

Optimization of phenolic compounds extraction and a study of the edaphic effect on the physicochemical composition of freeze-dried jaboticaba peel

Otimização da extração dos compostos fenólicos e estudo do efeito edáfico sobre a composição físico-química da casca de jaboticaba liofilizada

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ABSTRACT

Jaboticaba is a fruit with high nutritional potential with beneficial effects for health. The aims of this work were the optimization of the extraction of phenolic compounds (PhC) from freeze-dried jaboticaba peel (FJP) as a function of the stirring time (ST) and solvent volume (SV) and to study the edaphic effect on the physical-chemical composition of FJP from five orchards. In the first stage, a 2-factor, 2-level central composite designs combined with the Response Surface Methodology and desirability function was used. In the second stage, a one-way analysis of variance was used to investigate the edaphic effect on the responses. Total phenols (Tph), tannins (Ta), total monomeric anthocyanins (TMA), color index (CI) and tone responses (Ton) were analyzed. A second-order polynomial model was used for predicting of the first stage dates. The data from the second experiment were evaluated using analysis of variance, Tukey's test, and t-test. The optimal conditions for the PhC were 64 mL and 75 min. Under the optimum conditions, the corresponding predicted response values for PhC were 33.5, 7.91, and 5.57 mg g⁻¹ and 0.893 u.a. and 0.833 for Tph, Ta, TMA, CI, and Ton, respectively. According to the type of soil, different PhC values were found in FJP extracts. In nutrient-poor soils, highest levels of PhC, high CI and low tone were found.

Index terms: *Myrciaria cauliflora*; ultrasound; response surface methodology; physical-chemical properties.

RESUMO

A jaboticaba é uma fruta com alto potencial nutricional com efeitos benéficos para a saúde. No presente trabalho se estudou a otimização da extração de compostos fenólicos (PhC) da casca de jaboticaba liofilizada (FJP) em função do tempo de agitação (ST) e volume de solvente (SV), e se estudou o efeito edáfico na composição físico-química da FJP provenientes de cinco pomares. No primeiro estágio se aplicou um desenho central composto de 2 fatores e 2 níveis, combinado com a Metodologia de Superfície de Resposta e função desejabilidade. No segundo estágio se aplicou análise de variância para investigar o efeito edáfico nas respostas. Foram analisados os fenóis totais (Tph), taninos (Ta), antocianinas monoméricas totais (TMA), índice de cor (CI) e tonalidade (Ton). Um modelo polinomial de segunda ordem foi utilizado para prever os dados do primeiro estágio. Os dados do segundo experimento foram avaliados por meio de análise de variância, teste de Tukey e teste t. As condições ótimas para a extração de PhC foram 64 mL e 75 min. Nestas condições, as respostas preditas corresponderam a 33,5, 7,91 e 5,57 mg g⁻¹ e 0,893 u.a. e 0,833 de Tph, Ta, TMA, CI e Ton, respectivamente. De acordo com o tipo de solo, diferentes valores de PhC foram encontrados nos extratos de FJP. Em solos pobres em nutrientes foram encontrados níveis elevados de PhC, alto CI e baixa Ton.

Termos para indexação: *Myrciaria cauliflora*; ultrason; metodologia de superfície de resposta; propriedades físico-químicas.

INTRODUCTION

Tropical countries produce a large amount of native and exotic fruit species, which are potentially interesting to the food industry. The nutritional and therapeutic values of these fruits are mainly due to the

presence of bioactive compounds, especially polyphenols (Costa et al., 2013).

The jaboticaba (*Myrciaria cauliflora*) tree belongs to the family Myrtaceae, native to Brazil. Its fruits are the most economically important, as it has a sweet-sour taste (Reynertson et al., 2008).

Jaboticaba fruit is rich in carbohydrates (Lenquist et al., 2015), organic acids, terpenes, and phenolic compounds (Batista et al., 2014; Calloni et al., 2015; Lenquist et al., 2015; Plaza et al., 2016).

The jaboticaba fruit may contain a wide variety of nutrients; some of them are present low concentrations with great health benefits (Wu; Long; Kennelly, 2013), including being used as bioactive compounds.

