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ULTRASOUND-ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM JAMBOLAN (*SYZYGIUM CUMINI*) AND JABUTICABA (*MYRCIARIA JABUTICABA*) PULPS

EXTRAÇÃO ASSISTIDA POR ULTRASSOM DE COMPOSTOS FENÓLICOS DE POLPAS DE JAMBOLÃO (*SYZYGIUM CUMINI*) E JABUTICABA (*MYRCIARIA JABUTICABA*)

EXTRACCIÓN ASISTIDA POR ULTRASONIDO DE COMPUESTOS FENÓLICOS DE PULPAS DE JAMBOLÁN (*SYZYGIUM CUMINI*) Y JABUTICABA (*MYRCIARIA JABUTICABA*)

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ABSTRACT

Several variables influence the extraction of phenolic compounds, encompassing factors such as the choice of solvent, agitation, extraction duration, solute-solvent ratio, temperature, and mass transfer efficiency. Technological tools, including ultrasound, microwave, and centrifuge, offer avenues for enhancing efficiency, economizing time and resources, and augmenting yield. This study seeks to establish a standardized approach for extracting phenolic compounds from jambolan and jabuticaba pulps. The extraction protocol entailed the introduction of the designated solvent to the sample, followed by exposure to ultrasonic treatment across varying durations. Our findings indicate that a mere two extraction cycles might suffice to comprehensively elicit the extraction of polyphenols from both jambolan and jabuticaba. Furthermore, the temporal parameter displayed limited influence on outcomes. When focusing on jambolan, the zenith phenolic content was achieved through a dual-extraction procedure employing methanol as the solvent, executed sequentially over a 120-minute interval. This yielded a content of 1866.67 ± 15.7 mg GAE/100 g. Equally notable, the application of acetone in two consecutive extractions, each within a 60-minute span, yielded a comparable outcome of 1846.04 ± 11.02 mg GAE/100 g. In contrast, for jabuticaba, the most substantial phenolic content emerged through a triple-extraction regimen utilizing acetone as the solvent. This process, conducted successively over a 120-minute duration, culminated in a content of 2259.31 ± 0.89 mg GAE/100 g.

KEYWORDS

Anthocyanins; functional food; Polyphenol; proximal composition; nutrients.

RESUMO

Diversas variáveis influenciam a extração de compostos fenólicos, abrangendo fatores como escolha do solvente, agitação, duração da extração, relação soluto-solvente, temperatura e eficiência de transferência de massa. Ferramentas tecnológicas, incluindo ultrassom, micro-ondas e centrífuga, oferecem caminhos para aumentar a eficiência, economizar tempo e recursos e aumentar o rendimento. Este estudo busca estabelecer uma abordagem padronizada para extração de compostos fenólicos de polpas de jambolão e jabuticaba. O protocolo de extração envolveu a introdução do solvente designado na amostra, seguido pela exposição ao tratamento ultrassônico em durações variadas. Nossas descobertas indicam que apenas dois ciclos de extração podem ser suficientes para extrair de forma abrangente a extração de polifenóis tanto do jambolão quanto da jabuticaba. Além disso, o parâmetro temporal apresentou influência limitada nos resultados. Ao focar no jambolão, o conteúdo fenólico zenital foi obtido por meio de um procedimento de extração dupla empregando metanol como solvente, executado sequencialmente em um intervalo de 120 minutos. Isto rendeu um teor de 1866,67 ± 15,7 mg GAE/100 g. Igualmente notável, a aplicação de acetona em duas extrações consecutivas, cada uma dentro de um intervalo de 60 minutos, produziu um resultado comparável de 1.846,04 ± 11,02 mg GAE/100 g. Em contraste, para a jabuticaba, o conteúdo fenólico mais substancial surgiu por meio de um regime de tripla extração utilizando acetona como solvente. Esse processo, conduzido sucessivamente ao longo de 120 minutos, culminou com um teor de 2.259,31 ± 0,89 mg GAE/100 g.

PALAVRAS-CHAVE

Antocianinas, Alimentos funcionais, Polifenois, Composição proximal, Nutrientes.

RESUMEN

La extracción de compuestos fenólicos se encuentra influenciada por múltiples variables, como la elección del disolvente, el proceso de agitación, la duración de la extracción, la relación soluto-solvente, la temperatura y la eficiencia de la transferencia de masa. La incorporación de herramientas tecnológicas brinda oportunidades para aumentar la eficiencia del proceso, optimizar la utilización del tiempo y los recursos, y mejorar el rendimiento. Este estudio busca establecer un enfoque estandarizado para la extracción de compuestos fenólicos de las pulpas de jambolán y jabuticaba. El protocolo de extracción involucra la introducción del solvente designado en la muestra, seguido de una exposición a un tratamiento ultrasónico de diferentes duraciones. Nuestros resultados indican que la realización de dos ciclos de extracción puede ser suficiente para lograr una extracción com-

