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Optimized Ultrasound-Assisted Extraction of *Psidium laruotteanum* Roots: A Concentrated Source of Piceid from the Brazilian Savanna

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Abstract: *Psidium laruotteanum* is a subshrub Myrtaceae, native to the Brazilian savanna, and distributed in Central and South America. Here, the health beneficial compound piceid (resveratrol-3- β -mono-D-glucoside) is described for the first time in the genus *Psidium*. The ultrasound-assisted extraction from *P. laruotteanum* roots was optimized by a response surface methodology. A central composite design was used to assess six extraction variables. Optimal extractions were obtained with 62.5% MeOH in water at 69.1 °C, amplitude of 70%, cycle of 0.5 s⁻¹, pH 5.7 and solvent–solid ratio of 40:1, for 10 min. The method showed excellent recovery (90%–95%) and precision (CV: 0.69% for repeatability and 0.43% for intermediate precision). This species appears as the most concentrated plant source of piceid reported to date (29.15 mg·g⁻¹ in dry roots). Additionally, a very fast extraction (5 min) can afford 96% recovery of piceid from *Psidium laruotteanum* roots. It could be used as a natural and cheap source of piceid for several applications.

Keywords: *araçá-cascudo*; *Psidium laruotteanum*; *trans*-piceid; ultrasound-assisted extraction; experimental design



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1. Introduction

Piceid (resveratrol-3- β -mono-D-glucoside) is a stilbene and probably the most abundant form of resveratrol in nature [1]. Recently, this compound is gaining more attention because an increasing number of studies have shown physiological and pharmaceutical properties in common with resveratrol, including antioxidant [2], anti-cancer [3] and anti-inflammatory [4]. Additionally, after oral ingestion, piceid can be metabolized into *trans*-resveratrol, which becomes available in high concentrations. It has been also reported that resveratrol and phenolic compounds produced by several pine species may have an active role in protecting them against *Bursaphelenchus xylophilus* [5], also as active compounds in the response to mites by some plants, as *Vitis vinifera* [6].

In plants, the glycosylation of resveratrol protects it from enzymatic oxidation, so the glucoside form of piceid is more stable [7]. While it may be true, most studies still report low concentrations of this compound in plants and derivative foods. Some examples of food sources containing piceid are cocoa and chocolate products (0.35–7.14 $\mu\text{g}\cdot\text{g}^{-1}$) [8], peanut butter (0.067–0.225 $\mu\text{g}\cdot\text{g}^{-1}$) [9], tomato skin (0.1–50 $\mu\text{g}\cdot\text{g}^{-1}$) [10], hop cone (0.42–11.01 $\mu\text{g}\cdot\text{g}^{-1}$) [11],

grape berry skin ($42.19 \mu\text{g}\cdot\text{g}^{-1}$) [12] and wine ($1.19\text{--}22.08 \text{mg}\cdot\text{L}^{-1}$) [13]. Grapes and wine are widely studied, as resveratrol and its glycosides (e.g., piceid) have a potential role in the “French paradox”, the lower incidence of cardiovascular diseases associated with moderate wine consumption [14]. So, stilbenoids have been demonstrated to be interesting in food production [15].

Regarding vegetable sources, several stilbenoids have been determined even in grape stem, reaching up to $417 \mu\text{g}\cdot\text{g}^{-1}$ in some varieties [16], up to $30.9 \text{mg}\cdot\text{g}^{-1}$ was found in black spruce bark of *Picea Mariana* [17], and up to $4.4 \text{mg}\cdot\text{g}^{-1}$ was found in the bark of *Picea jezoensis* [18]. Stilbenoids, including piceid, have been proposed as natural products from agricultural wastes to be used as antimycotoxin agents [19], as it has been demonstrated that they are useful agents against several fungi. Therefore, several raw vegetable and waste materials can be proposed as a source of piceid and related stilbenoids to be used for different purposes.

During preliminary steps of investigation, we noticed piceid as an abundant compound in roots of *Psidium laruotteanum* Cambess. This species, known popularly as *araçá-cascudo*, occurs as subshrubs and belongs to the same genus of guava (*Psidium guajava*) in the family Myrtaceae [20]. Native to the Brazilian savanna, *P. laruotteanum* is also distributed in Paraguay, Bolivia, Colombia, Venezuela, Guyana, Suriname and Costa Rica. Although its fleshy fruits present exotic flavor and are consumed in natura or as handmade processed products, this plant is still underexploited. As far as we are concerned, there is only one investigation about the biological properties of this species (phytotoxicity of leaf extracts) with no assessment on its chemical composition [21].

Extraction techniques from solid samples are essential in analytical and industrial processes, enabling the isolation and purification of target compounds or analytes of interest [22]. These techniques play a crucial role in various applications, including environmental analysis, pharmaceutical research [23], and food production [24].

Several techniques have been used for the quantitative extraction of piceid and resveratrol from plant samples, including microwave-assisted extraction, accelerated solvent extraction and supercritical fluid extraction. In order to combine maximum extraction yield with short time of extraction and low solvent consumption, ultrasound-assisted extraction is a valuable alternative method. It is simple, efficient, environmental friendly and presents low instrumental requirements [25,26].

