Research, Society and Development, v. 14, n. 9, e4414949520, 2025
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v14i9.49520

Use of experimental design in the extraction of biactive compounds from the pitanga (*Eugenia uniflora L.*) leaf using ultrasound-assisted extaction

Aplicação do delineamento experimental na extração de compostos bioativos de folhas de pitanga (*Eugenia uniflora L.*) por extração assistida por ultrassom

Aplicación del diseño experimental en la extracción de compuestos bioactivos de hojas de pitanga (*Eugenia uniflora L.*) mediante extracción asistida por ultrasonido

Received: 09/06/2025 | Revised: 09/14/2025 | Accepted: 09/14/2025 | Published: 09/15/2025

Kayque Antonio Santos Medeiros[1]
ORCID: https://orcid.org/0000-0002-9325-0249
Maringá State University, Brazil
E-mail: Medeiros.kasm@gmail.com
Lucidio Molina Filho[1]
ORCID: https://orcid.org/0000-0002-2993-6895
Maringá State University, Brazil
E-mail: lmfilho2@uem.br
Otavio Akira Sakai
ORCID: https://orcid.org/0000-0002-3502-5107
Federal Institute of Education, Science, and Techology of Paraná, Brazil,
E-mail: otavio.sakai@ifpr.edu.br
Angela Maria Picolloto[1]
ORCID: https://orcid.org/0000-0002-8969-9844
Maringá State University, Brazil
E-mail: ampicolloto@uem.br

Abstract
The growing interest in natural bioactive compounds in human nutrition and food technologies has led to research on their inclusion as substitutes for synthetic compounds and their role in new analytical methods. In this context, pitanga (*Eugenia uniflora* L.), a native Brazilian fruit, has gained attention due to its astringent properties, pleasant aroma, and bioactive components found in its leaves and fruits. These leaves are rich in phenolic compounds such as hydroxycinnamic acids, flavonoids, and exhibit antimicrobial and antifungal activities. The present study aims to optimize the extraction of bioactive compounds from pitanga leaves using a hydroethanolic solvent and ultrasound-assisted extraction, combined with Response Surface Methodology (RSM) and Rotational Composite Design (DCCR) to quantify total phenolic content (TPC) and antioxidant activities (DPPH, FRAP, and ABTS). Experimental results demonstrated that the optimal extraction conditions, consisting of 50% ethanol and 68 minutes of ultrasound exposure, significantly increased both TPC and antioxidant activities compared to conventional extraction methods. The study highlights the potential of ultrasound-assisted extraction as a sustainable, efficient method for bioactive compound recovery, with promising applications in pharmaceuticals, cosmetics, and the food industry, aligning with the growing demand for eco-friendly alternatives. These findings also contribute to the advancement of methodologies for extracting bioactive compounds from native Brazilian plants, fostering the development of more sustainable processes Thus, this study provides an optimized protocol for extracting bioactive compounds from pitanga leaves through hydroethanolic ultrasound-assisted extraction, combined with RSM and DCCR, establishing a reliable methodology for quantifying phenolic content and antioxidant activity.
Keywords: Bioactive compounds; Extraction; Optimization; Pitanga; Response Surface Methodology (RSM).

Resumo
O crescente interesse em compostos bioativos naturais na nutrição humana e nas tecnologias de alimentos tem impulsionado pesquisas sobre sua utilização como substitutos de compostos sintéticos, bem como seu papel no desenvolvimento de novos métodos analíticos. Nesse contexto, a pitanga (*Eugenia uniflora L.*), fruto nativo do Brasil, tem despertado atenção devido às suas propriedades adstringentes, aroma agradável e à presença de compostos bioativos em suas folhas e frutos. As folhas são ricas em compostos fenólicos, como ácidos hidroxicinâmicos e flavonoides, além de apresentarem atividades antimicrobiana e antifúngica. O presente estudo teve como objetivo otimizar a extração de compostos bioativos de folhas de pitanga utilizando solvente hidroetanólico e extração assistida

[1] Environment Department, Maringá State University, Brazil.

por ultrassom, associadas à Metodologia de Superfície de Resposta (MSR) e ao Delineamento Composto Central Rotacional (DCCR) para quantificação do teor de fenólicos totais (TPC) e das atividades antioxidantes (DPPH, FRAP e ABTS). Os resultados experimentais demonstraram que as condições ótimas de extração — 50% de etanol e 68 minutos de ultrassom — proporcionaram um aumento significativo tanto do TPC quanto das atividades antioxidantes em comparação aos métodos convencionais. O estudo destaca o potencial da extração assistida por ultrassom como método sustentável e eficiente para recuperação de compostos bioativos, com aplicações promissoras nas indústrias farmacêutica, cosmética e de alimentos, atendendo à crescente demanda por alternativas ecologicamente corretas. Além disso, os achados contribuem para o avanço de metodologias voltadas à extração de compostos bioativos de plantas nativas brasileiras, favorecendo o desenvolvimento de processos mais sustentáveis. O presente estudo apresenta um protocolo otimizado para a extração de compostos bioativos de folhas de pitanga por meio de extração hidroetanólica assistida por ultrassom, combinada com MSR e DCCR, estabelecendo uma metodologia confiável para a quantificação do teor de fenólicos e da atividade antioxidante.
Palavras-chave: Compostos bioativos; Extração; Otimização; Pitanga; Metodologia de Superfície de Resposta (MSR).