pleta de polifenoles tanto del jambolán como de la jabuticaba. En el caso del jambolán, se alcanza el contenido fenólico óptimo mediante un procedimiento de extracción dual que utiliza metanol como solvente y se ejecuta secuencialmente durante un intervalo de 120 minutos. Esto arrojó un contenido de 1866,67 ± 15,7 mg GAE/100 g. Igualmente notable, la aplicación de acetona en dos extracciones consecutivas, cada una dentro de un lapso de 60 minutos, arrojó un resultado comparable de 1846,04 ± 11,02 mg GAE/100 g. En contraste, para jabuticaba, el contenido fenólico más sustancial surgió a través de un régimen de triple extracción utilizando acetona como solvente. Este proceso, realizado sucesivamente durante 120 minutos, culminó con un contenido de 2259,31 ± 0,89 mg GAE/100 g.

PALABRAS CLAVE

Antocianinas, Alimento funcional, Polifenoles, Composición Proximal, Nutrientes.

1 INTRODUCTION

Phenolic compounds are found in many fruits and have at least one phenol group in their chemical structure (HAMINIUK *et al.*, 2012; MARCHIOSI *et al.*, 2020). This group can be classified into polyphenols (lignin and tannins), oligophenols (flavonoids, stilbenes and coumarins), and monophenols (phenolic acids such as benzoic and cinnamic acids and their hydroxylated derivatives). Some are soluble in organic solvents, others in water, while large polymers, such as lignin, may be insoluble (VERMERRIS; NICHOLSON, 2006; MARCHIOSI *et al.*, 2020).

Proanthocyanidins and bi- and triflavonoids constitute the two dominant classes of plant phenolic compounds. Scientists have already identified more than 10,000 different structures characterized as flavonoids and their conjugates. These natural products can be split into three groups: flavonoids, isoflavonoids, and neoflavonoids (CHEYNIER *et al.*, 2013).

Phenolic compounds are white, although the complex electronic conjugation of some flavonoids results in a yellow, or even red, color. Anthocyanins, for example, represent a class of flavonoids that provide the orange, red, blue, and purple colors of many plant tissues (CHEYNIER *et al.*, 2013).

These compounds are not necessary for the key growth and development of plants. However, they are crucial for how plants interact with the environment, their ability to reproduce, and for their protection (CHEYNIER *et al.*, 2013). Thus, phenolic compounds can act as a defense mechanism against herbivores and pathogens; protect the cellular structure from chain reactions generated by free radicals; absorb ultraviolet light, protecting cell tissue against damage generated by solar radiation; present a structural function, by interconnecting cell wall polysaccharides and anchoring lignins; and aid in the functional development of pollen and the growth hormone auxin (CHEYNIER *et al.*, 2013; MARCHIOSI *et al.*, 2020).

The research on phenolics in fruits has been increasing over the years and has caught the interest of many researchers around the world. This interest is mainly because of discoveries related to potential benefits to human health (HAMINIUK *et al.*, 2012). These compounds are known for their antioxidant and antimicrobial properties, for exerting preventive activity against infectious and degenerative diseases, inflammation and allergies, acting as antimicrobials and modulating enzymes and other proteins (OZCAN *et al.*, 2014). It could be used to treat many diseases such as cancer, heart disease, and more (DIAS *et al.*, 2020).

Most research has focused on determining the content of total phenolics, flavonoids and anthocyanins (HAMINIUK *et al.*, 2012). While there are extraction methods that work for various solvents, creating a specific procedure for different foods is still necessary because of their diversity. Different factors affect the extraction of phenolic compounds, such as solvent, agitation, extraction time, solute-solvent ratio, temperature, and mass transfer efficiency (HAMINIUK *et al.*, 2012; DORTA *et al.*, 2013). The extraction techniques consist of simple maceration of the food in contact with a solvent (methanol, ethanol, acetone and ethyl acetate, and the combination of these solvents with water) (HAMINIUK *et al.*, 2012; LEFEBVRE *et al.*, 2021). Equipment such as ultrasound, microwave and centrifuge can be used in order to Reduce Time And Resources And Increase Yield (LEFEBVRE *et al.*, 2021).

Jambolan (*Syzygium cumini* (L.) Skeels, also known as *Eugenia jambolana* Lam.) is a fruit of the Myrtaceae family and Syzygieae tribe. Originally from Asia, the fruit is distributed all over the globe, and can be found in India, Bangladesh, Burma, Nepal, Pakistan, Sri Lanka, Indonesia, Malaysia, Thailand, Philippines, and even in countries in Africa and Latin America (SABINO *et al.*, 2018). Renowned for its pulp displaying a vivid spectrum ranging from red and purple to black, the fruit distinguishes itself through its substantial presence of phenolic compounds (995 to 1117 mg GAE/100 g, dry weight), predominantly anthocyanins (BRITO *et al.*, 2007; REYNERTSON *et al.*, 2008; RUFINO *et al.*, 2010; LESTARIO *et al.*, 2017).