Thus, the aim of this study was to assess the concentration of piceid in roots of *Psidium laruotteanum* and improve its extraction. An ultrasound-assisted method was optimized using a central composite design (CCD) at three levels with response surface methodology (RSM). The influence of six variables (type of solvent, temperature, amplitude, cycle, pH and solvent–solid ratio, on extraction) was discussed and an optimal condition was obtained. This procedure has shown very good results with similar compounds in other matrices [27,28].

2. Materials and Methods

2.1. Plant Samples

Roots of *P. laruotteanum* were collected from five individuals in the Brazilian savanna ($21^{\circ}58'5''$ S and $47^{\circ}53'12''$ W), São Carlos, Brazil. A voucher specimen was deposited at the Herbarium of the Federal University of São Carlos (number: 8720). The material was dried at 35°C (total loss of weight: 19.3%) and reduced to powder in an electric grinder and passed through a 1 mm screen mesh using a vibratory sieve shaker (AS 200, Retsch GmbH, Haan, Germany). Fat and wax were removed by washing 25 g of powdered roots with 100 mL of hexane for 15 min at $20\text{--}30^{\circ}\text{C}$ using a 45 kHz ultrasound bath (USC500T, VWR Collection, Radnor, PA, USA). This process was repeated four times in total. Later, the solid was filtered through Whatman filter paper ($3 \mu\text{m}$) under reduced pressure, dried at 35°C and stored at ambient temperature in the dark until extraction. The powdered roots had less than 0.01% of fat and wax.

2.2. Chemicals

Solvents were of HPLC-grade; hexane, methanol, acetic acid and acetonitrile were purchased from Panreac (Barcelona, Spain). Standard *trans*-piceid was obtained from Sigma Aldrich (St. Louis, MO, USA) and water was purified in a Milli-Q Integral system (Millipore, Billerica, MA, USA).

2.3. Ultrasound-Assisted Extraction (UAE) of Piceid

Powdered roots (0.25 g) were extracted in 27 mm × 80 mm (diameter × height) glass tubes under water bath with controlled temperature (Frigiterm, J.P. Selecta, Barcelona, Spain). An ultrasonic system with a 7 mm diameter probe (model UP200S, 200 W and 24 kHz, Hielscher Ultrasonics GmbH, Teltow, Germany) was placed at 5 mm from the bottom of the tube. A set volume and type of solvent were used under specific UAE conditions, following the design of experiment (DOE). After extraction, the solid phase was removed by centrifugation (J. P. Selecta, Barcelona, Spain) at 4000 rpm for 5 min. Then, the supernatant was adjusted to the initial volume of extraction and filtered through nylon filter (0.22 µm) before injection into the UPLC-PDA/MS system.

2.4. Determination of Piceid

To confirm piceid in *P. laruotteanum* roots, a 100% MeOH extract was analyzed in an Acquity UPLC H-Class system coupled to quadrupole-time-of-flight mass spectrometer (Q-ToF-MS, Synapt G2, Waters Corp., Milford, MA, USA). Masslynx software-version 4.1 was used to control the equipment. The injection volume was set to 2 µL, and the separation was performed on a reverse-phase C18 column (Acquity UPLC BEH, 2.1 × 100 mm, 1.7 µm, Waters). The mobile phase consisted of (A) water with 5% formic acid and (B) methanol with 5% formic acid at a flow rate of 0.45 mL·min⁻¹. The elution gradient was 0 min, 0% B; 1 min, 0% B; 3 min, 5% B; 4 min, 10% B; 4.5 min, 10% B; 5 min, 20% B; 7 min, 20% B; 8 min, 30% B; and 10 min, 30% B. Subsequently, the column was washed with 100% B for 2 min and equilibrated with 0% B for 2 min. An electrospray source operating in positive ionization mode was used under the following conditions: desolvation gas flow = 500 L·h⁻¹, desolvation temperature = 350 °C, cone gas flow = 10 L·h⁻¹, source temperature = 110 °C, capillary = 3.0 kV, cone voltage = 10 V and trap collision energy = 4 eV. Full-scan mode was used to measure the mass/charge ratios (*m/z*) between 50 and 1200 Da.

For quantification of piceid, extraction samples were analyzed on an Acquity UPLC H-Class system coupled to an Acquity UPLC Photodiode Array (PDA) Detector controlled by Empower version 3 Chromatography Data Software (Waters). Separations were performed in a reverse phase C18 column at 47 °C (Acquity UPLC BEH, 2.1 mm × 100 mm, 1.7 µm, Waters). The mobile phase consisted of phase A (water with 2% acetic acid) and phase B (acetonitrile with 2% acetic acid). The flow rate was 0.6 mL·min⁻¹ in a 4 min gradient as follows (%B): 0 min, 0%; 1 min, 0%; 1.1 min, 10%; 2 min, 10%; 3 min, 20%; 3.5 min, 60%; and 4 min, 100%. After this, the column was washed with 100% B for 3 min and equilibrated with 0% B for 3 min. The Photodiode Array (PDA) Detector was set in the wavelength range of 200–400 nm for the 3D scan with collection data rate at 40 points·s⁻¹ to identify the compound. However, for compound quantification, the PDA detector was set at fixed wavelength of 317 nm for 2D scan with collection data rate of 80 points·s⁻¹. These values fit the maximum absorbance of piceid (317 nm). The injection volume of the sample was 3 µL, and piceid was identified by spiking and comparing the retention time and maximum UV absorptions with the standard.

