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Enhancing Bioactive Compound Bioaccessibility in *Tapirira guianensis* Juices through Ultrasound-Assisted Applications

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Abstract: This study evaluated the chemical profile and bioactive compounds in *Tapirira guianensis* juice samples under high-intensity ultrasound (US) effects. *T. guianensis* juices were produced and processed using the US with varying ultrasound energy (0, 0.9, 1.8, 2.7, and 3.6 kJ·cm^{-3}) for 10 min. The treated juices were evaluated for total acidity, color, pH, total phenolic compounds, anthocyanins, carotenoids, antioxidant capacity, in vitro digestibility, as well as quantification of bioactive compounds by HPLC-DAD. The hydromethanolic extract obtained from *T. guianensis* exhibited the presence of two major categories of polyphenols, specifically galloylquinic acids and flavonols. Overall, US technology was responsible for inducing some negative changes, such as carotenoid degradation, but also some positive changes in the chemical profile of the beverages, such as increased phenolic content, improved antioxidant capacity, and increased anthocyanin content. However, the beneficial effects were prominent, thus opening opportunities to develop new functional beverages using this fruit with limited scientific studies.

Keywords: Anacardiaceae; "pau-pombo"; compounds phenolics; galloylquinic acids; flavonols

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1. Introduction

The family Anacardiaceae encompasses 800 species that span across 81 genera, primarily inhabiting tropical and subtropical regions. The *Tapirira* genus encompasses 35 acknowledged species from southern Mexico to various regions in South America. Among these species, four are exclusive to Brazil: *Tapirira obtusa* (Benth.) J. D. Mitch., *T. pilosa Sprague*, *T. retusa* Ducke, and *T. guianensis* Aubl. Among these indigenous species, *T. guianensis* is particularly prominent, displaying extensive distribution across all Brazilian biomes. Residents commonly refer to it as "pau-pombo" or "peito-de-pombo" [1]. In its composition, the species *T. guianensis* exhibits high antioxidant and nutraceutical potential, with the presence of alkylphenols, alkyl esters, triterpenes, steroids, norisoprenoids, and isolated flavonoids [2].

Although frequently found in the forests of French Guiana, this versatile tree has not been extensively utilized and is primarily consumed for its fruits. The widespread cultivation of *T. guianensis* is currently limited, but emerging prospects for its application, such as the production of colorless cosmetics or dietary supplements, hold the potential to support valuable endeavors [2].

Nutraceutical refers to isolated products from herbal remedies, dietary supplements (nutrients), specific diets, and processed foods. Lately, there has been an increased emphasis on the investigation of various plants, especially those possessing medicinal properties, aimed at discovering new and untapped sources of antioxidants. Among these natural antioxidant agents, phenolic compounds have gained significant prominence due to their association with decreased vulnerability to degenerative conditions, notably cardiovascular diseases and cancer [3]. Extraction is the primary stage in the chemical process and plays a crucial role in recovering, identifying, and purifying bioactive compounds from plant materials [4]. Various techniques have been reported for extracting polyphenolic compounds from different plants. These methods include maceration, Soxhlet extraction, microwave-assisted extraction, supercritical fluid extraction, accelerated solvent extraction, and US [2].

Recently, ultrasonication (US) has risen as an alternative to conventional solvent extraction methods. This technique augments the recovery of bioactive substances by amplifying mass transfer and aiding the penetration of solvents into the cellular structure [5]. The process of extracting phenolic compounds from plants is influenced by the chosen extraction method and the solvent used. Selecting the appropriate solvent for extracting polyphenolic compounds is crucial, as it directly influences the amount and variety of phenolic compounds obtained. The growing exploration of more effective solvents for extracting polyphenols from plants can catalyze the application of these inherent antioxidant properties in both the pharmaceutical and food sectors [6].

Phenolic compounds, secondary metabolites highly prevalent in plants, have garnered escalating interest from diverse audiences owing to their potent and varied health-enhancing effects in medicinal applications [7]. Phenolic compounds have been widely employed in direct applications to promote health and prevent diseases, spanning pharmaceuticals and nutraceuticals. Furthermore, they are notable in the food industry, acting as antibacterial agents, flavor enhancers, and color additives. Additionally, phenolics are prominent in cosmetic formulations as antioxidants, skin brighteners, fragrance components, and more [8]. As the extraction process is considered the initial and most important step in the identification, recovery, and isolation of bioactive compounds, it plays a crucial role in the development of biotechnological products with added value [5]. The meticulous choice of the extraction method is imperative to ensure precise and dependable analysis of bioactive compounds and to enhance comprehension of the chemical diversity within *T. guianensis* fruits. As a result, the present study delves into the exploration of ultrasound-assisted extraction (USAE) for bioactive compounds. The primary objectives of this study are as follows: (1) To undertake the chemical characterization of *T. guianensis* species under varying extraction power conditions; (2) To scrutinize the impact of ultrasound-assisted extraction (USAE) on the bioactive compound composition of the fruits, (3) To validate physicochemical analyses, including pH, titratable acidity, and color. Anticipated outcomes of this study encompass contributing insight into extraction techniques at different power levels from *T. guianensis* fruits, ultimately offering valuable insights for developing functional juice formulations.

2. Materials and Methods

2.1. Sample Preparation

The fruits of *Tapirira guianensis* were collected from the Manaquiri region (BR319, Km 150) in the Amazonas state. On 14 December 2022, undamaged and infection-free fruits were individually handpicked and promptly transported to the laboratory. Healthy and consistent fruits, characterized by uniform size and color, were chosen for seed extraction. These fruit samples were rapidly frozen at −18 °C and subjected to lyophilization using an Interprise I lyophilizer from Terroni, Brazil. The resulting lyophilized samples were ground and subsequently sieved through a mesh with an approximate size of 10. The fruits were blended in an industrial blender with a 1:1 (w/w) ratio of water. The juice was stored in glass bottles at −18 °C until the analyses were conducted.