Fruit of the same family (Myrtaceae) and species (*Eugenia*) of jambolan, jabuticaba sabará (*Eugenia jaboticaba* (Vell.) Kiaersk., or *Myrciaria jabuticaba*), is native to the south and southeast of Brazil, and distributed throughout Brazil, but also it can be found in Paraguay and Argentina (SALOMÃO *et al.*, 2018). The fruits are globoid, and standing out for their skin color, which varies between purple and black (NEVES *et al.*, 2018; SALOMÃO *et al.*, 2018). The jabuticaba skin and pulp, together, have their high levels of total phenolic compounds (744 mg GAE/100 g, f. w), mainly ellagic acid (311 mg GAE/100 g, f. w) (ABE *et al.*, 2011).

Hence, the objective of this study was to present a standardization for the phenolic compound extraction method from jambolan and jabuticaba pulps, with the intention of enhancing result comparability. Accordingly, an asymmetric factorial design was employed to validate the extraction methodology of these fruits. This involved a comparative analysis of diverse solvents, extraction frequencies, and durations to arrive at conclusive recommendations.

2 MATERIALS AND METHODS

2.1 SAMPLES

The collection of the fruits took place during their respective harvest periods, with jabuticaba being gathered in November and jambolan in December 2019, at Caldazinha - Goiás, Brazil (16°45'58.8"S, 48°55'04.9"W). Selection criteria encompassed the fruits reaching an optimal level of ripeness, characterized by a fully darkened skin hue. Any fruits displaying blemishes, diseases, or mechanical impairments were excluded from consideration. Subsequently, the fruits were carefully placed in plastic containers, sanitized with 2 ppm sodium hypochlorite, and manually pulped utilizing stainless steel knives.

The research focused solely on the edible segments of the fruits, aligning with local consumption norms. Hence, the jambolan's skin and pulp were meticulously separated from the seed, while the jabuticaba was employed in its entirety, encompassing the skin, pulp, and seed. The pulps were hermetically sealed within low-density polyethylene packaging under a vacuum environment, safeguarded against light exposure, and subsequently subjected to freezing conditions at a temperature of -20 ºC until the commencement of the analytical procedures.

2.2 PROXIMATE COMPOSITION

The moisture contents of jambolan and jabuticaba pulps were ascertained through gravimetric analysis (method No. 934.06), involving the drying of samples at 105°C, while the ash contents were determined by subjecting samples to combustion and incineration at 550°C until weight constancy (AOAC, 2019).

For the determination of nitrogen contents, the micro-Kjeldahl method (AOAC, 2019 - method No. 920.152) was employed. This encompassed subjecting the sample to acid digestion at 350°C, followed by neutralization with a sodium hydroxide solution (50%), subsequent distillation, and titration employing a hydrochloric acid solution (0.02 mol/L). The concentration of crude protein was calculated using the conversion factor 6.25.

The quantification of total lipid content was conducted according to the methodology outlined by Bligh and Dyer (1959). Extracts were obtained through the homogenization of samples utilizing a mixture of chloroform, methanol, and water in a rotary shaker. Subsequently, a natural phase separation process ensued at room temperature for a duration of 12 hours. The ensuing steps involved discarding the aqueous phase, filtering the lower layer through film into a petri dish, and facilitating solvent evaporation within a fume hood. The determination of lipid content was then established through the assessment of weight variance.

The total carbohydrate content was approximated by employing a difference-based approach, entailing the subtraction of values corresponding to moisture, crude protein, ash, and lipids from a sum of 100.

Calculation of the total energy value for the samples was executed utilizing the subsequent conversion factors: 4.0 kcal/g for total protein and carbohydrates, and 9.0 kcal/g for lipids (MERRIL; WATT, 1973).

2.3 EXTRACTION

The extraction procedures were executed in accordance with the methodology delineated by Chisté and collaborators (2011) and Escarpa and González (2000), albeit with certain adaptations. Within Teflon tubes, 1.0 g of pulp was combined with 10.0 mL of the chosen solvent (methanol; or methanol:acetone, 50:50, v/v; or acetone). Subsequent to this, the resultant extracts were subjected to ultrasonic treatment using an ultrasonic bath (UNIQUE – UltraSonic Cleaner – USC 2800) at 23 ºC, across various durations (30, 60, 90, and 120 minutes).

Following ultrasonic treatment, the extracts were meticulously filtered and subsequently transferred to 25.0 mL volumetric flasks, with the final volume being adjusted using the respective solvent. The requirement for additional extractions was evaluated based on the solid residue, with one, two, or three extraction cycles being applied as per the prescribed experimental design (Table 1).

The solid-liquid ratio during the extraction process varied in correspondence to the number of extraction cycles. Thus, extracts undergoing one, two, or three extraction cycles were associated with solid-liquid ratios of 1:10, 1:20, and 1:30 (g:mL, m/v), respectively.