2.5. Design of Experiments

Response surface methodology was used to establish the optimal condition to extract piceid from *P. laruotteanum* roots. A central composite design (CCD) was developed with six independent variables (x_1 , solvent; x_2 , temperature; x_3 , amplitude; x_4 , cycle; x_5 , pH; x_6 , solvent–solid ratio) at three levels. These levels were normalized and forced to fit values of −1, 0 and 1 so that all variables affected the response more evenly, independent

of the unit [29]. The complete design had 46 different experimental runs (Table 1). The resulting data was fit to a polynomial model to obtain the response surface. The effect of each variable and the interaction effects between variables were also explored. The general function of the CCD is described in the following equation:

$$y = b_0 + \sum_{i=1}^n b_i x_i + \sum_{i=1}^n \sum_{j=1}^n b_{ij} x_i x_j$$

where x_i are the studied variables; y is the extraction yield of piceid; and b are the coefficients obtained by the least square method.

Table 1. Central composite design with six variables at three levels of the UAE of piceid and the observed responses.

DOE Runs	Extraction Factors						Relative Values to Maximum Yield (%)
	x_1 : Solvent (MeOH %)	x_2 : Temperature (°C)	x_3 : Amplitude (%)	x_4 : Cycle (s ⁻¹)	x_5 : pH	x_6 : Solvent–Solid Ratio	
1	50 (−1)	30 (−1)	30 (−1)	0.7 (+1)	7 (+1)	20 (−1)	80.4
2	100 (+1)	30 (−1)	30 (−1)	0.7 (+1)	7 (+1)	40 (+1)	70.8
3	100 (+1)	70 (+1)	70 (+1)	0.3 (−1)	3 (−1)	40 (+1)	86.8
4	50 (−1)	30 (−1)	30 (−1)	0.3 (−1)	7 (+1)	40 (+1)	84.1
5	50 (−1)	70 (+1)	30 (−1)	0.3 (−1)	7 (+1)	20 (−1)	85.2
6	50 (−1)	70 (+1)	30 (−1)	0.3 (−1)	3 (−1)	40 (+1)	98.5
7	100 (+1)	70 (+1)	30 (−1)	0.7 (+1)	3 (−1)	40 (+1)	84.9
8	50 (−1)	70 (+1)	30 (−1)	0.7 (+1)	3 (−1)	20 (−1)	88.2
9	75 (0)	50 (0)	70 (+1)	0.5 (0)	5 (0)	30 (0)	93.0
10	100 (+1)	30 (−1)	70 (+1)	0.7 (+1)	7 (+1)	20 (−1)	73.7
11	75 (0)	30 (−1)	50 (0)	0.5 (0)	5 (0)	30 (0)	84.0
12	100 (+1)	30 (−1)	70 (+1)	0.3 (−1)	7 (+1)	40 (+1)	73.6
13	75 (0)	50 (0)	30 (−1)	0.5 (0)	5 (0)	30 (0)	92.7
14	75 (0)	50 (0)	50 (0)	0.3 (−1)	5 (0)	30 (0)	90.7
15	75 (0)	50 (0)	50 (0)	0.5 (0)	5 (0)	20 (−1)	88.7
16	50 (−1)	30 (−1)	70 (+1)	0.7 (+1)	3 (−1)	20 (−1)	87.0
17	75 (0)	70 (+1)	50 (0)	0.5 (0)	5 (0)	30 (0)	100.0
18	50 (−1)	70 (+1)	70 (+1)	0.3 (−1)	7 (+1)	40 (+1)	98.5
19	75 (0)	50 (0)	50 (0)	0.5 (0)	3 (−1)	30 (0)	89.1
20	50 (−1)	30 (−1)	70 (+1)	0.3 (−1)	7 (+1)	20 (−1)	83.6
21	100 (+1)	30 (−1)	70 (+1)	0.7 (+1)	3 (−1)	40 (+1)	79.0
22	100 (+1)	70 (+1)	70 (+1)	0.7 (+1)	7 (+1)	40 (+1)	91.3
23	100 (+1)	30 (−1)	30 (−1)	0.3 (−1)	3 (−1)	40 (+1)	67.0
24	100 (+1)	70 (+1)	70 (+1)	0.3 (−1)	7 (+1)	20 (−1)	84.6
25	100 (+1)	70 (+1)	30 (−1)	0.7 (+1)	7 (+1)	20 (−1)	77.1
26	50 (−1)	30 (−1)	30 (−1)	0.3 (−1)	3 (−1)	20 (−1)	80.8
27	75 (0)	50 (0)	50 (0)	0.7 (+1)	5 (0)	30 (0)	91.5
28	75 (0)	50 (0)	50 (0)	0.5 (0)	5 (0)	30 (0)	93.7
29	100 (+1)	30 (−1)	70 (+1)	0.3 (−1)	3 (−1)	20 (−1)	72.5
30	50 (−1)	30 (−1)	70 (+1)	0.7 (+1)	7 (+1)	40 (+1)	90.8
31	50 (−1)	30 (−1)	70 (+1)	0.3 (−1)	3 (−1)	40 (+1)	87.5
32	50 (−1)	70 (+1)	30 (−1)	0.7 (+1)	7 (+1)	40 (+1)	96.7
33	75 (0)	50 (0)	50 (0)	0.5 (0)	5 (0)	40 (+1)	95.4
34	100 (+1)	50 (0)	50 (0)	0.5 (0)	5 (0)	30 (0)	86.2
35	50 (−1)	70 (+1)	70 (+1)	0.7 (+1)	3 (−1)	40 (+1)	98.9
36	100 (+1)	30 (−1)	30 (−1)	0.7 (+1)	3 (−1)	20 (−1)	72.5
37	50 (−1)	50 (0)	50 (0)	0.5 (0)	5 (0)	30 (0)	95.1
38	50 (−1)	70 (+1)	70 (+1)	0.3 (−1)	3 (−1)	20 (−1)	87.5
39	50 (−1)	70 (+1)	70 (+1)	0.7 (+1)	7 (+1)	20 (−1)	88.8
40	50 (−1)	30 (−1)	30 (−1)	0.7 (+1)	3 (−1)	40 (+1)	91.5
41	100 (+1)	30 (−1)	30 (−1)	0.3 (−1)	7 (+1)	20 (−1)	67.8
42	100 (+1)	70 (+1)	30 (−1)	0.3 (−1)	7 (+1)	40 (+1)	87.3
43	100 (+1)	70 (+1)	70 (+1)	0.7 (+1)	3 (−1)	20 (−1)	78.4
44	75 (0)	50 (0)	50 (0)	0.5 (0)	5 (0)	30 (0)	93.8
45	75 (0)	50 (0)	50 (0)	0.5 (0)	7 (+1)	30 (0)	91.2
46	100 (+1)	70 (+1)	30 (−1)	0.3 (−1)	3 (−1)	20 (−1)	84.5