2.2. Ultrasound-Assisted Juice Processing

The *T. guianensis* juices (with a density of 0.5 g/mL) were subjected to treatment at five different power amplitudes: 0%, 20%, 40%, 60%, and 80% (corresponding to ultrasonic energy density levels of 0.0, 0.9, 1.8, 2.7, and 3.6 kJ·cm^{-3}), as outlined in Equation (1). These energy density values were determined through preliminary tests, considering the minimum and maximum nominal power of the equipment (Table 1). During the ultrasonic homogenization process, a 100 mL volume of juice was treated using a 25 mm diameter probe, operating at a frequency of 20 kHz and with a nominal power of 750 W (utilizing the VibraCell VCX 750 equipment from Sonics, Shawnee, OK, USA). The treatment duration was 10 minutes and conducted within an ice bath to prevent potential sample overheating [9].

$$\text{ED } (\text{J}\cdot\text{cm})^{-3} = \frac{\text{NAP} \times \text{t}}{\text{V}} \quad (1)$$

where, ED represents energy density; NAP represents applied nominal power (W); t is the processing time (s), and m V is the sample volume (cm^3).

Table 1. Sample treatment parameter.

Treatment US	Power (W)	Time (min)	Initial Temperature (°C)	Final Temperature (°C)
0%	-	-	-	-
20%	150	10	23.4 ± 0.4	57.9 ± 0.5
40%	300	10	23.8 ± 0.2	58.3 ± 0.3
60%	450	10	23.8 ± 0.2	57.5 ± 0.2
80%	600	10	24.0 ± 0.4	56.5 ± 0.2

2.3. Color Parameters

Color parameters, including lightness (L*), red-green (a*), and yellow-blue (b*), were assessed employing a colorimeter (Delta Color 71421, Delta Vista, Porto Alegre, Brazil). To quantify the alteration in color, the color variation (ΔE*) was computed using Equation (2). The divergence in color parameters (Δ) was defined as the dissimilarity between the attributes of juices treated with ultrasound and those left untreated.

$$\Delta\text{E}^* = \sqrt[2]{\Delta\text{L}^{*2} + \Delta\text{a}^{*2} + \Delta\text{b}^{*2}} \quad (2)$$

where, ΔE* = Color variation.

2.4. pH and Titratable Acidity

The pH assessment of the ultrasound-treated juices was conducted using a digital pH meter (K39-0014PA Kasvi, Xangai, China) at ambient temperature. Titratable acidity was determined through titration and expressed in grams of citric acid per 100 mL of juice [9].

2.5. Antioxidant Capacity

The assays were performed as described previously [10]. The assessment of the radical scavenging capacity resulting from various treatment processes of *T. guianensis* juices was carried out employing the DPPH• radical method. A 100 µM portion of methanolic DPPH• solution was prepared. Subsequently, the sample was prepared at 1 mg/mL and mixed with 1900 µL of the methanolic DPPH• radical solution. For comparison, Trolox was utilized as a positive control (ranging from 100 to 2000 µM). The mixture was then kept in darkness at room temperature for 30 min. Absorbance readings were taken at 515 nm using a Microplate Reader (Epoch 2, BioTek, Winooski, VT, USA). The antioxidant capacity was quantified in Trolox equivalents. The assay was conducted in triplicate. The relationship

was determined as y = −0.0004x + 0.7349, with an R^2 value of 0.9969, and the results were expressed in terms of micromolar Trolox Equivalents (μM Trolox/mL).

The $ABTS^{\bullet+}$ scavenging assay involves measuring the discoloration of the $ABTS^{\bullet+}$ solution in the presence of antioxidant extracts. After a reaction time of 6 min of the sample with the radical at a ratio of 1:10, absorbances were measured at 750 nm using a microplate reader. The standard curve was generated using Trolox (y = 0.0003x + 0.7483, R^2 = 0.9987), and the results were expressed in micromolar Trolox Equivalents (μM Trolox/mL).

The Ferric Reducing/Antioxidant Power Assay (FRAP assay) involves the evaluation of the extract's capacity to reduce Fe^{3+} to Fe^{2+} in the presence of a ferric-tripyridyltriazine complex. Following a 30-min interaction between the sample and the FRAP reagent at 37 °C, the absorbance was gauged using a UV spectrophotometer at 593 nm. A standard curve was constructed using $FeSO_4$ (with the relationship y = 0.0009x + 0.1664 and an R^2 of 0.9990). The outcomes were presented in terms of micromolar of Fe (II) equivalents (μM Fe(II)/mL) per gram of the sample.

The β-carotene mixture was composed of linoleic acid (20 μL), Tween 40 (200 μL), a β-carotene/chloroform solution (25 μL; 2 mg/mL), and chloroform (500 μL). Subsequently, the chloroform was evaporated under nitrogen conditions. Next, 25 mL of oxygen-saturated water was introduced and vigorously shaken. The resultant reaction mixture displayed absorbance values ranging from 0.6 to 0.7 at 470 nm (with the relationship y = −0.0003x + 0.8011 and an R^2 of 0.994). For the microplate experiment, 250 μL of this reaction mixture was combined with 10 μL of methanol (as a control), or the equivalent volume was added to *T. guianensis* juice samples, and the mixture was incubated at 45 °C. Absorbance readings were taken immediately and subsequently at 15-min intervals over 120 min. The outcomes were conveyed as the percentage inhibition of β-carotene.

2.6. Phenolics Compounds

The quantification of total phenolic compounds was performed using the Folin-Ciocalteu reagent. The reaction between the sample and the reagent was incubated for 5 min, and after that, sodium bicarbonate (6%) was added and incubated for an additional 90 min. Subsequently, the data interpretation was carried out using a microplate reader at 750 nm. Gallic acid was used as the standard (y = 0.0026x + 0.2738, R^2 = 0.9986). Results were expressed as mg of gallic acid equivalents for g of juice (mg GAE/g) [10].

2.7. Total Anthocyanin Content

The method employed to determine the total anthocyanin content in the species *T. guianensis* was based on the specific pH spectrophotometric approach. This method quantifies the total anthocyanin content expressed in milligrams of anthocyanins per 100 g sample. The Extinction Coefficient followed the methodology of adopting a value of 982 for the unique pH (pH 2.0). The absorbances for this method were evaluated using a microplate reader (BioTek, Elx800), with readings taken at a wavelength of 535 nm [11].