2.4 ASYMMETRIC FACTORIAL DESIGN

The optimization of optimal conditions for the extraction of phenolic compounds from the pulps was conducted through an asymmetric factorial study encompassing three distinct factors (number of extractions, time, and solvent). These factors were distributed across 3 and 4 levels. This specific application of asymmetric factorial design arises from experimental scenarios where factors are not characterized by an equal number of levels, as expounded by Addelman (1962).

The efficacy of this approach lies in its ability to amalgamate varying factors with their corresponding levels, allowing for the repeated testing of a particular factor at multiple levels by their concomitant combination. Consequently, this methodology facilitates the scrutiny of primary effects as well as interactions across diverse levels. This form of factorial study permits the judicious selection of the requisite number of trial runs, thereby enhancing the efficiency of assessing targeted effects, as elucidated by Oles (1993). Given that the accurate selection of factors and their corresponding levels is pivotal for a discerning analysis of the chosen variables' impact, the significance of this approach becomes evident (GUNST; MASON, 2009).

Within this specific experimental design, the significance of the examined factor is determined by its absolute value surpassing a predefined critical effect threshold. The critical effect itself is ascertained through comparative tests conducted between means. Furthermore, the utilization of graphical methodologies can prove beneficial in the evaluation of the factor's significance (HUND *et al.*, 2000).

Table 1 – Experimental model: independent variable levels (original and coded)

Independent variable (Factor)	Response	Levels			
		-1	0	+1	+2
Solvent	X_1	Methanol	Methanol:Acetone, 1:1, v/v	Acetone	
Number of extractions	X_2	1	2	3	
Time	X_3	30	60	90	120

Source: Research data

2.5 QUANTIFICATION OF TOTAL PHENOLIC COMPOUNDS

The determination of total phenolic compounds was executed employing the Folin-Ciocalteu test, a method grounded in the reduction of the phosphorus-wolframate-phosphomolybdate complex (GE *et al.*, 2020). To an aliquot of 0.25 mL of the extract, 0.25 mL of Folin-Ciocalteu reagent and 2.0 mL of distilled water were added. The resultant solutions were thoroughly mixed and allowed to stand at room temperature for a duration of 30 minutes. Subsequently, a 0.25 mL portion of 10% sodium carbonate solution (Na_2CO_3) was introduced to the solution, followed by an additional period of incubation at room temperature for 60 minutes. The measurement was then conducted at 750 nm utilizing a spectrophotometer (GENOVESE *et al.*, 2008). The obtained results were expressed as milligrams of gallic acid equivalent (GAE) per 100 grams of fresh sample (f.w.).

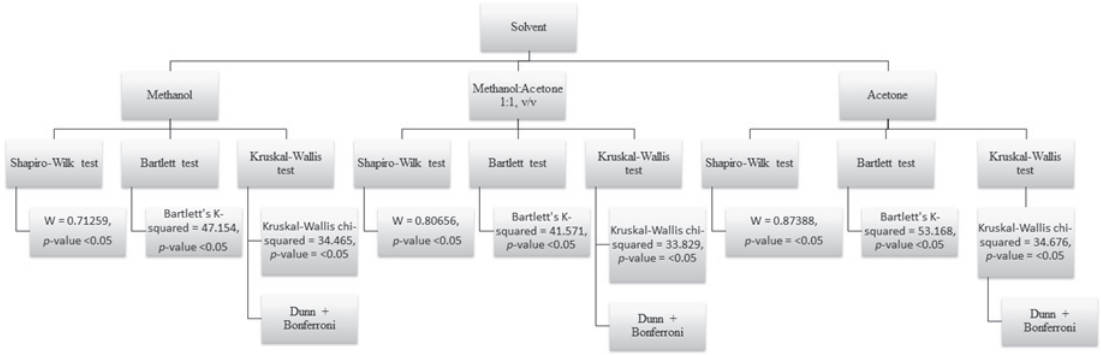
2.6 STATISTICAL ANALYSIS

The data underwent preliminary assessments of normality (Shapiro-Wilk test) and homogeneity of variances (Bartlett test) to determine the suitability of employing parametric or non-parametric inferential statistical methodologies. Subsequently, an analysis was conducted by comparing the number of extractions and extraction duration for each solvent, followed by a comparative evaluation of the optimal outcomes attained by each solvent to ascertain their respective performance levels.

For data displaying deviations from normal distribution (Shapiro-Wilk) and variance homogeneity (Bartlett), an evaluation was conducted through the Kruskal-Wallis test, supplemented by Dunn's post-hoc analysis and Bonferroni adjustment method, as illustrated in Figures 1 and 2.