The experimental design, analysis and surface response were developed using the software STATGRAPHICS Centurion (Trial Version 16.1.18, Statpoint Technologies, Inc., The Plains, VA, USA). Analysis of Variance (ANOVA) and the Least Significant Difference (LSD) test were used to determine the significance of differences between the means.

2.6. Performance of the Method

A calibration curve was developed to quantify piceid. Solutions of standard *trans*-piceid were prepared in triplicate at six concentrations ranging from 0.1 to 1 mg·L⁻¹. The linear regression equation between the solutions' concentrations and respective peak surface areas was used to quantify piceid in the extractions. Two extractions were performed to verify the amount of piceid in the optimal UAE condition.

The method for extraction of piceid from *P. laruotteanum* roots was validated according to procedures described in the ICH Guideline Q2 (R1) [30]. The considered parameters were precision and recovery. Precision was assessed considering repeatability (intra-day) and intermediate precision (extra-day). Repeatability was determined by the analyses of nine extractions under the same conditions in the same day. Intermediate precision was measured by three extractions on each of three consecutive days. Finally, precision was expressed as the Coefficient of Variance (CV) of piceid peak area.

The recovery of the method was determined by comparing the response in spiked and non-spiked root samples in duplicate. The standard solution of *trans*-piceid was spiked at a concentration of 0.6 mg·mL⁻¹.

2.7. Extraction Kinetics

Kinetics was analyzed at the UAE optimal condition of piceid from *P. laruotteanum* roots. Six extraction times were assessed in duplicate: 5, 10, 15, 20, 25 and 30 min.

3. Results

3.1. Identification of Piceid

A 100% MeOH extract was analyzed in an Acquity UPLC H-Class system coupled to quadrupole-time-of-flight mass spectrometer. Figure 1 shows the UPLC–MS spectrum of *trans*-piceid peak acquired in positive ion mode.

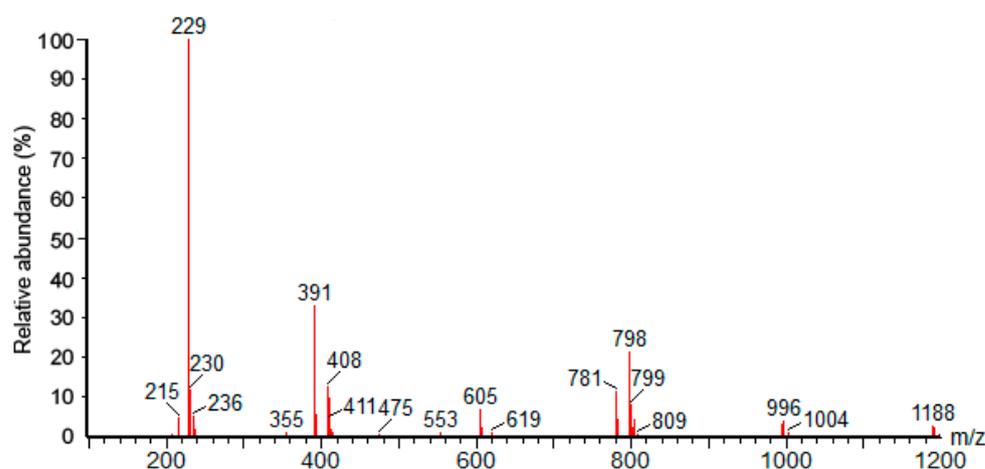


Figure 1. UPLC–MS spectrum of *trans*-piceid peak acquired in positive ion mode of 100% MeOH extract of *Psidium laruotteanum* roots. Retention time: 5.518 min.