Dried jaboticaba peel product is traditionally employed in the treatment of hemoptysis, cough, bronchitis, asthma and inflammation of the tonsils. The fruit peel and leaves are used for diarrhea and dysentery as well, due to their high astringency (Souza-Moreira et al., 2011).

Other biological activities were also evaluated for these species. Ethanolic extracts showed inhibitory effects against *K. pneumoniae* (Haminiuk et al., 2011). The aqueous extracts of seed and peel inhibited the growth of three Gram-negative bacteria: *S. aureus*, *L. monocytogenes* and *E. coli* (Silva et al., 2014).

Research has demonstrated that phenolic compounds extracted from parts of the jaboticaba tree assist to prevent or reduce oxidative stress and increase one's antioxidants.

Batista et al. (2014) studied the effect of an extract prepared from the jaboticaba peel, used as an indicator of oxidative stress, in obese rats. Due to the peculiar polyphenol content, this type of extract could be an alternative to minimize the oxidative stress induced by a high fat diet and circulating saturated lipids.

Calloni et al. (2015) determined the macronutrient and phenolic compounds of pulp and jaboticaba peel. In addition, an extract of jaboticaba peel was evaluated for its in vitro antioxidant activity and its capacity to modulate oxidative stress, as well as mitochondrial function in human lung fibroblast cells (MRC-5). These authors showed a new role for jaboticaba peel as a mitochondrial protectant in pathological conditions in which mitochondrial dysfunction is involved.

To achieve greater efficiency of the bioactive compounds, an efficient extraction must be performed to maximize the extraction parameters with minimum degradation of the phenolic compounds, resulting in an extract with high antioxidant activity using clean technology with minor environmental impact, less toxic raw materials and low cost (Santos; Veggi; Meireles, 2010).

The ultrasound method used for the extraction of antioxidant compounds facilitates the liberation of the extractable compounds. It occurs due to the increase in solvent mass transfer from the continuous phase into the

plant cells where the bioactive compounds are present, mainly in the initial minutes of the extraction (Zhang et al., 2009).

Furthermore, the ultrasound method shortens the time of extraction, decreases the solvent consumed, heightens the productivity and improves the extract quality (Wang; Weller, 2006).

Under natural conditions, plants produce bioactive compounds as secondary metabolites. The biosynthesis of bioactives may be influenced by micronutrients, such as K, Ca, Mn, Mg, Cu, Zn and Co (Treutter, 2010). Some of these act as enzyme cofactors that regulate the biosynthesis pathway of phenolic compounds.

Micronutrients are important in the biosynthesis of other metabolites. In leaves of *Eugenia uniflora* and *Myrciaria cauliflora*, flavonoids, total phenols, and hydrolysable tannins correlated strongly with Cu and Mn (Duarte et al., 2010).

Cu and Mn are cofactors for peroxidases and polyphenol oxidases, enzymes involved in lignin biosynthesis (Lin; Chen; Lui, 2005). The equilibrium between lignification and the production of phenolic compounds, flavonoids and tannins in the leaves depends on the supply of these two metals.

In jaboticaba fruit, this tendency was not so evident; it was only observed that nutrient-poor soils showed higher levels of all phenolic compounds (Duarte et al., 2012).

The relation between edaphic factors and chemical composition of the jaboticaba peel, as well as an effective method for the extraction of its phenolic compounds, has been poorly explored. Thus, the aim of this study is to optimize the extraction process of the phenolic compounds of the jaboticaba peel as a function of the stirring time and solvent volume of ethanol/hydrochloric acid (EtOH/HCl) and to apply the best response to study the edaphic effect on the composition of physicochemical properties of jaboticaba peel.

MATERIAL AND METHODS

Plant material

Jaboticaba fruits (*Myrciaria cauliflora*) were collected from the Jaboticabal farm (Hidrolândia, Goiás). Ripe fruits were harvested from five orchards containing different characteristics of soils (named S1 to S5, as shown in Table 1). Five jaboticaba trees were selected from each orchard, and 2 kg of ripe fruits was harvested. The total quantity of fruit collected was 10 kg. Two kilograms of all fruits harvested from each orchard was separated, obtaining five samples (one sample per orchard).

Table 1: Characteristics of soils from different orchards selected to collect samples of jaboticaba fruits.