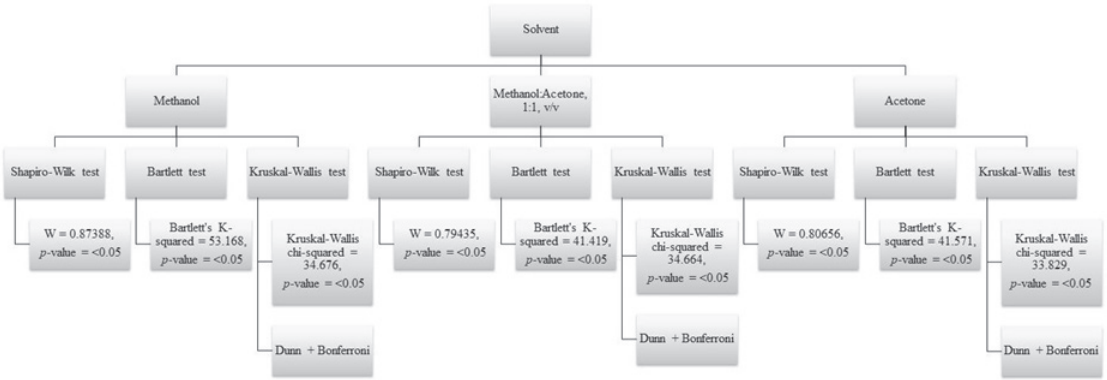
All statistical evaluations were carried out at a significance level of 0.05 (= 5%). When dealing with data adhering to a normal distribution, analysis of variance (ANOVA) with Tukey's post-hoc testing was administered. This step was essential to satisfy the prerequisites necessary for conducting parametric statistical analyses, which encompassed the assumptions of normality and variance homogeneity. The entire statistical analysis process was executed using Graphpad Prism version 6.0.0 (Graphpad Software).

Figure 1. Statistical analysis flowchart of jambolan pulp.



Source: Research data

Figure 2 – Statistical analysis flowchart of the jabuticaba whole fruit pulp



Source: Research data

3 RESULTS AND DISCUSSION

3.1 PROXIMATE COMPOSITION

Both fruits are distinguished by their elevated water content and significant sugar concentrations, as outlined in Table 2. The jambolan pulp subjected to scrutiny in this study demonstrates moisture contents akin to those documented by Brito and collaborators (2017) (87.2 g/100 g), Seraglio and collaborators (2018) (84.7 to 88.6 g/100 g), and Vital and collaborators (2020) (84.0 to 85.3 g/100

g), encompassing examinations of jambolan originating from the states of Pará, Santa Catarina, and Minas Gerais, respectively.

Similarly, the entire jabuticaba fruit exhibits levels in congruence with those reported by Inada and collaborators (2015) (87.4 g/100 g) and Lima and collaborators (2008) (79.41 g/100 g) for *M. jaboticaba* (cv. Sabará). Upon comparative analysis of the fruits in this study with counterparts bearing akin characteristics, such as juçara (*Euterpe edulis*) (76.6 g/100 g) and açaí (*Euterpe oleracea*) (82.7 to 92.0 g/100 g), it becomes discernible that the humidity levels remain commensurate. These results are within what is expected for fresh fruit.

It is desirable to retain the inherent moisture content of fruits and vegetables to preserve their organoleptic attributes, including texture and flavor (LEVI *et al*., 1983). Understanding fruit moisture content is crucial for predicting the growth and propagation of microorganisms, with the goal of devising preservation methods that extend shelf life. Additionally, identifying the specific microorganisms that thrive in this environment is of paramount importance. This knowledge can be leveraged advantageously, as certain transformations, such as fermentation, may be considered desirable. Fruit can then be utilized as a raw ingredient for the production of fermented products like yogurt or liqueurs (HAMAD, 2012).

In terms of ash content, no statistically significant disparity was discernible amongst the fruits subjected to analysis within this investigation, as delineated in Table 2. Aggregate ash concentrations surpassed those reported for jambolan pulps as indicated by Brito and collaborators (2017) (0.23 g/100 g) and Vital and collaborators (2020) (0.32 g/100 g), y*et al*igned with values documented by Barcia and collaborators (2012) (0.42 g/100 g). Comparable levels were reported by Inada and collaborators (2015) (0.39 g/100 g) for jabuticaba. Moreover, it is noteworthy that the fruits scrutinized in this study exhibit lower ash concentrations in comparison to juçara pulps (1.2 g/100 g) (INADA *et al*., 2015) and açaí (0.42 to 0.94 g/100 g) pulps, as reported by Minighin and collaborators (2020).

The jambolan pulp exhibits statistically superior protein content in comparison to the entire jabuticaba fruit. Nevertheless, both samples present protein values lower than those noted in juçara (2.4 g/100 g) (INADA *et al*., 2015) and açaí (1.12 to 1.51 g/100 g) (MINIGHIN *et al*., 2020). Similar protein concentrations were reported for jambolan by Brito and collaborators (2017) (0.85 g/100 g) and Vital and collaborators (2020) (0.75 g/100 g), and for jabuticaba (0.63 g/100 g) by Inada and collaborators (2015).

