heavy rainfall. This, in turn, maintained a stable growing environment for the okra plants, enabling them to grow better with higher fruit yield. This supports previous research by Mohammed et al. (2022) whom stated that the application of *Leucaena leococephala* leafy biomass as soil amendment could bind loosed soil particles together thereby preventing them from leaching and run-off which could provide favorable conditions for optimum okra growth and yield compared to the control. More so, organic matter in the soil acts like a sponge, retaining moisture in the soil for longer periods (Li et al., 2022; Pradhan et al., 2023). This increased water availability might have ensured a consistent water supply to the okra plants, preventing stress and promoting continuous growth, which is reflected in increased plant growth and yield. Similar results were reported by Mohammed et al. (2023) when *Gliricidia sepium* leafy biomass significantly improved the growth and yield of tomato plants compared to the control. In addition, leafy biomasses from *A. zygia* and *A. lebbeck* are known to contain bioactive compounds that can stimulate plant growth. Leafy biomasses from *A. zygia* and *A. lebbeck* are recognized for their content of bioactive compounds that possess the capability to stimulate plant growth. These compounds contribute to the overall health and vigor of plants, promoting robust growth and development. According to Iqbal et al. (2023), and Mekonnen & Kibret (2021), these compounds, through various mechanisms, might have promoted cell elongation and division, leading to increased height, stem diameter, fruit yield, and fruit dry weight in okra plants. This finding confirms the suggestion by Aggangan (2019) that the higher availability of nutrients increases succulent growth of plants. It might have therefore been for this reason that plant height, stem diameter, fruit yield/ha and dry weight of fruits (kg/ha) increased slowly through the experimental period than in the unamended soils (control). Organic amendments like *A. lebbeck* and *A. zygia* leafy biomasses can possess natural weed-suppressive and pest-repelling properties (Bamboriya et al., 2022). By minimizing weed competition and deterring pests, the okra plants have reduced stress and can allocate more energy and nutrients towards growth and fruit production, resulting in increased fruit yield and dry weight (Chauhan et al., 2023).

In the control (T1) there is a lower nutrient content in the soil compared to the other treatments which might have attributed to the lowest growth and yield performance. Nutrient deficient soils result in retarded growth and lower yields of plants (Elavarasan & Premalatha, 2019; Füllgrabe et al., 2022; Narayan et al., 2022; Yadav et al., 2021). Also, plants that received amendments at T2, T3 and T4 were not significantly different in height, stem diameter, fruit yield/ha and dry weight (kg/ha). This might imply that, plants with amendment at T2 and T3 received enough nitrogen for growth; hence the excess in nitrogen received by plants with amendment at T4 did not result in significant difference in the growth and yield of the okra.

CONCLUSIONS

The use of *A. lebbeck* and *A. zygia* leafy biomass increases the growth in height and stem diameter of okra plants. Furthermore, it increases the production and dry weight of okra fruits. However, it is important that research is conducted to determine the most effective and efficient application rates of *A. lebbeck* and *A. zygia* leafy biomass to optimize growth and improve okra yield.

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