2.8. Carotenoid Content

The quantification of carotenoid content in *T. guianensis* juices was conducted through the spectrophotometric method. In a concise summary, 1 mL of *T. guianensis* juice was combined with 6 mL of distilled water and 5 mL of hexane, followed by vigorous vortexing for 1 min. The resulting supernatant (hexane phase), which contained the lipid fraction, was analysed at 452 nm using a Microplate Reader (BioTek, Elx800) with hexane as the reference. The outcomes were presented in terms of carotenoid content, determined by referring to a calibration curve (y = 0.0045x − 0.0011, R^2 = 0.9998) that had been previously constructed using a β-carotene standard [11].

2.9. Digestibility Assay

To assess the impact of ultrasonic treatment on the bioactive compound bioaccessibility in *T. guianensis* juices, an in vitro digestibility simulation was performed according to the

INFOGEST protocol, with the oral phase excluded. The concentration of antioxidants was analyzed following the digestion step.

2.10. Quantification of Bioactive Compounds by HPLC-DAD

The methodology for quantitative analysis of polyphenols entails the determination of response factors for standard polyphenol derivatives employing a gradient elution system. These relative response factors facilitate polyphenol analysis using only a single reference standard per class.

Each sample (500 µL) was homogenated with 500 µL methanol HPLC grade and filtered using FPTE filter 0.45um. Standard of pyrogallol, Gallic acid, Cyanidin, Cyanidin 3-glicoside, Delphinidin 3-glicoside, Epicatechin and Quercetin were purchased from Sigma Aldrich.

A stock solution of each reference standard was dissolved in methanol (1 mg/mL). Working standard solutions were prepared by 0.015–0.5 mg/mL fold dilution of the stock solution with methanol before HPLC analysis.

The mobile phase to polyphenols quantification for HPLC-DAD (Shimadzu Corporation Co., Ltd., Kyoto, Japan) analyses consisted of water (A) and methanol (B) at pH 3 with phosphoric acid. Gradient: 0.0–1.0 min in isocratic mode at 10% (B), 10–40% (B) in 13 min, 40–70% (B) in 6 min, 70–100% (B) in 7 min, followed by elution with 100% methanol for 5 min. A Shimadzu Prominence LC-20AD, equipped with a DGU-20A5 degasser equipped with an SPD-20A (PDA) detector, was employed. The temperature oven was maintained at 30 °C. The Linearity was evaluated by analysis of external standard stock solution from 0.5 to 0.0156 mg/mL (n = 3). The equation parameters (slope and intercept) of each standard curve were used to obtain the sample concentrations. The limits of detection (LOD) and quantitation (LOQ) were calculated from a calibration curve by dividing the standard deviation of the calibration curve by its slope multiplied by 3.3 and 10.0, respectively [12].

Quantification by Relative Response Factor

Quantitative analysis of anthocyanin, flavonol, catechin, gallic acid and pyrogallol derivatives was performed by establishing response factors from quercetin, cyanidin, gallic acid and pyrogallol standard selected as reference [13]. The response factors (RF) for the phenolic derivatives were calculated as a ratio of the concentration in relation to the corresponding area of the standard sample [14]. The relative response factors (RRF) were calculated as the ratio of the RF for each analyte to that of the chosen reference. The quantification of phenolic derivatives content in the sample was carried out according to the following equation:

$$\text{Content (\%, } w/w) = [A_{samp} \times RRF \times Rf \times V_{samp} \times 100]/[W_{samp} \times 1000]$$

where: A_{samp}: area due to the phenolic in the sample (mAU*s); RRF: the average relative response factor of that phenolic derivative to the reference phenolic; Rf: response factor of the phenolic standard [(µg/mL)/mAU*s]; V_{samp}: volume of sample solution (mL); W_{samp}: sample weight (µg).

2.11. Statistical Analysis

A completely randomized experimental design was conducted to evaluate the effect of the US at five levels of energy density (0, 20%, 40%, 60%, and 80%). All results were obtained in triplicate and evaluated using Analysis of Variance (ANOVA). The Duncan's test was applied with a confidence level of 95%.

3. Results and Discussion

3.1. Physicochemical Properties

The color attributes of fruit juice play a crucial role in shaping consumers' perceptions of its overall quality. Furthermore, the attractive hue of the end product can substantially enhance its acceptance levels among consumers.

The color of the juice is primarily dictated by the presence and makeup of anthocyanidins, phenols, and carotenoids within it, which exert a highly beneficial influence on safeguarding probiotic cells [15].

Significant differences were observed among the *T. guianensis* juices produced at different ultrasonic energy densities in terms of ΔE ($p < 0.05$) (Figure 1).

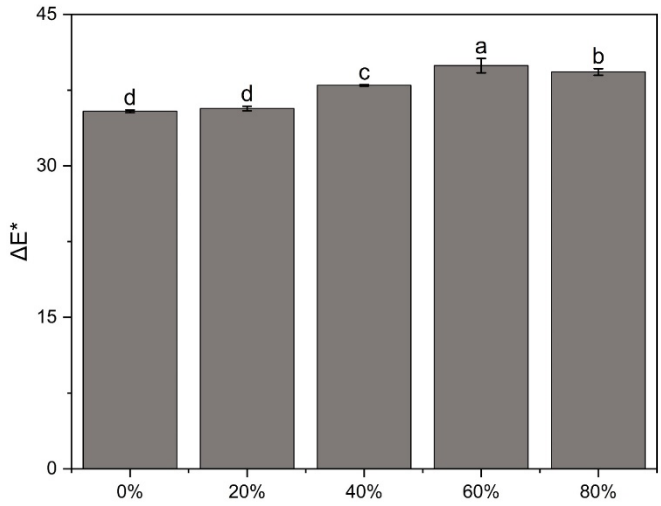

Figure 1. Color parameters for *T. guianensis* juices treated with ultrasound. (a–d) Means with the same letter in the same column are not significantly different according to the Duncan test (p-value > 0.05).

In [16], the L* values were found to vary between 16.4 and 21.9, with the highest values recorded at the 20% mark. This pattern of yellow-orange color is commonly associated with citrus fruit peels, resulting from the breakdown of green chlorophyll and the emergence of the distinctive red-yellow hue.

The a* value, indicating redness, is significant among the color parameters for *T. guianensis* juice. To comprehend the cumulative impacts of the L*, a*, and b* color parameters, ΔE values were computed. The maximum ΔE value was observed at 60% and 80%, primarily due to the elevated b* value. This phenomenon is attributed to the substantial presence of anthocyanins in these samples, as the principal anthocyanin compounds in fruits are recognized for imparting the red color.