Both fruits subjected to analysis within the current study (as shown in Table 2) exhibit minimal lipid levels, markedly lower than those documented in juçara (3.32 g/100 g) (INADA *et al*., 2015) and açaí (2.94 to 10.67 g/100 g) (MINIGHIN *et al*., 2020). While Vital and collaborators (2020) report similar lipid concentrations for jambolan (0.27 g/100 g), Brito and collaborators (2017) indicate slightly higher contents (0.49 g/100 g). The lipid concentrations in the whole jabuticaba fruit, as analyzed by Inada and collaborators (2015), mirror those observed in the current study (0.23 g/100 g).

The complete jabuticaba fruit exhibits heightened concentrations of total carbohydrates in comparison to the observed values for jambolan. However, both fruits manifest lower levels than juçara (41.22 g/100g, w.b.) (INADA *et al*., 2015), while maintaining concentrations higher than those found in açaí (2.88 to 5.24 g/100 g) (MINIGHIN *et al*., 2020). Proximate values were reported by Brito and collaborators (2017) for jambolan (11.4 g/100 g) and by Inada and collaborators (2015) for jabuticaba (11.35 g/100 g).

Given the augmented protein and carbohydrate content in jabuticaba, this is mirrored in its total energy content, which in turn surpasses that of jambolan pulp. However, both fruits present lower values than juçara (66.0 Kcal/100 g). Comparable outcomes for jambolan were documented by Brito and collaborators (2017) (48.0 Kcal/100 g), and higher values by Barcia and collaborators (2012) (61 to 68 Kcal.100-1 g). In comparison, the total energy value of the whole jambolan fruit, as delineated by Inada and collaborators (2015), stands at a lower value (31 Kcal/100 g) than what is reported in this study (as demonstrated in Table 2).

Table 2 – Nutrients (g/100 g) and total energy value (Kcal/100 g) of jambolan and jabuticaba pulps

Fruit	Moisture	Ash	Protein	Lipid	Carbohydrate	Total energy value
Jambolan	$88.9^{a} \pm 0.2$	$0.4^{a} \pm 0.0$	$0.9^{a} \pm 0.0$	$0.3^{a} \pm 0.0$	$9.5^{b} \pm 0.2$	$44.2^{b} \pm 0.7$
Jabuticaba	$85.6^{b} \pm 0.1$	$0.4^{a} \pm 0.0$	$0.8^{b} \pm 0.0$	$0.2^{b} \pm 0.0$	$13.0^{a} \pm 0.2$	$57.1^{a} \pm 0.4$

*Results expressed as mean ± standard deviation of three replicates. Different letters in the same column indicate statistical difference (p-value <0.05).
Source: Research data

3.2 PHENOLIC COMPOUNDS

When subjected to individual statistical analysis, the data acquired for both jambolan and jabuticaba demonstrated a lack of adherence to a normal distribution pattern (as illustrated in Figures 1 and 2), rendering direct result comparisons challenging. Nonetheless, upon accounting for the absolute values, analyzing graphical representations (Figures 3 and 4), and employing non-parametric tests, it becomes evident that optimal outcomes were attained following two or three extraction cycles for both fruits. As elucidated in a prior study (CHISTÉ *et al.*, 2011), the proposed model lacks the capability to comprehensively elucidate the behavior exhibited by the experimental data.

Notably, in several instances, the disparity in total compound content resulting from two extractions closely approximated, and sometimes even surpassed, the yield obtained from three extractions. This implies that two extraction cycles could potentially suffice to elicit the comprehensive extraction of polyphenols from jambolan and jabuticaba.

Regarding the duration of each extraction phase, its influence on outcomes did not reach statistical significance, with variations being more attuned to other parameters (number of extractions and solvent composition). Thus, the temporal parameter did not exhibit a substantial impact on the results. A parallel observation can be made in relation to the solvent type or composition employed for extraction. The most substantial levels of extracted compounds from both jambolan and jabuticaba displayed variations contingent upon the amalgamation of all observable parameters, underscoring the complex interplay between these factors.

Upon contrasting the most substantial polyphenol concentrations in jambolan (as illustrated in Figure 4), achieved through the utilization of distinct organic solvents (methanol/60 min/2 extractions; acetone/120 min/2 extractions; methanol:acetone/120 min/3 extractions), the optimal yield was realized under two distinctive conditions. Firstly, the implementation of acetone as the solvent, encompassing consecutive dual extractions within a 120-minute interval, yielded the highest content (1846.04 ± 11.02 mg GAE/100 g) (Table 3). Secondly, the use of methanol yielded comparable outcomes when subject to a sequence of two extractions executed within 60 minutes (1866.67 ± 15.7 mg GAE/100 g).

Similarly, upon juxtaposing the peak concentrations of jabuticaba polyphenols (as depicted in Figure 4), acquired via diverse organic solvents (methanol/90 min/3 extractions; acetone/120 min/3 extractions; methanol:acetone/120 min/3 extractions), the superior yield materialized when acetone was employed as the solvent. This outcome was achieved through three consecutive extractions over a 120-minute interval, resulting in a content of 2259.31 ± 0.89 mg GAE/100 g.