Resumen
El creciente interés en los compuestos bioactivos naturales en la nutrición humana y en las tecnologías alimentarias ha impulsado investigaciones sobre su uso como sustitutos de compuestos sintéticos, así como sobre su papel en el desarrollo de nuevos métodos analíticos. En este contexto, la pitanga (*Eugenia uniflora L.*), fruto nativo de Brasil, ha despertado atención debido a sus propiedades astringentes, su aroma agradable y la presencia de compuestos bioactivos en sus hojas y frutos. Las hojas son ricas en compuestos fenólicos, como los ácidos hidroxicinámicos y flavonoides, y presentan actividades antimicrobiana y antifúngica. El presente estudio tuvo como objetivo optimizar la extracción de compuestos bioactivos de hojas de pitanga utilizando un solvente hidroetanólico y extracción asistida por ultrasonido, combinadas con la Metodología de Superficie de Respuesta (MSR) y el Diseño Compuesto Central Rotacional (DCCR), para cuantificar el contenido fenólico total (TPC) y las actividades antioxidantes (DPPH, FRAP y ABTS). Los resultados experimentales demostraron que las condiciones óptimas de extracción —50% de etanol y 68 minutos de ultrasonido— aumentaron significativamente tanto el TPC como las actividades antioxidantes en comparación con los métodos convencionales. El estudio resalta el potencial de la extracción asistida por ultrasonido como un método sostenible y eficiente para la recuperación de compuestos bioactivos, con aplicaciones prometedoras en las industrias farmacéutica, cosmética y alimentaria, en línea con la creciente demanda de alternativas ecológicas. Además, los hallazgos contribuyen al avance de metodologías para la extracción de compuestos bioactivos de plantas nativas brasileñas, favoreciendo el desarrollo de procesos más sostenibles. Este estudio proporciona un protocolo optimizado para la extracción de compuestos bioactivos de hojas de pitanga mediante extracción hidroetanólica asistida por ultrasonido, combinada con MSR y DCCR, estableciendo una metodología confiable para la cuantificación del contenido fenólico y la actividad antioxidante.
Palabras clave: Compuestos bioactivos; Extracción; Optimización; Pitanga; Metodología de Superficie de Respuesta (MSR).

1. Introduction

The interest in alternatives for incorporating active natural compounds into human food or food technologies has been the focus of research and studies, as well as their use in new analytical methods for quantification and the replacement of various synthetic compounds. Moreover, the pursuit of sustainability and the rational use of these resources enables us to utilize various plant species, such as leaves and fruits, with great bioactive potential.

Pitanga (*Eugenia uniflora L.*) is a fruit native to Brazil and belongs to the Myrtaceae family. It is particularly distinguished by the astringent properties and pleasant aroma of its leaves and fruit. (Amorim *et al.,* 2009; Mesquita *et al.,* 2017). They are of great economic interest in Brazil, standing out in the production of juices, pulps, syrups, and jams (Costa, Garcia-Diaz, Jimenez and Silva, 2013). Pitanga leaves are considered non-traditional edible plants (PANCs) and are widely used in folk medicine to treat inflammations, fevers, and stomach issues, in addition to being recognized as a medicinal herb under Brazilian legislation (ANVISA, 2005, Amorim *et al*, 2009).

Its leaves have been the subject of studies by various researchers due to their notable bioactive characteristics. (Lorenzo *et al*., 2018; Tessaro, *et al.,* 2021). Such as being rich in polyphenols, including hydroxycinnamic acids (cinnamic and caffeic acids, tyrosol, hydroxycoumarins, hydroxyphenylpropens) and flavonols (quercetin, myricetin, and myricitrin),

among others (Lorenzo *et al.*, 2018) Moreover, they exhibit antimicrobial activities. (Vargas *et al*., 2019) and antifungal (Silva-Rocha *et al.*, 2015) and several other pharmacological properties.

In this context, pitanga leaf extracts are presented as significant sources of natural antioxidants, widely used in the substitution of synthetic compounds or in technological applications. (Vargas, *et al*., 2016, Lorenzo, *et al*. 2018; Tessaro*, et al*., 2021;). They studied the application of pitanga leaf extracts, using different extraction methods, in pork burgers and found that the extracts helped with coloration, reduced lipid content during storage, and inhibited protein oxidation levels. Solid-liquid extraction is a mass transfer process in which the chemical compounds of interest are extracted through contact with a solvent. Its efficiency depends on several factors, such as solvent choice, technique, extraction time, temperature, pH, and potential interferences. (Ghenabzia *et al*., 2023). The use of conventional methods in solid-liquid extraction has some disadvantages, such as the chemical transformation of the extracts, which may generate toxic residues, long extraction times, and the plant structure's resistance to solvent penetration, hindering mass transfer (Gil-Martín *et al.,* 2022).

The use of ultrasonic bath in the extraction of these bioactive compounds has been gaining attention as an innovative technology aimed at sustainable extraction.

Ultrasonic system consists of a transducer, which converts electrical energy into sound energy, mechanically vibrating at ultrasonic frequencies. (Rostagno and Prado, 2013; Tiwari, 2015) it provides greater solvent penetration into cellular materials and enhances mass transfer through acoustic cavitation generated by the driving force of the sonication extraction effect. (Picó, 2013; Tiwari, 2015).

It provides greater solvent penetration into cellular materials and enhances mass transfer through acoustic cavitation generated by the driving force of the sonication extraction effect. (*E, uniflora L*using a hydroethanolic solvent and ultrasound-assisted extraction for the quantification of CFT and antioxidant activities (DPPH, FRAP e ABTS^{o}+) using the statistical planning of rotational composite design (DCCR) allied to Response Surface Methodology (MSR).