Variations in anthocyanin content induced by sonication can be influenced by various factors. Typically, ultrasound treatment enhances the extractive capacity of bioactive compounds, leading to the potential elevation of anthocyanin levels following the exposure of plant tissue to ultrasound waves [15].

Grape juice, rich in anthocyanins, displays a broad spectrum of colors that can be affected by factors including grape ripeness, cultivation practices, and processing methods. Studies suggest that extended sonication treatment (exceeding 2 min) applied to grape juice has the potential to result in a decrease in the L* value [17]. On the other hand, it was discovered that ultrasound treatment during grape must maceration resulted in enhanced grape juice color compared to commercial enzymatic maceration.

Ensuring appropriate pH and acidity levels in food items is crucial, given that these characteristics substantially affect their physical, chemical, and microbial stability. An acidic milieu (with a pH below 4.6) can impede the proliferation of various bacterial strains. Additionally, the degradation of vitamins is influenced by the pH of the processed food [9].

Following the US (ultrasonic-assisted extraction) processing, the pH and total acidity of each sample were measured, revealing no significant differences between the various treatments (Table 2). These findings align with a previous study conducted by [18], which similarly reported no significant shifts in pH and acidity in ultrasonicated "açaí" and "buriti" juices. The consistent pH and acidity levels across the different treatments indicate that these parameters contributed equally to each sample. Consequently, any alterations in the nutritional composition of the beverage cannot be ascribed to variations in pH or acidity.

Table 2. Physicochemical analysis values.

Treatment	pH	Titratable Acidity
0%	4.5 ± 0.2	7.0 ± 0.1
20%	4.7 ± 0.1	7.0 ± 0.1
40%	4.8 ± 0.2	7.5 ± 0.1
60%	4.7 ± 0.2	7.8 ± 0.1
80%	4.8 ± 0.1	7.9 ± 0.2

The observed alterations in the yellow hue of the samples are evident from the decrease in carotenoid content in the analyzed juice samples (as depicted in Figure 2). These outcomes can be attributed to the concept that our perception of color is a psychological response influenced by the visible spectrum emitted or reflected by an object.

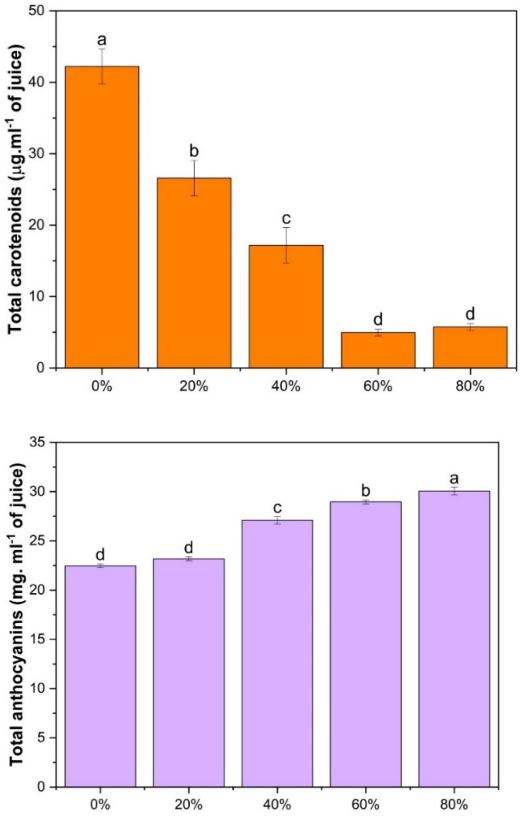

Figure 2. Effect of US energy density on total monomeric anthocyanin and carotenoid content. (a–d) Means with the same letter in the same column are not significantly different according to the Duncan test (p-value > 0.05).

In the context of ultrasound-treated juice, the comprehensive perception of color likely originates from various modifications induced by ultrasound techniques. These encompass potential isomerization reactions of existing anthocyanins, which may be triggered by the formation of free radicals generated during the sonication process [9].

All extracts had negative ΔL* values. The ΔL* value in the color space indicates the blue or yellow hue, ranging from −30 to 30. Negative ΔL* values represent blue. Anthocyanins derived from delphinidin mainly develop shades of blue color. This is apparent from the outcomes of the chemical analysis, which demonstrate a greater prevalence of anthocyanins derived from delphinidin in all the extracts [19].

3.2. Effect of Sonication on Total Carotenoids and Total Anthocyanins

Carotenoids are the pigments responsible for the vibrant colors in fruits and vegetables. They hold significant functions within the human body, serving as provitamin A (in the form of β-carotene), antioxidants, modulators of immune function and carcinogen metabolism, and regulators of cell proliferation and differentiation [15]. Earlier research has established a robust link between the consumption of carotenoids and enhancements in the immune system, along with a diminished susceptibility to numerous cardiovascular diseases, cancer, cataracts, and macular degeneration [20].

The results on the effect of sonication treatments on the total carotenoids of *T. guianensis* juice are presented in Figure 2. A significant reduction (p-value < 0.05) in carotenoid concentration was observed mainly at 60% and 80% (4.9 and 5.7 µg/mL β-carotene equivalents), confirming that high levels of ultrasound treatments can induce chemical decomposition of carotenoids.

The carotenoid levels in the ultrasound-treated Chokanan mango juice [21] and carrot juice [22] decreased according to the results obtained in the present study. This shearing effect of sonication after longer exposure can cause carotenoid isomerization [21], leading to decreased carotenoid levels observed for *T. guianensis* juice.

Upon analyzing the results, it is evident that an increase in ultrasound energy density during juice processing correlates with a rise in total anthocyanin content. The 80% juice sample exhibited the highest number of anthocyanins, reaching 15.05 mg/100 mL. This indicates that sonication power enhances the extraction capacity of anthocyanins. The phenomenon can be explained by the fact that ultrasound treatment facilitates the release of bioactive components from the juice by disrupting and breaking down the material [23]. Moreover, another contributing factor to the increased anthocyanin content is the deactivation of enzymes responsible for anthocyanin degradation by sonication. Specifically, higher-powered sonication contributed to the inhibition of β-D-glucosidase, one of the enzymes primarily responsible for anthocyanin degradation. These enzymes break the covalent bond between the aglycone and the glycosyl residue, resulting in the formation of unstable anthocyanidins. Thus, by deactivating these enzymes, sonication helps preserve the anthocyanins in the juice and prevents their breakdown [15].