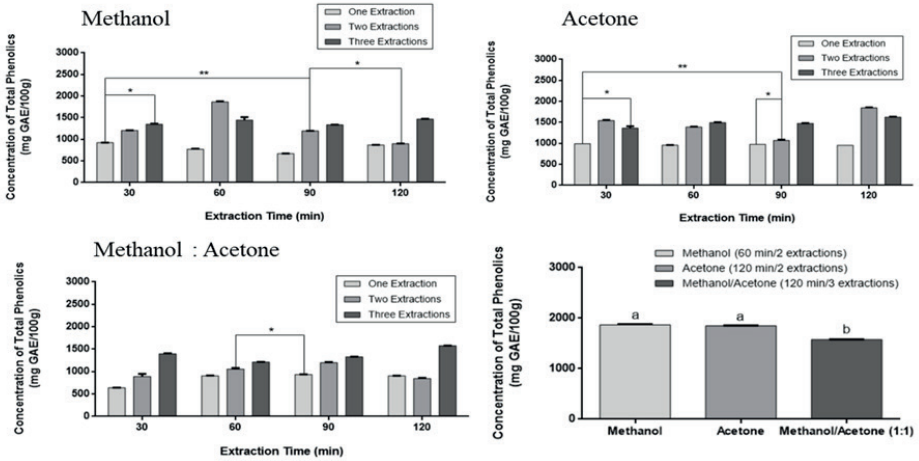
Table 3 – Experimental model and total concentration of phenolic compounds (mg of GAE/100 g) from jambolan pulp and the whole fruit of jabuticaba

Run	Independent variables			Total of phenolic compounds of jambolan pulp	Total of phenolic compounds of the whole fruit of jabuticaba
	X_1	X_2	X_3		
1	-1	-1	-1	922.82 ± 2.07	884.53 ± 0.75
2	-1	-1	0	770.25 ± 13.45	872.49 ± 0.45
3	-1	-1	+1	669.50 ± 4.00	882.99 ± 0.78
4	-1	-1	+2	869.23 ± 2.32	874.29 ± 1.37
5	-1	0	-1	1204.03 ± 5.04	1959.49 ± 5.42
6	-1	0	0	1866.67 ± 15.70	1980.97 ± 1.33
7	-1	0	+1	1192.56 ± 7.30	1990.69 ± 1.80
8	-1	0	+2	899.17 ± 4.80	1761.69 ± 9.20
9	-1	+1	-1	1348.19 ± 15.50	1721.90 ± 14.09
10	-1	+1	0	1444.76 ±70.23	1417.26 ± 14.85
11	-1	+1	+1	1336.09 ± 2.12	2073.52 ± 5.98
12	-1	+1	+2	1465.74 ± 6.82	2067.30 ± 1.09
13	0	-1	-1	639.37 ± 3.92	895.78 ± 1.00
14	0	-1	0	904.07 ± 6.81	903.84 ± 3.90

Run	Independent variables			Total of phenolic compounds of jambolan pulp	Total of phenolic compounds of the whole fruit of jabuticaba
	X_1	X_2	X_3		
15	0	-1	+1	936.61 ± 1.21	889.93 ± 1.68
16	0	-1	+2	906.84 ± 1.95	904.39 ± 0.23
17	0	0	-1	888.21 ± 59.30	1064.51 ± 21.51
18	0	0	0	1056.85 ± 23.91	2020.44 ± 0.44
19	0	0	+1	1196.20 ± 18.05	2027.38 ± 2.09
20	0	0	+2	846.33 ± 15.40	1932.04 ± 0.56
21	0	+1	-1	1396.94 ± 9.93	1252.75 ± 20.13
22	0	+1	0	1211.38 ± 4.18	1848.05 ± 9.99
23	0	+1	+1	1328.62 ± 4.32	1975.46 ± 27.03
24	0	+1	+2	1576.62 ± 4.70	2097.37 ± 3.40
25	+1	-1	-1	990.99 ± 0.57	916.29 ± 0.67
26	+1	-1	0	958.19 ± 2.16	910.14 ± 1.60
27	+1	-1	+1	976.07 ± 0.88	912.21 ± 0.87
28	+1	-1	+2	952.82 ± 0.02	898.69 ± 1.12
29	+1	0	-1	1540.67 ± 18.25	1537.28 ± 28.32
30	+1	0	0	1389.56 ± 10.30	2008.01 ± 2.12
31	+1	0	+1	1071.58 ± 17.38	2031.12 ± 1.65
32	+1	0	+2	1846.04 ± 11.02	2015.55 ± 0.18
33	+1	+1	-1	1364.59 ± 40.84	2001.98 ± 29.19
34	+1	+1	0	1494.54 ± 9.83	2164.66 ± 0.63
35	+1	+1	+1	1476.58 ± 7.48	2200.04 ± 8.68
36	+1	+1	+2	1627.00 ± 8.45	2259.31 ± 0.90