The present study needs to optimize the extraction of bioactive compounds from pitanga leaves using a hydroethanolic solvent and ultra-sound assisted extraction, combined with Response Surface Methodology (RSM) and Rotational Composite Design (DCCR) to quantify total phenolic content (TPC) and anti oxidant activities (DPPH, FRAP and ABTS).

2. Methodology

An experimental research was conducted, of a mixed nature: part in the field and part in the laboratory, in a study of a quantitative nature (Pereira *et al*.., 2018), employing simple descriptive statistics with data classes, mean values, and standard deviation (Shitsuka *et al.*., 2014), as well as statistical analysis (Vieira, 2021).

2.1 Materials

The reagents hydrochloric acid (HCl), gallic acid, acetic acid, Folin-Ciocalteu reagent, methanol, ethanol and potassium persulfate was purchased from Dinâmica (Indaiatuba, São Paulo, Brazil). Ferric chloride and sodium carbonate where acquires from Labsynth (Diadema, São Paulo, Brazil), 2,2-diphenyl-1-picrylhydrazil (DPPH), 2,4,6-tris(2-pyridil)-s-triazine (TPZ), 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX) where acquires from Sigma-Aldrich (St. Louis, CO, USA). All solutions were prepared using analytical grade reagents and distilled water.

2.2 Sampling and preparation of pitanga leaf

Leaves of the pitanga plant (*Eugenia uniflora L harvest of* 2020*)* were collected in the city of Umuarama. (Paraná – Brasil), at the coordinates. 23º47'17''S 430 meters above sea level. The leaves were sanitized in a sodium hypochlorite solution (5%) in distilled water for 15 minutes and then placed in a forced-air drying oven. (MARCONI, MA035 - SP) at 35 ºC by 24 hours. The dried leaves were ground and then the extraction was performed.

2.3 Extraction process

In a Falcon tube, pitanga leaf powder was added (w/w) and hydroethanolic solution in the ratio of 1:10 (dry leaf/extracting solution). It was then subjected to agitation and grinding in a Turratec. (Tecnal, TE-102, Poracicaba, Brazil) for 5 minutes, taken to the ultrasound bath. (ECO-SONICS, Q3.8, 40 kHz, 88 Watts, São Paulo, SP) and then centrifuged. (MTD III PLUS) a 3000 rpm by 5 min. Ultrasonic extraction time (A) and the amount of ethanol used in the process. (B) were considered as independent variables according to the experimental design presented in Table 1. The supernatant was filtered and collected in an amber bottle, then stored in-10 ºC until the day of the analyses. A control extraction in boiling water was performed for 10 minutes, in the proportion 1:10 (leaf: water) and then filtered and stored in an amber bottle.

Table 1 - Concentration of total phenolics and antioxidant activity of samples dry leaf of Pitanga.

	Variables		Responses			
	A:	B:	TPC	DPPH	FRAP	ABTS°⁺
Trials	Extraction time (min)	Ethanol (% w/w)	(mg GAE/g sample)	(µM Trolox/g sample)	(µM Trolox/g sample)	(µM Trolox/g sample)
1	20 (-1)	20 (-1)	146.23 ± 1.73	355.44 ± 5.60	820.66 ± 0.00	233.08 ± 4.85
2	60 (+1)	20 (-1)	208.15 ± 0.87	459.71 ± 2.31	1116.61 ± 3.27	480.51 ± 4.37
3	20 (-1)	80 (+1)	183.28 ± 2.09	474.43 ± 10.54	890.12 ± 8.16	275.66 ±4.85
4	60 (+1)	80 (+1)	219.14 ± 2.85	517.43 ± 5.67	1114.20 ± 8.15	654.38 ± 0.97
5	12 (-1,41)	50 (0)	172.93 ± 1.24	419.18 ± 0.60	914.06 ± 0.00	386.77 ± 0.73
6	68 (+1,41)	50 (0)	395.17 ± 0.86	642.23 ± 5.93	1424.95 ± 3.26	751.35 ± 4.83
7	40 (0)	8 (-1,41)	182.10 ± 0.86	393.93 ± 2.09	970.62 ± 9.78	250.07 ± 3.87
8	40 (0)	92(+1,4)	252.16 ± 2.27	544.04 ± 1.70	1039.36 ± 6.53	501.60 ± 1.94
9	40 (0)	50 (0)	326.59 ± 1.76	512.16 ± 3.75	636.87 ± 0.00	509.42 ± 3.88
10	40 (0)	50 (0)	326.38 ± 1.24	511.37 ± 3.00	634.44 ± 3.26	510.69 ± 1.94
11	40 (0)	50 (0)	325.87 ± 0.20	513.90 ± 3.00	636.87 ± 0.00	510.79 ± 0.00
water extraction			120.08 ± 2.34	258.87 ± 4.89	346.00 ± 6.53	209.92 ± 1.82

Abbreviations: TPC, total phenolic compounds, expressed as mg gallic acid equivalent (GAE)/100g. Source: Research data (2025).