3.3. Antioxidant Capacity

Anthocyanins can interrupt oxidative chain reactions by scavenging free radicals, thereby reducing oxidative stress [15]. Given the altered anthocyanin profile after US treatment, an analysis of antioxidant capacity was performed on the samples. In this study, all experimental frequencies contributed positively to antioxidant capacity. As shown in Figure 3, US treatment at 80% exhibited the highest antioxidant potential in the juice. These results demonstrate that the increase in electron-donating antioxidants is important in determining the ultrasound-treated *T. guianensis* juice samples.

Anthocyanins, especially cyanidin and its glycosides, are included in the list of known natural compounds that act as powerful antioxidants. This is due to their unique chemical structure, which is capable of accepting unpaired electrons, such as reactive oxygen/nitrogen species, and inhibiting oxidative stress [24].

Like other "superfruits", Jabuticaba exhibits a potent in vitro antioxidant capacity (DPPH, ABTS). In investigating the effect of ingesting lyophilized extract from *Myrciaria jaboticaba* peels on antioxidant activity in rat plasma, the results revealed an increase in plasma antioxidant capacity (1.7-fold by the TEAC method and 1.3-fold by the ORAC method). Anthocyanins such as cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside appear responsible for the reported effect [25].

The hydroalcoholic extract of *Plinia trunciflora* leaves was evaluated for its antioxidant potential using the FRAP and DPPH methods. It was found to exhibit high activity, primarily due to the presence of cyanidin and cyanidin-3-*O*-glucoside [26].

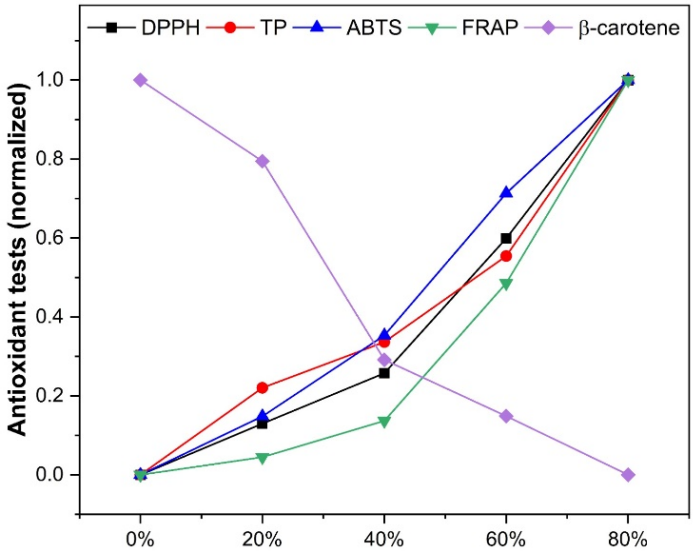

Figure 3. The antioxidant potential of *T. guianensis* juices subjected to high-intensity ultrasound was evaluated through total phenolic compounds, DPPH, ABTS, FRAP, and β-carotene assays.

A positive and significant effect (p-value < 0.05) was observed with the increase in ultrasound energy density. Considering the untreated juice, there was a 41% increase in the content of phenolic compounds (Table 3).

Table 3. Results are expressed as mean ± standard deviations (n = 3).

Treatment	DPPH (μM Trolox/mL)	ABTS (μM Trolox/mL)	FRAP (μM Fe(II)/mL)	Total Phenolics (mg GAE/g)	β-Carotene (% Inhibition of β-Carotene)
0%	1003.9 ± 5.2 ^{e}	1274.3 ± 6.7 ^{e}	1168.2 ± 4.0 ^{e}	466.9 ± 0.2 ^{e}	78.6 ± 1.8 ^{a}
20%	1051.4 ± 8.8 ^{d}	1373.2 ± 0.2 ^{d}	1190.1 ± 4.2 ^{d}	509.3 ± 0.1 ^{d}	66.8 ± 0.3 ^{b}
40%	1098.1 ± 2.9 ^{c}	1509.9 ± 8.4 ^{c}	1234.9 ± 2.2 ^{c}	531.6 ± 0.2 ^{c}	37.9 ± 1.4 ^{c}
60%	1223.1 ± 6.3 ^{b}	1749.9 ± 7.7 ^{b}	1404.9 ± 1.1 ^{b}	573.5 ± 0.3 ^{b}	29.8 ± 2.8 ^{d}
80%	1369.7 ± 6.6 ^{a}	1941.0 ± 12.0 ^{a}	1655.6 ± 4.2 ^{a}	659.3 ± 0.2 ^{a}	21.2 ± 2.9 ^{e}

(a–e) Means with the same letter in the same column are not significantly different.

The results regarding the effects of treatments with *T. guianensis* juice on bioactive compounds are displayed in Table 3. It was observed that ultrasound significantly affected all bioactive compounds. The lowest losses were exhibited in US80% (659.3 ± 0.2) and US60% (573.5 ± 0.3), followed by US40% (531.6 ± 0.2), US20% (509.3 ± 0.1), and US0% (466.9 ± 0.2), respectively.

Physalis minima Linn demonstrated that the leaf extract exhibited the highest total phenolic content value of 1125.42 ± 14.60 mg of gallic acid equivalent (GAE) per gram of plant (dry extract) [27]. Ultrasound-assisted extraction (UAE), utilizing aqueous ethanol as the solvent, was applied for the first time to extract phenolic compounds from *Terminalia chebula* Retz. fruits, where the total phenols yield was 448.7 ± 2.15 mg GAE/g DW, in agreement with the anticipated value (447.8 mg GAE/g DW) [28]. The pulp of the tropical fruit araticum (*Annona crassiflora*) exhibited a total phenolic yield of 433.80 mg GAE/g [29].

Phenolic compounds are typically associated with the cell wall, such as pectin, cellulose, hemicellulose, and lignin residues. Undergoing ultrasound (US), processing can enhance mass transfer rates and create microcavities that may disrupt the cell wall, resulting in the liberation of antioxidant content [9]. Furthermore, augmenting the power during the cavitation process intensifies the breakdown of the cell wall, leading to the generation of

hydroxyl radicals. These radicals play a pivotal role in the hydroxylation of the aromatic ring of phenolic compounds at the ortho, meta, and para positions [30].