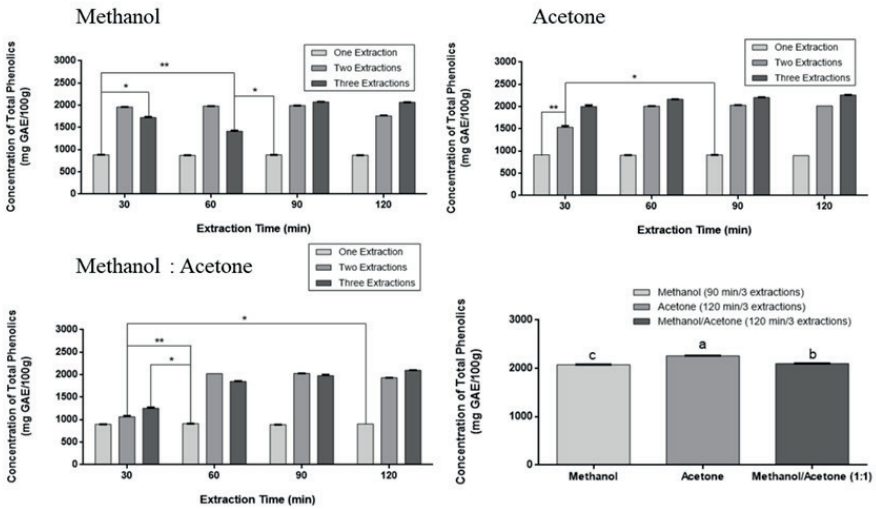
*Results expressed as mean ± standard deviation of three replicates.
Source: Research data

Figure 3 – Total concentration of phenolic compounds from jambolan pulp extracted through the association of three different independent variables (number of extractions, time and solvent). The asterisk indicates the results that showed statistical difference. The greater the number of asterisks, the greater the statistical difference. The letters (a, b) indicate statistical difference by the ANOVA test



Source: Research data

Figure 4 – Total concentration of phenolic compounds from the whole fruit of jabuticaba extracted through the association of three different independent variables (number of extractions, time and solvent). The asterisk indicates the results that showed statistical difference. The greater the number of asterisks, the greater the statistical difference. The letters (a, b, c) indicate statistical difference by the ANOVA test



Source: Research data

Upon scrutinizing the impact of ultrasound application on the extraction of phenolic compounds from *Myrciaria jaboticaba* peel, Tarone and collaborators (2021) expounded that the extracts yielded from this procedure displayed elevated levels of total phenolic compounds (18.36 ± 0.36 mg/g) and gallic acid derivatives (9.23 ± 0.32 mg/g). This outcome was in contrast to extracts acquired through conventional methods, which exhibited comparatively lower levels (total phenolic compounds: 16.53 ± 1.19 mg/g; gallic acid derivatives: 4.89 ± 0.65 mg/g). Yet, in terms of flavonol derivatives (ultrasound extraction: 0.24 ± 0.01 mg/g; solvent extraction: 0.32 ± 0.02 mg/g) and anthocyanin derivatives (ultrasound extraction: 7.81 ± 0.06 mg/g; solvent extraction: 10.22 ± 0.06 mg/g), the contents were reported to be diminished in ultrasound-assisted extraction (1.17 mg/g).

Similarly, the influence of ultrasound was evaluated while investigating the phenolic compound profile, which demonstrated decreased levels across all analyzed compounds (TARONE *et al.*, 2021). However, certain disparities surfaced in the method employed by the researchers, notably in the variance of solvents deployed for each process (ultrasound extraction: 70% ethanol in ultrapure water; v/v; containing 1% formic acid; solvent extraction: 50% ethanol in ultrapure water; v/v). Additionally, deviations existed in the solid-liquid ratio (ultrasound extraction: 1 g in 25 mL; solvent extraction: 0.1 g in 1.5 mL, equivalent to 1 g in 15 mL) and the number of extractions conducted (ultrasound extraction: 1 extraction; solvent extraction: 3 extractions). As a consequence, it becomes challenging to conclusively attribute the divergent outcomes solely to the utilization of ultrasound technology.

4 CONCLUSION

The devised methodology to assess the multifarious factors influencing the extraction of phenolic compounds from jambolan and jabuticaba proved effective in validating the extraction process. In the context of jambolan, the zenith phenolic content was achieved through a dual-extraction modality employing methanol as the solvent, carried out in sequence over a 120-minute timeframe, resulting in a content of 1866.67 ± 15.7 mg GAE/100 g. Similarly noteworthy, the utilization of acetone in two consecutive extractions, each spanning 60 minutes, yielded a commensurate result of 1846.04 ± 11.02 mg GAE/100 g. In contrast, for jabuticaba, the most pronounced phenolic content surfaced through a triple-extraction regimen utilizing acetone as the solvent. This consecutive process, spanning 120 minutes, culminated in a content of 2259.31 ± 0.89 mg GAE/100 g. Furthermore, it is evident that the variation of solvents is the foremost determinant significantly influencing the ultimate extract yield.

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