3.2. Development of the UAE Method

To optimize the extraction of piceid from *P. laruotteanum* roots, a central composite design was developed (Table 1). The analyzed variables were solvent composition (x_1 : 50, 75, 100% methanol in water), temperature (x_2 : 30, 50, 70 °C), amplitude (x_3 : 30, 50, 70%),

cycle (x_4 : 0.3, 0.5, 0.7 s⁻¹), solvent pH (x_5 : 3, 5, 7) and solvent–solid ratio (x_6 : 20:1, 30:1, 40:1). Relative values to the maximum peak surface area of piceid were used as the responses.

The design had 46 runs with 12 center points and their data were used to obtain a mathematical model. The significance of each effect was determined using ANOVA, comparing the mean square against an estimate of the experimental error. The absolute values of the estimated effects divided by their standard values are plotted in a Pareto chart (Figure 2). Variables or combination of variables crossed by the vertical line had a significant effect on the response ($p < 0.05$).

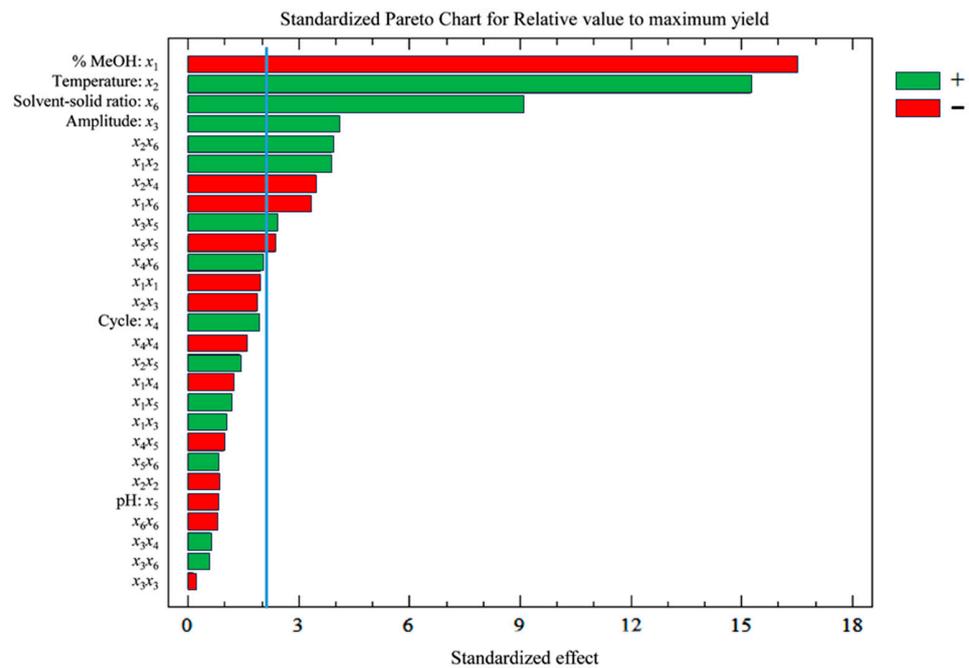


Figure 2. Effects of variables and their interactions on the extraction of piceid from *Psidium laruotteanum* roots.

The square coefficient of regression (R^2) statistic indicated that the model explained 97.9% of the variability in the relative value to maximum yield. The standard error of the predicted value showed a standard deviation of the residuals of 0.0192. For this reason, the model could be used to estimate the responses for the optimization of UAE of piceid.

3.3. Response Surface and Optimization of the Method

The model produced in the experimental design was used to build 3D surface plots and determine the optimal levels of the variables for the UAE of piceid. While examining a pair of variables, the others were kept constant in the central point (Figures 2–7).

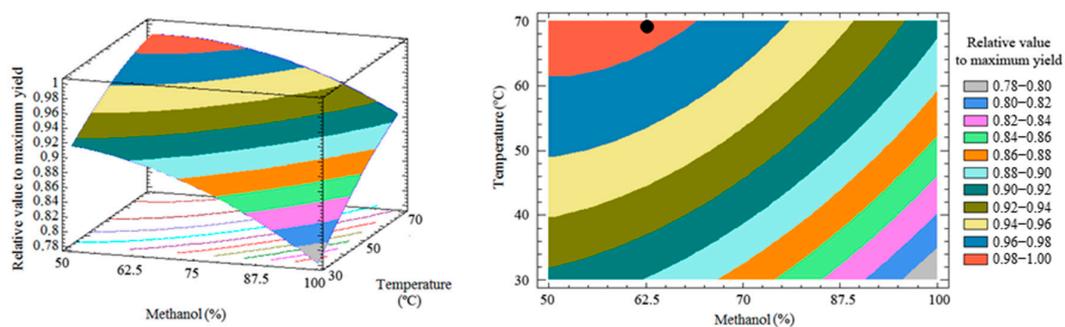


Figure 3. Response surface and contour plots between solvent (MeOH %) and temperature. Black dot: final optimal condition considering all variables.