The application of the US for extraction purposes has been extensively discussed in the literature. For instance, Ref. [17] demonstrated that using ultrasound at 35 kHz with an ultrasonic bath significantly enhanced the extraction of total phenolic compounds and antioxidant capacity from red grape peel extracts compared to conventional extraction methods (with mechanical agitation, without specified conditions). The ultrasound-assisted approach achieved concentrations 1.8 and 1.6 times higher, respectively. Similarly, Ref. [31] observed a notable increase of 30 to 40% in the total phenolic content and antioxidant capacity of orange peel extracts when employing ultrasound at 25 kHz with 90 W power, using an ultrasonic transducer plate.

A novel study [32] assessed the contrast between ultrasound-assisted extraction (performed using a 25 kHz frequency and 150 W power in an ultrasonic bath) and conventional extraction (employing mechanical agitation without specific conditions) for extracting apple pomace extracts. The researchers observed concentrations 1.2 times higher for phenolic content, 1.4 times higher for proanthocyanidins, and 1.3 times higher for flavan-3-ols in the ultrasound-assisted extraction experiments.

Ref. [33] described a significant effect of ultrasound ($\approx 260 \pm 6$ W/L, with a 3.8 cm^2 surface area ultrasonic probe) on the extraction rate of total phenolic compounds from olive leaves, with a value of 1.9 times higher than conventional extraction (with mechanical agitation, 170 rpm).

3.4. Quantification of Bioactive Compounds by HPLC-DAD

The quantification was carried out using the integration areas of the peaks of the molecules and by interpolation in the calibration curve constructed with a solution of analytical standards similar to each phenolic compound quantified. The identification of different phenolic compounds in this study makes it relevant since the presence of these compounds has a range of bioactivities, as already reported in previous studies.

The total polyphenol content of *T. guianensis* (TG) tea leaf samples was measured in 100% methanol, 75% methanol, 50% methanol, and water extracts, with values of 78.98 ± 0.43 mg GAE/g DW, 29.6 ± 0.97 mg GAE/g DW, 50.72 ± 1.99 mg GAE/g DW, and 56.38 ± 2.72 mg GAE/g DW, respectively [34].

In a recent study, extracts of phenolic compounds with ultrasound revealed that this treatment can enhance the extraction of polyphenols from fruits [35]. The extraction of thermostable bioactive compounds such as gallic acid derivatives can be enhanced up to a certain temperature level. When there is an increase in temperature, the contents tend to decrease. On the other hand, thermolabile molecules such as flavonoids have a disadvantage when there is an increase in US power and temperature [36,37].

The hydromethanolic extract obtained from *T. guianensis* indicated the existence of two primary categories of polyphenols: galloylquinic acids and flavonols. Nonetheless, for an accurate identification of the sugar's composition and the positioning of the flavonoid aglycone and galloylquinic acids, additional validation and structural clarification of the polyphenolics are necessary. These can be accomplished through techniques such as ^{13}C and ^{1}H NMR [34].

The identification and matching of peaks related to phenolic compounds in *T. guianensis* juice samples were carried out by comparing their retention times and absorption spectra with those of established standards (Table 4; Figure 4). A total of thirty-three phenolic compounds were detected in the samples. The flavonol profile of *Tapirira* is complex, with the presence of twelve flavonol derivatives displaying intense signals. Regarding anthocyanins, they were tentatively identified as cyanidin, cyanidin-3-glucoside, and derivatives based on their profiles compared to standard compounds.

Table 4. HPLC-DAD data to characterization and quantification of phenolic compounds from *T. guianensis* using their spectral characteristics in HPLC-DAD by retention time and absorption spectrum (λ max).