2.4 Optimization of the pitanga leaf extract

Extraction parameters were optimized applying a statistical planning of rotational composite design (DCCR) allied to Response Surface Methodology (MSR) using the Software Statistica 7.0 (Realese 7, StatSoft, INC, Tulsa, USA), with 4 factorial points (+ 1 and -1), 4 axial points (+ 1.41 and -1.41) and 3 central points (0), totalizing 11 experiments. The independent variables were ultrasound extraction time (A) and ethanol concentration (B). The dependent variables (responses) evaluated were the total phenolic content (TPC) and antioxidant capacity by DPPH, ABTS and FRAP assays. The experiments were processed in a random order. The following general form of model was used to fit the observed data in DCCR.

$$Y_i = \beta_0 + \beta_1 A + \beta_2 B + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{12} A.B \quad (1)$$

Where Y_i is the response, β_0 is the intercept, A and B are the independent variable; $\beta_1, \beta_2, \beta_{11}, \beta_{22}, \beta_{12}$ are coefficients.

2.5 Determination of total phenolic compounds (TPC)

The determination of total phenolic compounds (TPC) in the extracts was performed using the Spectrophotometric method of Folin-Ciocalteu. Analysis was performed mixing 0.1 mL of the sample, 6 mL of distilled water, 0.5 mL of the Folin-Ciocalteu reagent and 1.5 mL sodium carbonate aqueous solution (20%) in test tube and supplemented the volume to 10 mL with distilled water. The solution was left at rest for 20 minutes at room temperature and then read the absorbance at 765 nm using UV-vis spectrophotometer (Thermo Scientific, GENESYS 10S UV-VIS, EUA). The TPC were quantified using calibration curve with gallic acid (GAE) and the results expressed in mg of GAE per g of dry pitanga leaf (Mussi, Pereira, 2022).

2.6 Determination of antioxidant activity of the pitanga leaf extract

For the determination of antioxidant activity of extracts, three methods were performed: the method of 2.2-defenil-1-picril-Hidrazil (DPPH), the ferric antioxidant Power reducer (FRAP) both described by Mussi and Pereira (2022). Measurements of the DPPH, FRAP and radical ABTS°+ methods were based on 6-hydroxy-2, 5, 7, 8-Tetramethylchromano-2-carboxylic acid (TROLOX) calibration curves and the results expressed in M M of Trolox equivalents per g of dry pitanga leaf.

2.7 Statistical analyses

Data expressed in mean ± standard deviation was statistically analyzed by an analysis of variance (ANOVA) and Tukey's test at a 5% significance level, using Statistic 7.0 software version 7.0 (StatSoft, Inc, Tulsa, USA).

3. Results and Discussion

3.1 Total phenolic compounds (TCP) and antioxidant activity of the pitanga leaf obtained Optimization of the pitanga leaf extract

In Table 1 demonstrate the results of the optimization of the pitanga leaf extraction and the control extraction (water extraction). Values of TCP ranged from 183.28 to 395.17 mg GAE/g of dry sample, whereas for the antioxidants activity fluctuated between 355.44 to 642.23 µM Trolox/g sample, de 634.44 para 1424.95 µM Trolox/g sample and 233.08 to751.35 µM Trolox/g sample to DPPH, FRAP e ABTS°+ respectively The highest quantification values, both for TCP and for antioxidant activities (DPPH, FRAP and ABTS°+) were identified in the same trial, a trial with 68 minutes of ultrasonic extraction and 50% (w/w) of ethanol. The control extraction was performed as a comparison, as in several places, pitanga leaves are sold and used as tea for empirical treatment, which offers various benefits.

Denardin *et al*. (2015) describe a relationship between the polyphenolic content and antioxidant activity in pitanga leaves and fruits, showing that the antioxidant activities are due to the high content of phenolic compounds in the plant material Studies reveal that the antioxidant activity of phenolic acids may depend on the number of hydroxyl groups and their positions on the molecules, based on the delocalization of electrons from the aromatic ring, resulting in high antioxidant activity through the flavonoids present, such as hydroxycinnamic acids, quercetin, and cyanidin. (Denadin *et al.,* 2015) compounds that are abundantly present in pitanga leaves (Denadin *et al*., 2015; Lorenzo *et al*., 2018; Vargas, *et al.,* 2019).

Lorenzo *et al*., (2018) conducted a study on the extraction of pitanga leaves concentrated in a rotary evaporator, using 60% (w/w) of ethanol and 45 min in an ultrasonic-assisted extraction bath, still stirred for 30 minutes at 80 ºC in a mechanical shaker, finding TPC of 229.38 mg GAE/g of extract and of 570.97 mg Trolox/g of ABTS °+, lower values found in this study using similar methodologies.

Proper choice of solvent and extraction method can effectively influence the efficient extraction of plant material, as phenolic compounds are much more soluble in less polar organic solvents than in water (Kim and Lee, 2003; Russel *et al.,* 2009).

Values obtained by hot water extraction were lower than the lowest values obtained through optimization., being 30% lower in TCP, e 27%, 45% e 10% lower for DPPH, FRAP e ABTS respectively. These lower values were likely found due to the extraction method used, as the use of ultrasound and extracting solutions composed of solvents with different polarities can influence the extraction of these active compounds, as well as increase their efficiency (Gil-Martín*, et al*., 2022). Other factors, such as process time and solvent concentration, also play an important role in the extraction of bioactive compounds. An important factor was also the chosen temperature, approximately. 100 ºC thus potentially degrading bioactive compounds through heat, mainly due to the high heterogeneity of the polyphenolic compounds involved and the combined effect of time/temperature on their degradation. (Garcia-Perez *et al*., 2010; Suvarnakuta *et al*., 2011; Djendoubi *et al.,* 2012).