Soil type (ST)	Characteristics of soils
S ₁	Sandy/clayey (white sand).
S ₂	Sandy (gravel), low nutrient.
S ₃	Clayey (with organic fertilization), greater balance of nutrients.
S ₄	Sandy/clayey (gravel).
S ₅	Sandy/clayey, low nutrient.

Freeze-dried jaboticaba peel sample preparation

The fruits in each sample per orchard were washed for three minutes in water containing 200 ppm of active chlorine, followed by manual crushing of the fruits for the separation of the peel. Part of the fresh peel of each sample was mixed together. Then, 150 g of mix was packed in polyethylene bags, frozen at -20 °C for 24 h. The frozen peel was homogenized with 50 mL of distilled water using a portable blender, subsequently freeze-dried in a Thermo Heto Power Dry LL3000 lyophilizer for 48 h at -50 °C and low pressure (approximately 10⁻¹ atm). The lyophilized product was called freeze-dried jaboticaba peel (FJP).

Experimental part

The experiment was divided into two stages. During the first stage, optimization of the extraction process of phenolic compounds from FJP by response surface methodology (RSM) was studied. In the second stage, improved parameters of optimization were applied to the extraction of the phenolic compounds of each orchard sample. Herein, the effect of the soil type on the phenolic compound quantity was evaluated.

Extraction of phenolic compounds optimization

To minimize the stirring time and solvent volume used in the process of phenolic compounds extraction, in a randomized experimental design, a central composite design (CCD) was used (Table 2). The stirring time (θ) ranged from 9 min to 51 min. The solvent volume (V) was a binary solvent mixture of ethanol and hydrochloric acid (EtOH/HCl, 9:1 v/v) and ranged from 4 mL to 16 mL (Table 2).

To extract a large quantity of phenolic compounds from FJP and obtain the best color parameter, the θ and V values were applied to the CCD and then extracted three more times together with extraction volumes, each

being half the time and the same amount of volume initially applied. The total process quantities of extraction parameters are shown in Table 3.

Table 2: Central composite design 2² with coded levels and corresponding absolute levels: Stirring time (θ) and solvent volume (V).

Factor	Coded levels and corresponding absolute levels				
	-1,414	-1	0	+1	+1,414
θ (min)	9	15	30	45	51
V (mL)	4	6	10	14	16

Extracts from each experimental assay were prepared by ultrasonic-assisted stirring, UAS (Maxiclean, Merse 750), according to Escarpa and González (2001). Extraction was performed in test tubes to which 1 g of FJP was added and one volume of EtOH/HCl and stirred for a time set previously. After each stirring, the extracts were centrifuged, and the liquid was separated by decantation. Extracts from each assay were transferred to 25 mL volumetric flasks. When the extract volume was less than 25 mL, it was filled with EtOH/HCl. In contrast, the extract was concentrated with a rotary evaporator until filling the 25 mL volumetric flask.

Soil effect on the phenolic compounds of FJP

The remains of fresh peel samples from each orchard were used to study the soil (edaphic) effect on the phenolic compounds of FJP. Extracts of the five samples were obtained by UAS. The θ and V parameters were determined by applying the procedure of simultaneous optimization responses of the optimization algorithm (described in the statistical analysis) on the results of the first stage.

Total phenols (Tph), tannins (Ta), total monomeric anthocyanin (TMA), color index (CI) and tone (Ton) were analyzed from the extracts of FJP obtained from the two experimental stages.

Physico-chemical analysis

Total phenolic content

The amount of total phenolics was determined using Folin–Ciocalteu reagent (Escarpa; González, 2001). Initially, the extract was diluted in distilled water (1:4 v/v). Then, 0.3 mL of the diluted sample was transferred into a 25 mL volumetric flask containing 0.5 mL Folin–Ciocalteu reagent (Sigma-Aldrich). After 1 min, 4 mL of 20% (w/v)

Na_2CO_3 was added and brought to volume with distilled water. The contents were mixed and allowed to rest for 30 min. The absorbance at 750 nm was measured in a UV – Vis spectrophotometer (Perkin Elmer, Lambda 35 UV/Vis). The amount of total phenolics was calculated as tannic acid equivalent (TAE) in mg per g of fresh weight (FJP).