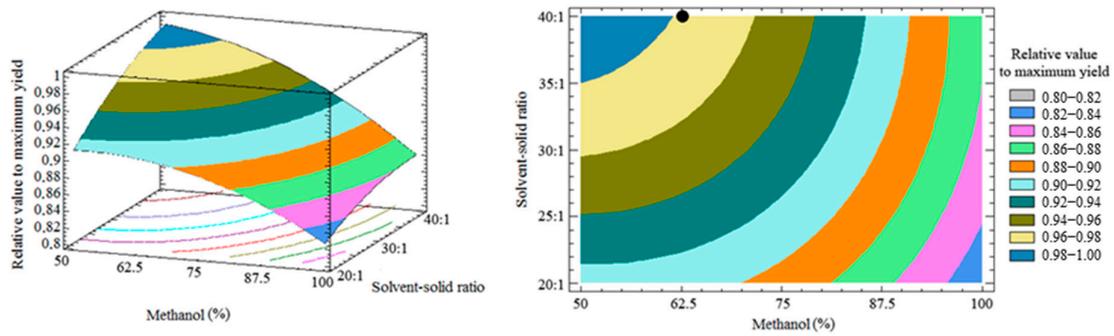


Figure 4. Response surface and contour plots between MeOH % and solvent–solid ratio. Black dot: final optimal condition considering all variables.

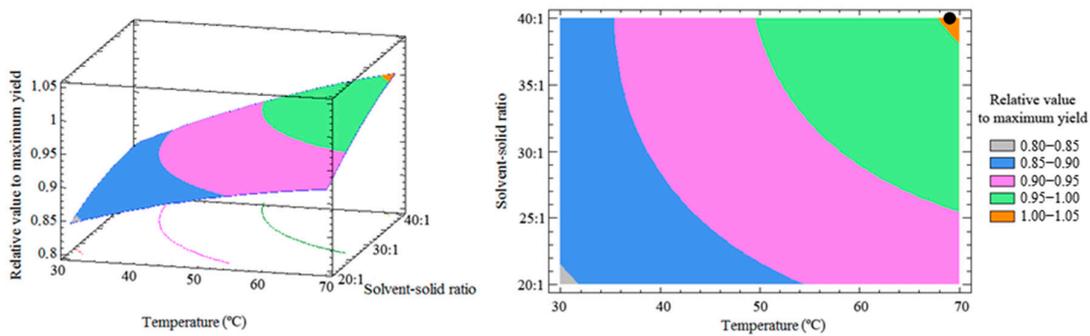


Figure 5. Response surface and contour plots between temperature and solvent–solid ratio. Black dot: final optimal condition considering all variables.

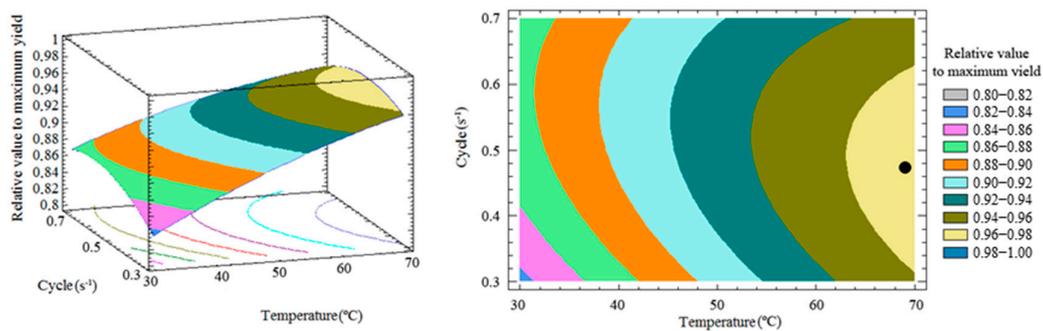


Figure 6. Response surface and contour plots between temperature and cycle. Black dot: final optimal condition considering all variables.

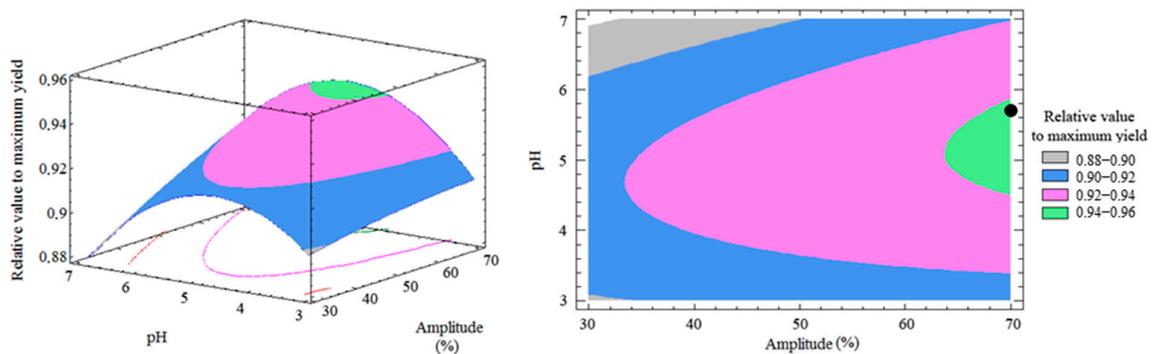


Figure 7. Response surface and contour plots between amplitude and pH. Black dot: final optimal condition considering all variables.