3.2 Analysis of variance (ANOVA) and quadratic response surface model (MSR) for dried pitanga leaf extracts

To validate the fit of the proposed model with the obtained results, an analysis of variance test was performed (ANOVA) by the central composite rotational design (CCRD), which are showed of Table 2. Valures of F-Calculated were higher than those of F-Tabulated for all the experiments conducted (TCP, DPPH, FRAP and ABTS^{o+}), indicating that the model is not only significant ($p < 0.05$) but also useful for predictive purposes. The linear regression coefficients (A-Time (min) and B-EtOH (% w/w)) The trials showed half of the effects and were also obtained with a significance level of. $p < 0.001$, demonstrating significant effects for the independent variables, concluding that the model fits the experimental data well.

The values of the coefficients of determination (R^2) showed a good relationship with the model being predictive, being acceptable for all the trials (TCP = 0.80; DPPH = 0.86; FRAP = 0.95 and ABTS^{o}+= 0.96), thus, the fitted models were statistically significant. According to Govaerts *et al*, (2020) in the analysis of variance of a model, a portion of the total variation of the observations around the mean can be described by the regression equation, while the remainder is part of the residuals. The higher or closer to 1 the value of the coefficient of determination (R^2), the better the model's fit to the observed data.

Table 2 – ANOVA for responses surface quadratic model.

Source	Df	Sum of Square			
		TPC	DPPH	FRAP	ABTS^{o+}
		(mg GAE/g sample)	(µM Trolox/g sample)	(µM Trolox/g sample)	(µM Trolox/100g)
A-Time (min)	1	21111.16***	26662.16***	192665.64***	163102.24***
B-EtOH (% w/w)	1	2695.67***	18896.88***	3366.24***	40817.93***
AB	1	169.63***	938.29**	1290.91**	4309.18***
A^2	1	7611.23***	57.10*	324796.61***	1088.24***
B^2	1	27983.90***	6622.30***	139224.82***	39256.78***
Residual	5	13325.07	8296.54	29732.79	11134.15
Lack of Fit	3	13324.80***	8293.20***	29728.84***	11132.98***
Pure Error	2	$0,27^{ns}$	$3,34^{ns}$	$3,95^{ns}$	$1,17^{ns}$
Variance analysis of quadratic square					
R-Squared		0.8011	0.8655	0.9505	0.9584
F-Tabulated		5.05	5.05	5.05	5.05
F-Calculated		13.41	19.23	66.72	66.97

Df - degree of freedom; *Significant at ($p < 0.05$); ** Significant at ($p < 0.01$); ***Significant at ($p < 0.001$); ns – not significant at ($p < 0.05$)
Source: Research data (2025).

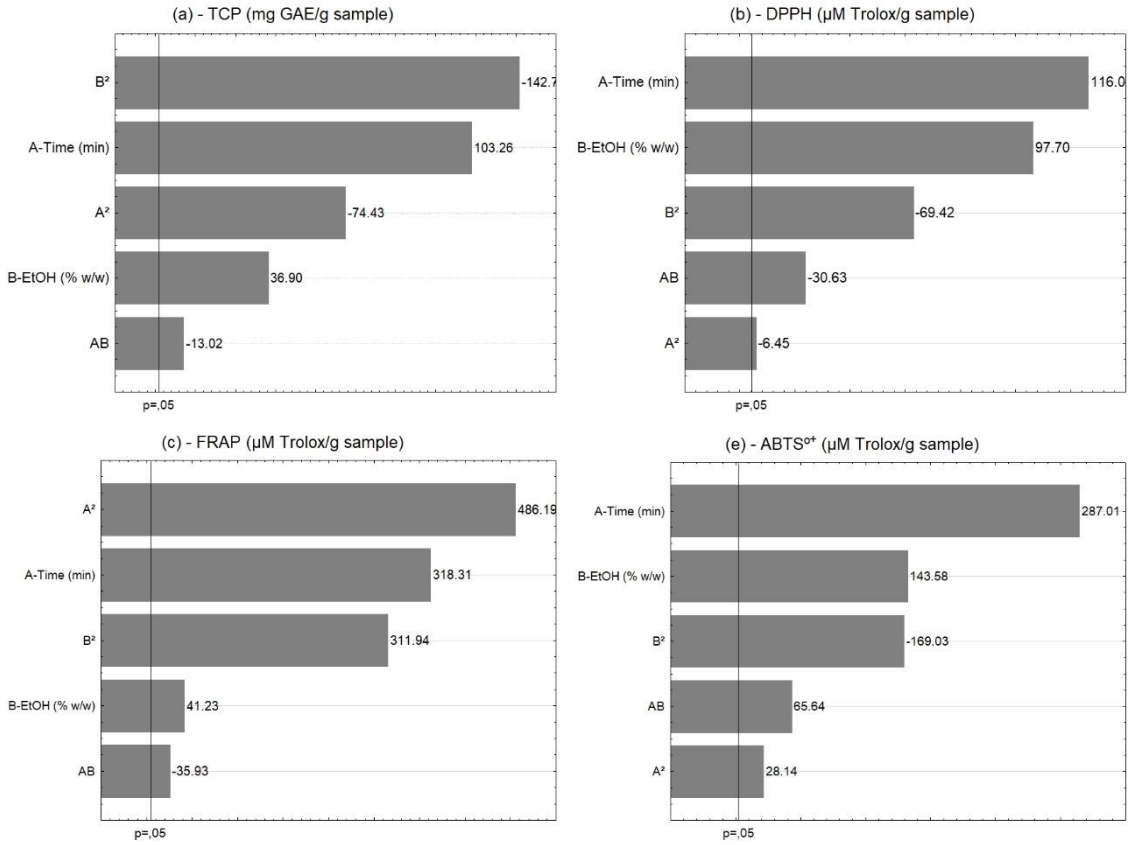
Equations representing the fitted model to describe TCP, DPPH, FRAP and ABTS^{o}+ in the extraction of bioactive compounds from pitanga leaves" (*Eugenia uniflora L.)* are described in the Eq. 2, 3, 4 and 5, in which , A is the ultrasound time (min) and B is the ethanol percentage (% w/w):