Total tannin content

Tannins were determined as described by Waterman and Mole (1994). Extract samples of 1.0 mL were evaporated up to half of their volume and then diluted with 0.5 mL distilled water. Next, 2.0 mL serum bovine albumin solution (1.0% w/v) was added. This solution was stirred and kept at rest for 15 min, followed by centrifugation (Centribio, 80-2B) for 15 min at 4000 rpm. The supernatant was discarded, and the precipitate dissolved with 4.0 mL SDS-triethanolamine-isopropanol solution plus 1.0 mL the 20% ferric chloride reagent. After 15 min, the absorbance was measured at 510 nm on the spectrometer. The blank was prepared with SDS-triethanolamine-isopropanol solution and 20% ferric chloride solution. The amount of tannins was calculated as tannic acid equivalent (TAE) in mg per g of FJP.

Total monomeric anthocyanins

Total monomeric anthocyanins (TMA) were determined according to the differential pH method described by Reynertson et al. (2008). An extract volume of 0.5 mL was transferred to two 25 mL volumetric flasks. The first flask was filled with buffer pH 1.0, and the second

flask was filled with buffer pH 4.5. The flasks were kept at rest 15 min under the light, and the solutions were read on the spectrometer. The range of the absorbance was between 520-700 nm for both solutions. TMA was expressed as cyanidin-3-glucose in mg g^{-1} and calculated according to Equation 1:

$$A = (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH}1.0} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH}4.5} \quad (1)$$

where A is the absorbance, and $A_{520\text{nm}}$ and $A_{700\text{nm}}$ are absorbances for both buffers pH 1.0 and 4.5 measured at 520 and 720 nm, respectively. MM is equal to 449 g mol^{-1} (molar mass of cyanidin-3-glucose), and DF is the dilution factor, equal to $26.900 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $b = 1$.

Color and tone index

The color and tone index were determined using the methodology described by Somers and Evans (1977). An extract volume of 0.5 mL was transferred to 10 mL volumetric flasks. The flask was filled with distilled water, then stirred and kept at rest for 15 min in the dark. The solutions were read on the spectrometer. The absorbance was measured from 420 nm to 620 nm every 100 nm using distilled water as reference in a cuvette with the same optical thickness, in order to establish the baseline or the water line.

The color (CI) and tone (Ton) were calculated by Equations 2 and 3, respectively:

$$CI = A_{420} + A_{520} + A_{620} \quad (2)$$

Table 3: Total phenolic (Tph in mg g^{-1}), tannin (Ta in mg g^{-1}), total monomeric anthocyanin (TMA in mg g^{-1}), color index (CI) and tone (Ton) from freeze-dried jaboticaba peel.

Assay	θ_t (min) ¹	V_t (mL) ²	Tph	Ta	TMA	CI	Ton
1	37.5	24	17.1 ± 0.2	4.32 ± 0.03	3.15 ± 0.03	0.411 ± 0.01	0.808 ± 0.01
2	37.5	56	20.3 ± 0.2	4.67 ± 0.05	3.45 ± 0.09	0.461 ± 0.01	0.833 ± 0.01
3	112.5	24	31.4 ± 0.1	6.06 ± 0.04	5.15 ± 0.03	0.920 ± 0.01	1.133 ± 0.01
4	112.5	56	29.6 ± 0.2	5.26 ± 0.18	4.69 ± 0.03	0.999 ± 0.01	0.901 ± 0.01
5	22.5	40	15.9 ± 0.2	3.42 ± 0.02	2.33 ± 0.03	0.257 ± 0.01	0.760 ± 0.02
6	127.5	40	27.5 ± 0.2	4.96 ± 0.04	4.10 ± 0.41	1.125 ± 0.02	0.982 ± 0.01
7	75.0	16	24.3 ± 0.1	5.33 ± 0.01	4.25 ± 0.15	0.633 ± 0.03	0.961 ± 0.01
8	75.0	64	33.5 ± 0.05	7.91 ± 0.08	5.57 ± 0.03	0.893 ± 0.01	0.833 ± 0.01
9	75.0	40	27.2 ± 0.28	6.64 ± 0.09	5.13 ± 0.06	0.879 ± 0.01	1.035 ± 0.02