3.4. Optimal Condition and Method Validation

The following optimal condition was obtained according to the RSM: ultrasound-assisted extraction using 62.7% of MeOH in water, temperature of 69.1 °C, amplitude of 70%, cycle of 0.47 s⁻¹, pH of 5.7 and a solvent–solid ratio of 40:1. The model equation estimated an extraction yield of 29.13 mg·g⁻¹. Thus, two extractions were performed in similar conditions (Table 2), and the resulting yield was 29.15 ± 0.17 mg·g⁻¹, which fits perfectly the predicted value.

Table 2. Optimal and used extraction conditions with respective piceid yields.

	MeOH in Water (%)	Temperature (°C)	Amplitude (%)	Cycle (s ⁻¹)	pH	Solvent–Solid Ratio	Extraction Yield of Piceid (mg·g ⁻¹)
Optimal condition	62.7	69.1	70	0.47	5.7	40:1	29.13 *
Used condition	62.5	69.1	70	0.50	5.7	40:1	29.15 ± 0.17

*: estimated value by the model's equation.

The same conditions were used to validate the method, considering precision and recovery. Precision was evaluated by the repeatability (analysis of nine extractions in the same day) and intermediate precision (six extractions in each of three consecutive days). Table 2 shows the results.

3.5. Extraction Kinetics

Time is another key factor in determining the viability of extraction methods. Defining the shorter efficient extraction is essential in order to minimize energy costs and avoid the oxidation of compounds. The result is shown in Figure 8.

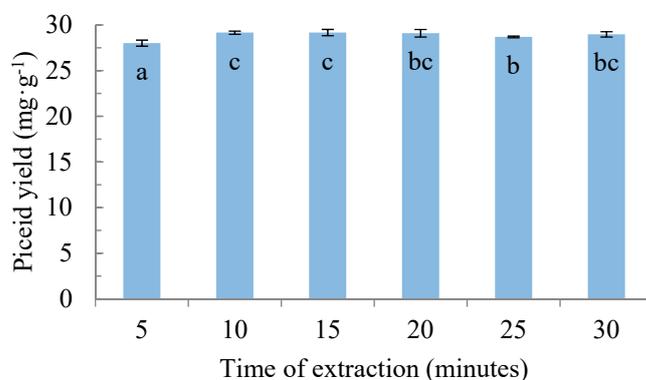


Figure 8. Piceid yield under different times of ultrasound-assisted extraction. Different letters: significant difference ($p < 0.05$).

4. Discussion

Piceid was confirmed in *Psidium laruotteanum* roots by mass spectra in positive ion mode (Figure 1). The fragmentation of the ion 391 [M + H]⁺ into the ion 229 [M + H]⁺ due to loss of 162 Da (glucose) indicates the presence of piceid. Additionally, the chromatographic peaks of the extraction solutions were checked by comparing their retention time and UV–vis spectra (λ_{\max} : 232.9 and 317.3 nm) with the standard before quantifications. To the best of our knowledge, this is the first time that piceid is described and quantified in the genus *Psidium*.

The most significant variables on the extraction of piceid were solvent (x_1), temperature (x_2), solvent–solid ratio (x_6) and amplitude (x_3). The extraction solvent had a negative effect so a higher recovery was achieved on decreasing the percentage of methanol in water. In contrast, temperature, solvent–solid ratio and amplitude had a positive effect as the recovery was higher on increasing their values. Studies on *Polygonum cuspidatum* roots have also demonstrated better recovery of piceid when increasing the solvent polarity and the

temperature [31]. This probably happens because piceid contains a glucoside that increases its polarity, and heating favors the extraction by increasing the solubility and diffusion coefficient of solutes [32,33].

Greater responses found using higher solvent–solid ratios are explained by the principle of mass transference, where the driving force is the concentration difference between the solid and the liquid [25]. This ratio could be increased using higher solvent volumes or less solid. However, the reduction of solid could increase the error and increasing the volume affects the viability due to longer procedures to evaporate the solvent and concentrate the solute. Consequently, no higher ratios were investigated. The amplitude also presented a positive effect on the extraction of piceid. The formation and collapse of cavitation bubbles are proportional to this variable. Thus, the higher the amplitude, the higher is the ultrasonic intensity that breaks the cells of the plant matrix and releases the compounds into the extraction medium [16]. Degradation of piceid due to the high amplitude was not found.