TCP (mg GAE/g sample) = -175.69+10.56A-0.09A²+8.97B-0.08B²-0.01AB. (2)
DPPH (µM Trolox/g sample) = 155.20+4.82A-0.01A²+6.51B-0.04B²-0.02AB. (3)
FRAP (µM Trolox/g sample) = 1645.83-39.32A+0.61A²-15.80B+0.18B²-0.03AB. (4)
ABTS^{o}+ (µM Trolox/g sample) = 35.38+1.62A+0.04A²+9.60B-0.10B²+0.05AB. (5)

Lack of fit was significant for all the models presented (TCP, DPPH, FRAP, and ABTS^{o}+); however, its reproducibility is good due to the very small value of the pure error, resulting in a false indication of lack of fit, as the calculated F-value is also high (Silva-Júnior *et al*., 2021).

Effects of the linear, quadratic factors, and extraction time interactions (A-Time (min) e quantidade de etanol (B-EtOH (w/w)) are represented in the Pareto diagram Figure 1. The vertical line indicates the significance of the data at p = 0.05, and the horizontal bars represent the values of the effects of the variables and their interactions.

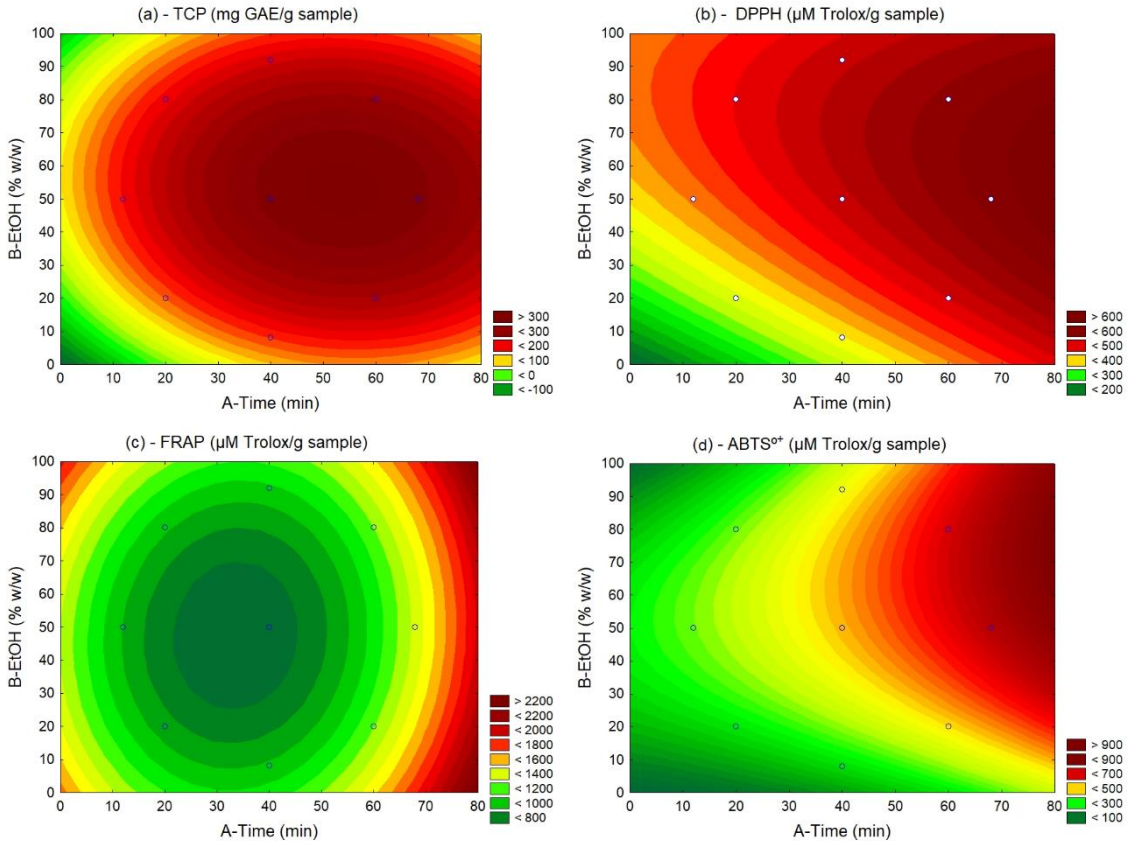
Figure 1 - Pareto chart of standardized effects from pitanga leaf.



Source: Research data (2025).