Values are means ± SD; n = 3 samples. ¹Total stirring time. ²Total solution volume.

$$Ton = A_{420} / A_{520} \quad (3)$$

Statistical analysis

The statistical treatment of the first experimental data consisted in fitting a polynomial function to the set of experimental data collected from a full-factorial 2^2 central composite design. Multiple regression analysis was used to fit Equation 4 to the experimental data by means of the least squares method:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i<j}^k \beta_{ij} X_i X_j + \varepsilon \quad (4)$$

where Y represents the predicted response, β_0 is the model intercept, β_i , β_{ii} and β_{ij} are linear and interaction coefficients, respectively, and X is the independent variable. ε corresponds to the model residue.

The statistical significance of the model was determined by evaluating the p-value, F-value and lack of fit with a 95% confidence level obtained from the analysis of variance (ANOVA). The extent of fitting of the experimental results to the polynomial model equation was expressed by the adjusted coefficient of determination ($R_{adj.}^2$). The response surface was obtained by using the fitted model and keeping one independent variable constant and varying the other two variables.

Based on the model (Equation 4), the best combination between the levels of stirring time and solvent volume that achieved the desired objectives (Table 1) was fitted to each variable, using the procedure of simultaneous optimization of responses proposed by Derringer and Suich (1980). In this procedure, the desirability function is represented by D, obtained by the geometric mean of the estimates d_j ($j=1, \dots, p$), in which p is the total number of variables.

The data from the second experiment were subjected to analysis of variance to test the significant influence of type of soil (edaphic effect) on the phenolic compounds of FJP. When significant, the average values were compared using Tukey's test at 5%. Calculated average values from phenolic compounds of the edaphic effect examination and the results of the desirability function were compared for paired samples by t-tests. All calculations and graphs were obtained using Statistica software.

RESULTS AND DISCUSSION

Extraction of phenolic compounds

Total phenolic, tannin and monomeric anthocyanin contents

The first set of experimental data is summarized in Table 3, where different types of data are presented. Tph, Ta and TMA values varied from 15.9 to 33.5 mg g⁻¹, 3.42 to 7.91 mg g⁻¹ and 2.33 to 5.57 mg g⁻¹, respectively, indicating that the amount of phenolic compounds that can be extracted from the FJP depends on the solvent volume and the ultrasound-assisted stirring time.

The most significant changes were shown using analysis of variance (Table 4). The high variability of the experimental data was due to the stirring time effects. However, this factor depends on the solvent volume and vice-versa, such as shown in Table 4. It shows an interaction among θt and Vt with Tph, Ta, TMA and Ton with $R_{adj.}^2 > 0.90$ for Tph, TMA, CI and Ton, but $R_{adj.}^2 = 0.786$ for Ta, indicating that the model for physico-chemical analysis showed a good fit with the experimental data.

A three-dimensional surface was generated based on Equation 4 and the regression coefficient from Table 4. Figure 1 shows these surfaces where clearly shows that the response values increased when levels of the solvent volume and stirring time were higher. The maximum limit of the experimental extraction was achieved at 64 mL of solvent volume with 75 min of stirring time (Table 3). In this experimental assay, Tph, Ta, and TMA were 33.5, 7.91, and 5.57 mg g⁻¹, respectively.

The reduction in the Tph, Ta and TMA values with times greater than 75 min may be due to the action of the polyphenol oxidase activity that degrades the phenolic compounds (Esmaili; Ebrahimzadeh; Abdi, 2017) and the monomeric anthocyanin polymerization, causing a decrease in their concentration (Malacrida; Motta, 2005).

Comparing the results of the physicochemical analyses of jaboticaba peel extracts obtained in the present study with other studies, the binary solvent mixture EtOH/HCl used for each stirring time was very efficient during the extraction process of polyphenolic compounds.

Methanol has been reported as the most efficient solvent for extracting anthocyanins (Kapasakalidis; Rastall; Gordon, 2006). In fact, this solvent may be 20% more effective than ethanol in extracting anthocyanins from grape marc (Castañeda-Ovando et al., 2009); however, food industries prefer ethanol because of the higher toxicity of methanol.