RT (min)	Compound	λ (nm)	Tg0 (μg/L ± SD)	Tg20 (μg/L ± SD)	Tg40 (μg/L ± SD)	Tg60 (μg/L ± SD)	Tg80 (μg/L ± SD)
3.40	Py_D	271	131.61 ± 22.96 ^{cd}	112.51 ± 4.92 ^{c}	15.05 ± 2.00 ^{b}	7.49 ± 0.17 ^{a}	7.13 ± 0.95 ^{a}
5.56	Py_D	269	262.44 ± 0.00 ^{c}	259.67 ± 36.73 ^{c}	58.16 ± 2.81 ^{a}	185.69 ± 82.07 ^{b}	344.98 ± 7.19 ^{d}
7.00	Pyrogallol	266	305.47 ± 23.22 ^{d}	299.14 ± 31.08 ^{d}	58.29 ± 2.03 ^{c}	41.28 ± 0.49 ^{b}	37.51 ± 1.65 ^{a}
7.19	Py_D	271	283.59 ± 30.55 ^{a}	970.90 ± 46.60 ^{c}	445.79 ± 62.41 ^{b}	391.60 ± 94.42 ^{b}	400.38 ± 59.61 ^{b}
7.69	Gallic acid	269	155.15 ± 20.64 ^{c}	252.33 ± 98.24 ^{d}	79.49 ± 1.73 ^{b}	57.63 ± 5.35 ^{a}	397.76 ± 32.28 ^{e}
8.24	G_D	266	162.13 ± 11.55 ^{c}	171.18 ± 3.45 ^{c}	68.18 ± 5.24 ^{b}	36.00 ± 3.31 ^{a}	213.78 ± 23.98 ^{d}
8.79	G_D	266	279.79 ± 54.56 ^{c}	121.30 ± 33.36 ^{a}	255.88 ± 6.47 ^{c}	201.26 ± 7.33 ^{b}	381.02 ± 37.53 ^{d}
9.30	G_D	277	147.91 ± 22.54 ^{c}	186.01 ± 19.33 ^{d}	126.18 ± 2.90 ^{b}	97.69 ± 8.37 ^{a}	127.99 ± 21.63 ^{b}
10.51	G_D	524	297.19 ± 12.00 ^{b}	296.38 ± 19.52 ^{b}	253.04 ± 10.12 ^{a}	267.44 ± 23.28 ^{a}	354.20 ± 32.39 ^{c}
11.28	Cy_D	524	206.80 ± 26.98 ^{b}	248.32 ± 1.04 ^{c}	275.16 ± 65.35 ^{b}	167.94 ± 38.66 ^{a}	189.03 ± 28.47 ^{a}
11.84	Cy_D	510	114.81 ± 4.42 ^{b}	112.07 ± 1.74 ^{a}	132.78 ± 9.13 ^{c}	133.78 ± 27.80 ^{d}	137.80 ± 1.05 ^{e}
12.15	Cy_D	361	84.09 ± 44.08 ^{a}	117.24 ± 17.65 ^{b}	118.00 ± 10.64 ^{b}	224.39 ± 98.67 ^{c}	252.93 ± 29.21 ^{d}
12.49	F_D	514	255.28 ± 14.72 ^{b}	244.24 ± 32.68 ^{b}	308.06 ± 57.02 ^{cd}	93.61 ± 3.43 ^{a}	299.62 ± 36.59 ^{c}
12.55	Cyanidin	356	6.38 ± 0.35 ^{a}	184.73 ± 11.76 ^{d}	11.56 ± 0.01 ^{b}	108.95 ± 11.92 ^{c}	178.72 ± 27.81 ^{d}
12.89	F_D	354	342.35 ± 13.01 ^{a}	351.02 ± 25.50^{a}	374.17 ± 2.95 ^{a}	320.11 ± 24.87 ^{a}	503.43 ± 70.09 ^{b}
13.29	Cy_D	514	36.45 ± 1.76 ^{a}	38.83 ± 3.60 ^{a}	64.82 ± 15.70 ^{c}	52.93 ± 4.15 ^{b}	65.44 ± 1.86 ^{d}
13.37	Delphinidin 3-glucoside	354	71.44 ± 0.00 ^{a}	72.08 ± 3.01 ^{a}	134.07 ± 5.07 ^{b}	<LOD	<LOD
13.47	F_D	524	401.85 ± 24.74 ^{c}	429.96 ± 44.34 ^{d}	243.77 ± 61.69 ^{b}	227.63 ± 17.70 ^{b}	197.29 ± 1.19 ^{a}
13.75	Cy_D	524	0.45 ± 0.00 ^{a}	0.59 ± 0.12 ^{a}	181.27 ± 0.13 ^{b}	228.76 ± 42.64 ^{d}	208.86 ± 7.59 ^{c}
14.52	Cy_D	351	0.33 ± 0.00 ^{a}	0.46 ± 0.05 ^{b}	0.60 ± 0.02 ^{c}	0.40 ± 0.03 ^{b}	<LOD
15.73	Cth_D	300	4.68 ± 0.86 ^{a}	11.73 ± 2.02 ^{b}	281.92 ± 57.42 ^{e}	64.31 ± 5.19 ^{c}	95.59 ± 2.35 ^{d}
16.32	Cth_D	300	30.33 ± 4.04 ^{b}	25.53 ± 0.00^{a}	44.52 ± 8.48 ^{c}	25.52 ± 0.02 ^{a}	66.13 ± 0.08 ^{d}
16.53	Cth_D	368	36.12 ± 5.12 ^{a}	76.14 ± 1.14 ^{c}	448.44 ± 18.14 ^{d}	39.23 ± 0.27 ^{a}	58.92 ± 5.02 ^{b}
18.24	F_D	357	6.61 ± 0.09 ^{a}	8.39 ± 0.84 ^{b}	12.74 ± 0.65 ^{c}	11.79 ± 0.42 ^{c}	11.27 ± 1.13 ^{c}
19.48	F_D	360	134.52 ± 5.91 ^{a}	150.60 ± 14.98 ^{b}	208.32 ± 9.97 ^{d}	208.50 ± 14.59 ^{d}	205.83 ± 15.07 ^{c}
19.78	F_D	357	25.25 ± 1.04 ^{a}	29.91 ± 3.62 ^{b}	42.68 ± 2.04 ^{c}	42.75 ± 2.58 ^{c}	42.28 ± 3.75 ^{c}
20.12	F_D	366	2606.37 ± 60.95 ^{c}	2357.27 ± 184.14 ^{b}	1680.91 ± 38.82 ^{a}	1717.23 ± 51.18 ^{a}	1791.89 ± 75.83 ^{a}
20.60	F_D	361	21.93 ± 2.43 ^{a}	26.81 ± 2.95 ^{b}	41.61 ± 2.11 ^{cd}	40.32 ± 2.60 ^{cd}	39.89 ± 2.01 ^{c}
20.94	F_D	366	17.08 ± 1.07 ^{a}	22.06 ± 2.30 ^{b}	33.49 ± 1.76 ^{de}	33.06 ± 1.30 ^{c}	32.42 ± 2.26 ^{d}
22.34	F_D	366	21.23 ± 0.69 ^{a}	22.67 ± 1.71^{b}	33.92 ± 1.15 ^{c}	55.33 ± 3.98 ^{d}	62.15 ± 3.84 ^{e}
22.71	F_D	371	8.05 ± 0.10 ^{a}	9.09 ± 0.58 ^{b}	12.40 ± 0.06 ^{c}	21.05 ± 0.54 ^{d}	24.99 ± 0.72 ^{e}

RT = retention time; Cy_D = Cyanidin derivative; F_D = Flavonol derivative; Cth_D = Catechin derivative; G_D = Gallic acid derivative; Py_D = Pyrogallol derivative; ANOVA: minimal detectable difference = 117.57; F > Fcritical; Tukey: confidence = 95%; Means with the same letter in the same line are not significantly different. % RSD values = <16–30>; R^2 = <0.997–0.999>.

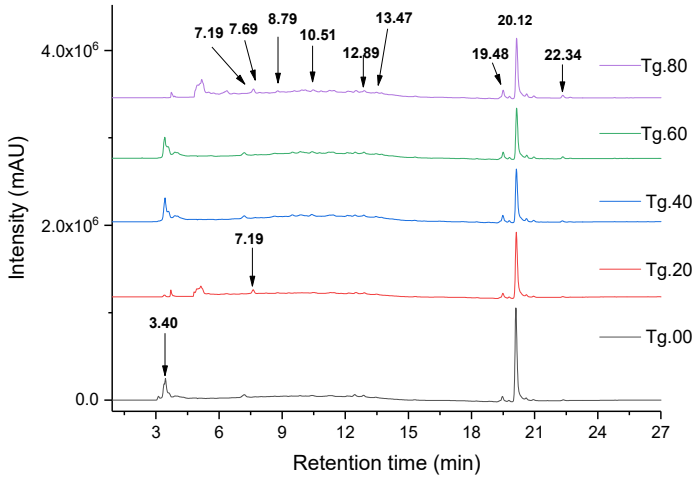

Figure 4. HPLC chromatograms of polyphenols in *Tapirira guianensis* juices. Peak assignment in Table 1.