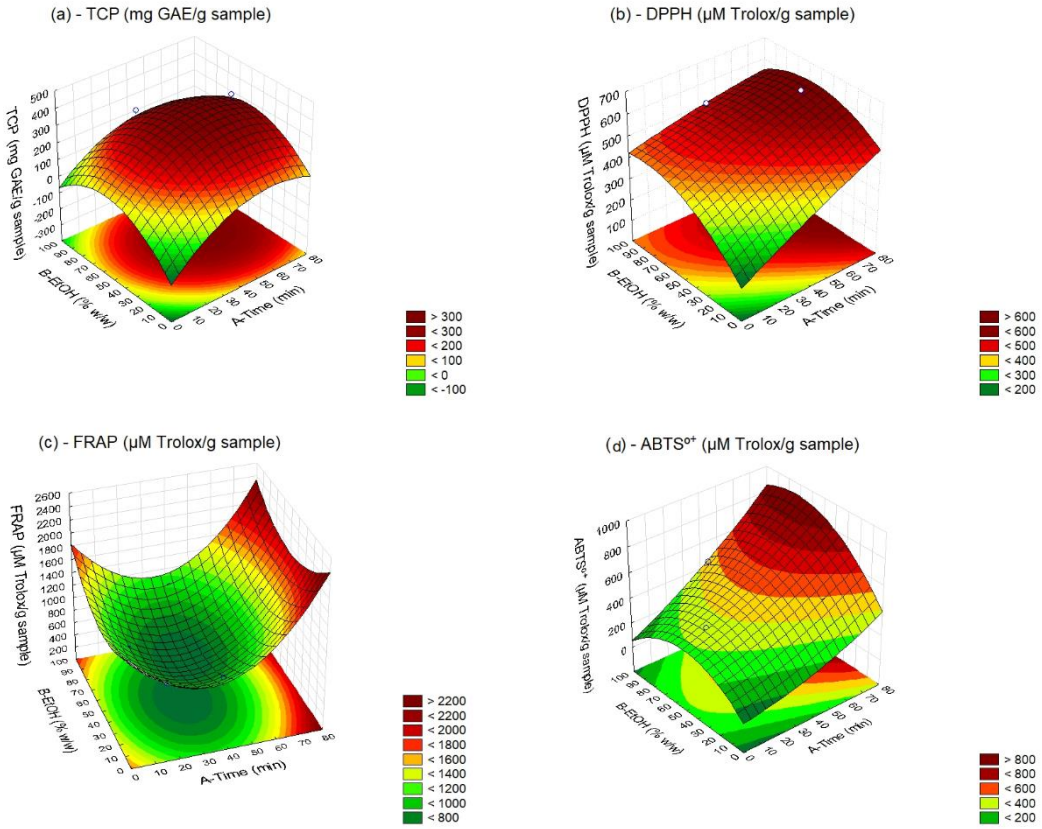
All linear, quadratic factors, and interactions of the effects of the variables were statistically significant for TCP, DPPH, FRAP, and ABTS^{o}+ ($p < 0.05$). The linear effects of ultrasound extraction time (A-Time (min)) were positive for all analyses, indicating that the exposure time of the plant material to ultrasound positively impacted the extraction of bioactive compounds from pitanga leaves. This can also be observed through the response surface of the analyses, Figures 2 and 3, which show that the best results occur at high ultrasound extraction times. This outcome was expected, as several studies indicate that ultrasound-assisted extraction (US) enhances the efficiency of extracting bioactive compounds from plant matrices .

Figure2 - Thermal surface 2D to extraction pitang (a) by TCP. (b) By DPPH. (c): by FRAP. (d) by ABTS.



Source: Research data (2025).

Figure 3 - Thermal surface 3D to extraction pitang (a) by TCP. (b) By DPPH. (c): by FRAP. (d) by ABTS.



Source: Research data (2025).

The values of the linear effects of ethanol concentration (B-EtOH (% w/w)) were also positive in all analyses. However, the range of effect values for the DPPH and ABTS°+ analyses were greater than those for TCP and FRAP, indicating a stronger influence of ethanol on the identification of AA in these analyses, since both processes use the same antioxidant activity mechanism based on electron transfer. This likely occurred due to the ultrasound, which facilitated bubble generation, thereby aiding the unblocking of the cavitation area and intensifying the sonochemical reactions.

All interaction effects showed low values compared to the values of the other effects, while the quadratic effects of time and ethanol concentration yielded different values for each analysis, without following a clear trend.

4. Conclusion

Results demonstrated that process optimization, through the experimental design (DCCR), enabled the maximization of total phenolic compounds concentration and antioxidant activity. The combination of 50% ethanol and 68 minutes of extraction proved to be the most efficient condition, outperforming traditional methods such as conventional hot water extraction.

This study contributes to the advancement of bioactive compound extraction methodologies, enabling a more efficient and sustainable use of natural resources. Pitanga, with its proven antioxidant properties, can be explored as a valuable source for the pharmaceutical, cosmetic, and food industries, especially as a substitute for synthetic compounds, aligning with more eco-friendly and efficient practices. Furthermore, the application of ultrasound-assisted extraction offers great potential for

developing more efficient processes with low environmental impact, fostering innovation in the use of native plants from Brazil.

References

Amorim, A.C.L., Lima, C.K.F., Hovell. A.M.C., Miranda, A.L.P. , Rezende, C.M. & Rezende. (2009). Antinociceptive and hypothermic evaluation of the leaf essential oil and isolated terpenoids from *Eugenia uniflora* L. (Brazilian Pitanga). *Phytomedicine*, 16 (10), 923-928.

Costa, A.G.V., Garcia-Diaz , D.F. , Jimenez, P. , & Silva, P.I. (2013). Bioactive compounds and health benefits of exotic tropical red–black berries. *J Funct Foods*, 5 (2), 539-549.

Denardin, C.C., Hirsch, G. E., da Rocha, R. F., Vizzotto, M. , Henriques, A.T., Moreira, J. C., & Emanuelli, T. (2015). Antioxidant capacity and bioactive compounds of four Brazilian native fruits. JFDA. 23 (3), 387-398.