Additionally, six interactions between variables affected the response and were better analyzed using 3D surface plots. The regression coefficients for the variables and their interactions were calculated. Therefore, the equation for the fitted model is given below:

$$\begin{aligned}
 y = & 0.931 - 0.054x_1 + 0.050x_2 + 0.013x_3 + 0.007x_4 - 0.002x_5 + 0.029x_6 - 0.024x_1^2 + 0.013x_1x_2 \\
 & + 0.003x_1x_3 - 0.004x_1x_4 + 0.004x_1x_5 - 0.011x_1x_6 - 0.010x_2^2 - 0.006x_2x_3 - 0.012x_2x_4 \\
 & + 0.005x_2x_5 + 0.013x_2x_6 - 0.002x_3^2 + 0.002x_3x_4 + 0.008x_3x_5 + 0.002x_3x_6 - 0.019x_4^2 \\
 & - 0.003x_4x_5 + 0.007x_4x_6 - 0.029x_5^2 + 0.003x_5x_6 - 0.010x_6^2
 \end{aligned} \quad (1)$$

where y is piceid yield; x_1 , solvent; x_2 , temperature; x_3 , amplitude; x_4 , cycle; x_5 , pH; and x_6 , solvent–solid ratio.

Following the model results, solvent of extraction had a significant relationship with temperature and solvent–solid ratio (Figure 2). A decrease in MeOH from 100 to 50%, with an increase in temperature up to 70 °C (Figure 3) or in solvent–solid ratio to 40:1 (Figure 4), resulted in higher extraction yields of piceid. No higher temperatures than 70 °C were applied because of solvent evaporation during the extraction. In addition, polyphenolics can oxidize or degrade, decreasing the yield and conformation of the compounds [34], and it was also demonstrated that stilbenoids are stable up to 70 °C [16].

Temperature of extraction also had significant relationship with solvent–solid ratio and cycle (Figure 2). Increasing temperature from 30 to 70 °C, with an increase in solvent–solid ratio from 20:1 to 40:1 (Figure 5) favored the extraction yield of piceid. Temperature had a slightly higher effect on the response than solvent–solid ratio. Increasing temperature until 70 °C at a solvent–solid ratio of 20:1 reaches 95% of response, while an increase of solvent–solid ratio until 40:1 at 30 °C reaches 90% of response. Nevertheless, these variables present a direct correlation where the maximum response is achieved when both variables are increased. Figure 6 explores the interaction of temperature with ultrasound cycle. Increasing the temperature from 30 to 70 °C and keeping the cycle in the range of 0.32 and 0.62 resulted in higher responses.

The effect of amplitude interacting with pH of extraction is shown in Figure 7. Higher amplitudes associated with pH between 4.5 and 5.9 resulted in higher recoveries of piceid. This also illustrates the significant quadratic relation of pH (x_5x_5) with response (Figure 2).

The main properties of the extraction method at the optimum point were calculated. The method is precise with coefficient of variation for repeatability and intermediate precision of just 3.9% and 4.3%, respectively (Table 2). The recovery of piceid ranged from 90% to 95%, and it was estimated by comparing the response in samples spiked and non-spiked with standard piceid. These results are then consistent for piceid extraction using the UAE method.

Finally, after checking the results of the kinetic study (Figure 8), it can be concluded that in 10 min the maximum amount of piceid was extracted from roots of *P. laruotteanum* using the optimal UAE conditions. However, five minutes seem more valuable considering a difference of just 1.15 mg·g⁻¹ (lower than 4%) in half of the time.

Many studies have focused on the quantification and extraction of piceid from other plant sources, like grape berry skin [35], tomato skin [36], hop cone [11] and cocoa powder [8], but just achieved concentrations ranging from 0.042 to 50 $\mu\text{g}\cdot\text{g}^{-1}$ in longer extraction procedures (around 30 min). The richest natural source of piceid reported so far is *Polygonum cuspidatum*, the Japanese knotweed. Kuo et al. [31] obtained 10.77 $\text{mg}\cdot\text{g}^{-1}$ in optimized ultrasound-assisted extraction of its roots. However, our study shows a much higher concentration in roots of *P. laruotteanum*, 29.15 $\text{mg}\cdot\text{g}^{-1}$, and adds valuable knowledge about the chemical potential of this species. Further investigations should also be encouraged to explore the cultivation of *P. laruotteanum* and the influence of abiotic and biotic variables on its secondary metabolism since there is no information about these aspects in the literature.

5. Conclusions

Piceid was described for the first time in the genus *Psidium*. A simple, fast and efficient UAE method was obtained to recover this compound from roots of *Psidium laruotteanum*. Solvent, temperature, solvent–solid ratio and amplitude strongly influenced the extraction. Cycle and pH had minor effects but might be considered to achieve a higher recovery due to interactions with the other variables. The proposed method showed excellent precision and recovery. Under optimal extraction conditions, the yield of piceid was 29.15 $\text{mg}\cdot\text{g}^{-1}$ in dry roots. For this reason, *P. laruotteanum* appears as the most concentrated plant source of the health beneficial compound piceid. It highlights the exploration of native species from the Brazilian savanna and opens a new commercial opportunity, as a very fast extraction of just 5 min can still afford for 96% of this recovery of piceid from *P. laruotteanum* roots. Specific growing conditions should be studied to determine their effects on the levels of piceid as well as other phenolics. Related plants should be also studied to allow for the evaluation of additional sources of piceid and stilbenoids.

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