The polyphenolic value in the samples was significantly different from each other (ANOVA: minimal detectable difference = 117.57; $F > F_{critical}$; Tukey: confidence = 95%). The increase in the process of US value did not favor the extraction of pyrogallol or pyrogallol derivatives. The extractive content of gallic acid increased as the 'process' increased, except to 40, 60 and 80. The process with 'US80' was more efficient to extract the derivatives of Gallic acid.

The stability of gallic acid was observed to be impacted by a range of ultrasonic parameters, encompassing factors like solvent type and concentration, ultrasonic power, frequency, temperature, and extraction duration in the simulated extraction setup. These results underline the importance of not only assessing extraction yield but also accounting for potential phenolic compound degradation during ultrasonic polyphenol extraction. In the ultrasonic extraction sector, achieving optimum outcomes necessitates meticulous optimization of extraction parameters to enhance yield while mitigating the risk of phenolic compound degradation induced by ultrasound [38].

Process variation was not crucial to improving the extraction of some cyanidin derivatives. The Delphinidin 3-glucoside content was below the limit of detection to process in 60 and 80. However, process 40 was more efficient to increase the yield. For flavonol at 13.47 min, as the process value is decreased, the efficiency of the extraction process increases only to 80. In turn, the flavonoid of 12.89 had its yield increased by 80. Figure 5 shows that process 40 was efficient for extracting catechin derivatives, and processes 20, 40 and 60 were more efficient for glycosylated cyanidin derivatives.

An initial exploration was undertaken to analyze the kinetics of extracting overall polyphenols and their fractions to optimize extraction duration through sonication. Methanol was chosen as the preferred solvent owing to its efficiency in extracting catechin, epicatechin, and epigallocatechin [30].

When subjected to various processing methods, the concentrations of delphinidin and petunidin exhibited a decline. Among the techniques used, ultrasound treatment showed the least impact, resulting in reductions of delphinidin by 52.56% and petunidin by 73.82% in just 1 min [39]. Ref. [40] also found that anthocyanin structure impacted their thermal stability. Moreover, the microwave treatment had the most influence on the degradations of delphinidin and petunidin, while the ultrasonic treatment had the lowest impact.

When the degradation kinetics of cyanidin-3-glucosylrutinoside before and after exposure to ultrasonic treatment were compared, Ref. [41] found that the rate constant k increased and the half-life $t_{1/2}$ decreased with increasing ultrasonic power. In addition, the greatest degree of degradation of cyanidin-3-glucosylrutinoside was 44.2% and was obtained at 600 W for 60 min.

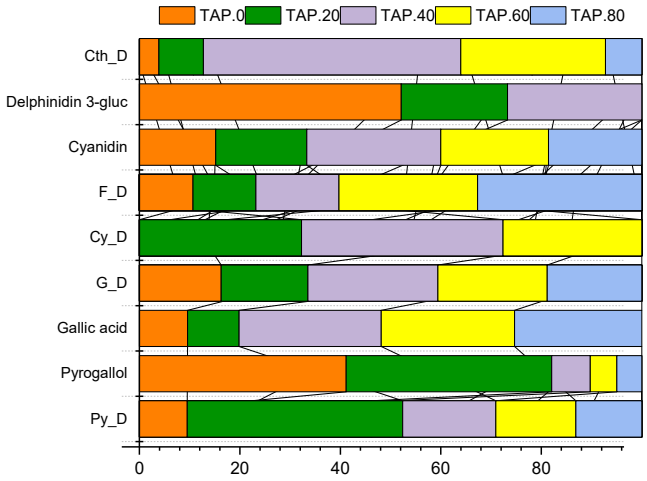

Figure 5. The graphic content of phenolic compounds (Cth_D = Catechin derivative; F_D = Flavonol derivative; Cy_D = Cyanidin derivative; G_D = Gallic acid derivative; Py_D = Pyrogallol derivative) in each experiment of the ultrasound method (TAP = *Tapirira* juice and the subsequent numbers represent ultrasound power or time).

The diverse nature of flavonols led to varied responses in different processes concerning the percentage of extraction of these flavonoids. Among the processes studied, processes 20 and 80 demonstrated the highest extractive potential overall.

Regarding the extraction of phenolic compounds from grape seeds defatted by US and solvent (S) using maceration (M) and (US), a T-Test analysis showed significant effects of maceration on grape seeds defatted by ultrasound (US-M). Specifically, this process had a significant impact on the content of total polyphenols, total tannins, total anthocyanins, cinnamic acids, flavonols, and antioxidant activity ($p < 0.05$) [30].

The utilization of ultrasound extraction significantly boosted the bioactive component content and overall extraction efficiency. Notably, the duration and power amplitude of ultrasound treatment exerted a noteworthy influence on the content of specific bioactive compounds, underscoring their crucial role in the extraction process [23].

4. Conclusions

Based on the findings of the current study, the utilization of ultrasound (US) technology brought about notable modifications in the nutritional composition of *T. guianensis* juices. Some observed changes were negative (such as β-carotene degradation), while others were positive and beneficial (including increased phenolic content, enhanced antioxidant activity, and elevated anthocyanin levels). Notably, ultrasound power emerged as a crucial determinant of these nutritional alterations. The treatment employing the maximum power (US 80%) seemed to be the optimal approach to mitigate the adverse effects of excessive ultrasound processing.

Furthermore, the content of extracted gallic acid displayed an increase with the progression of the process duration, except for the 40, 60, and 80 durations. The 'US80' extraction process showcased enhanced efficiency in extracting gallic acid derivatives. A pivotal contribution of this study lies in the identification and quantification of over 30 phenolic compound derivatives within *T. guianensis* juice sourced from the Amazon fruit cultivar, utilizing High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD).

As a result, this research offers a method to enhance the extraction of bioactive compounds previously identified in earlier studies through the application of ultrasound. Nonetheless, further investigations are warranted, with a particular focus on mitigating potential unfavorable ultrasound effects, alongside conducting sensory analyses involving consumers.

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