Djendoubi, N. M.; Boudhrioua, N.; Kechaou, N.; Courtois, F.; & Bonazzi, C. (2012). Influence of air drying temperature on kinetics, physicochemical properties, total phenolic content and ascorbic acid of pears. *FBP*. 90(3), 433-441.

Garcia-Perez, J. V.; García-alvarado, M. A.; Carcel, J. A.; & Mulet, A. (2011). Extraction kinetics modeling of antioxidants from grape stalk (Vitis vinifera var. Bobal): Influence of drying conditions. *J Food Eng*. 101(1), 49-58.

Ghenabzia. I., Hemmami, H., Ben-Amor, I., Zeghoud S., Seghir, B.B., & Hammoudi, R. (2023). Different methods of extraction of bioactive compounds and their effect on biological activity: A review. *Int. j. Second. Metab*. 10 (4), 469–494.

Gil-Martín, E., Forbes-Hernández, T., Romero, A., Giampieri, D.C.F., & Battino,M. (2022). Influence of the extraction method on the recovery of bioactive phenolic compounds from food industry by-products. *Food Chem*. 378,131918.

Govaerts, B., Francq, B., Marion, R., Martin, M., & Thiel, M. (2020). The Essentials on Linear Regression, ANOVA, General Linear and Linear Mixed Models for the Chemist. *Module in Chemistry, Molecular Sciences and Chemical Engineering*. doi:10.1016/b978-0-12-409547-2.14579-2

Kim D. O., & Lee C. Y. (2003). Extraction and isolation of polyphenolics. Current Protocols in Food Analytical Chemistry: John Wiley & Sons. https://doi.org/10.1002/0471142913.fai0102s06

Lorenzo, J. M., Vargas, F. C., Pateiro, S. I., Furtado, M., Sant`Ana, M.M., Rocchetti, A. S., Barba, G., Dominguez, F. J., Lucini, R., & Sobral , L. A. (2018). .Influence of pitanga leaf extracts on lipid and protein oxidation of pork burger during shelf-life, *Food Res Int.*, 114 (May) : 47-54.

Mesquita,P.R.R. ,Nunes, E.C., Santos,F.N. , Bastos,L.P. ,Costa, M.A.P.C. ,Rodrigues, F.M. Rodrigues, & Andrade, J. B. (2017). Discrimination of Eugenia uniflora L. biotypes based on volatile compounds in leaves using HS-SPME/GC–MS and chemometric analysis Microchemical. *Revista Microquímica*. 130, 79-87.

Mussi, L.P., & Pereira, N. R. (2022). Storage stability of freeze-dried powder of jambolan (Syzygium cumini (L.)) fruits at different degrees of maturity and packages. *BJFT*. 25, e2021096.

Picó, Y. (2013). Ultrasound-assisted extraction for food and environmental samples. *Trends in Analytical Chemistry*. 43, 84-99.

Rostagno, M. A.; & Prado, J. M. (2013).Natural Product Extraction: Principles and Applications. RSC PUBLISHING, Cambridge, UK, 2013.

Russell W. R., Labat A., Scobbie L., Duncan G. J., & Duthie G.G. (2009). Phenolic acid content of fruits commonly consumed and locally produced in Scotland. *Food Chem.* 115, 100- 104.

Silva-Junior, M. E; Araújo, M. V. R. L; Santana, A. A; Silva, F. L. H; & Maciel, M. I. (2021). Ultrasound -assisted axtraction of bioactive compounds from cciriguela (Spondias purperea L.) peel: Optimization and comparison with conventional extraction and microwave. *Arab J Chem*. 14 (8),103260.

Silva R.W.P. , Brito,V.L. ,Lemos, M.R.A., Ferreira, L.A.L. Soares, T.I.E. Svidzisnki, E.P. Milão, E. P., & Chaves, G. M. (2015). Effect of the crude extract of Eugenia uniflora in morphogenesis and secretion of hydrolytic enzymes in Candida albicans from the oral cavity of kidney transplant recipients. *BMC Complement. Altern. Med*. 15 (1), 6.

Suvarnakuta, P.; Chaweerungrat, C.; & Devahastin, S. (2011). Effects of drying methods on assay and antioxidant activity of xanthones in mangosteen rind. *Food Chem*. 125 (1), 240-247.

Tessaro, L.; Luciano, C. G.; Bittante, A.M.Q.B., Lourenço, R.V.; Martelli-Tosi, M.; & SobraL, P.J.A. (2021). Gelatin and/or chitosan-based films activated with "Pitanga (Eugenia uniflora L.) leaf hydroethanolic extract encapsulated in double emulsion.``. ***Food Hydrocoll***, *[S.L.],* 113, 106523-10254

Tiwari, B.K. (2015). Ultrasound: A clean, green extraction technology. *Trends in Analytical Chemistry*. 71, 100–109.

Vargas, F. C., Gómez, B., Khaneghah, A. M., Strozzi, I., Gavahian, M., Barba, F. J. Sobral, P. J. A., & Lorenzo, J. M. (2019). Assessment of the suitability of pitanga leaf extract as a natural antioxidant for enhancing canola oil stability: Monitoring lipid oxidation parameters. *Eur. J. Lipid Sci. Technol.* 121 (5), 1800447.

Vargas, F.C. , Pereira, L.A., Costa, P.A., Melo, M.P. , & Sobral, P.J.A. (2016). Rosemary and Pitanga aqueous leaf extracts on beef patties stability under cold storage. *Braz. Arch. Biol. Technol*. 59, e16160139.