Table 4: Analysis of variance results of the first experimental stage.

Factor ^a	Tph ^b		Ta		TMA		CI		Ton	
	F-test ^c	RC	F-test	RC	F-test	RC	F-test	RC	F-test	RC
Int.	-	-1.199	-	-2.60	-	-2.21	-	-0.58	-	-6.9 10 ⁻³
θ_t	179.5**	0.529	21.4**	0.17	130.5**	0.13	844.5**	0.02	503.97**	0.02
θ_t^2	20.9**	-0.002	39.8**	-9.4 10 ⁻⁴	80.3**	-6.9 10 ⁻⁴	38.1**	-7.4 10 ⁻⁵	131.24**	-5.5 10 ⁻⁵
V_t	24.3**	0.059	11.6**	0.08	12.3**	0.06	41.4**	0.02	150.92**	0.02
V_t^2	1.4 ^{n.s.}	0.003	0.1 ^{n.s.}	-2.3 10 ⁻⁴	0.9 ^{n.s.}	-3.5 10 ⁻⁴	15.0**	-2.2 10 ⁻⁴	91.18**	-2.2 10 ⁻⁴
$\theta_t V_t$	5.6*	-0.002	2.8*	-4.8 10 ⁻⁴	4.6*	-3.2 10 ⁻⁴	0.3 ^{n.s.}	1.2 10 ⁻⁵	133.06**	-1.1 10 ⁻⁴
R_{adj}^2	0.908		0.786		0.910		0.973		0.978	

^a θ_t , total stirring time; V_t , total solution volume; R_{adj}^2 , adjusted coefficient of determination; ^b Tph, total phenolic; Ta, tannin; TMA, total monomeric anthocyanin; CI, color index; Ton, tone; ^c F-test; RC, regression coefficient; *P<0.05; **P<0.01; ^{n.s.} Non-significant.

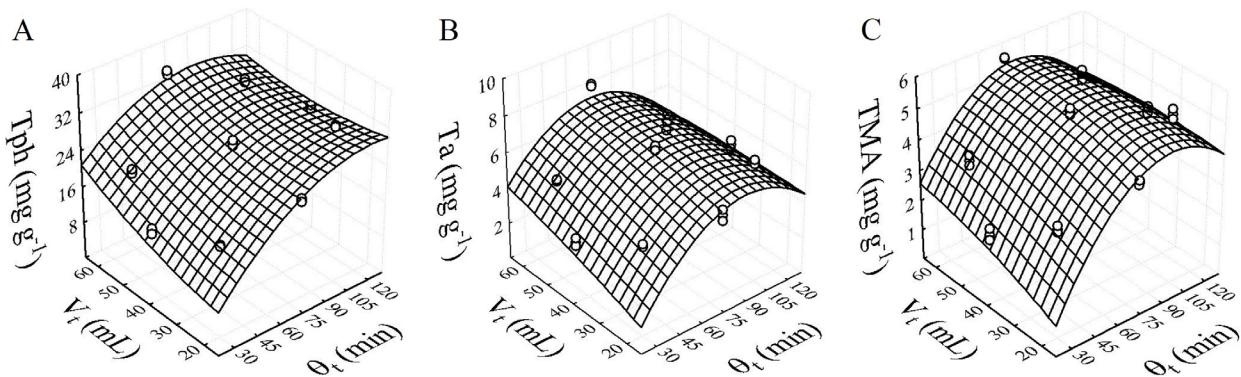


Figure 1: Response surfaces for results of the physical-chemical analysis of freeze-dried jaboticaba peel extracts obtained as a function of the total stirring time (θ_t) and total solvent volume (V_t) of the binary solvent ethanol and hydrochloric acid in proportion 9:1 (v/v). A) Total phenols (Tph), B) tannins (Ta), and C) total monomeric anthocyanins (TMA).

The binary solvent mixture EtOH/HCl is more efficient than ethanol, since the Tph and TMA values of assay 8 were higher than those obtained by Santos et al. (2010), who extracted Tph=26 mg g⁻¹ and TMA=4.7 mg g⁻¹ in jaboticaba peels using ethanol and two hours of ultrasonic stirring.

Fortes et al. (2011) obtained 12.5 mg g⁻¹ TMA from Jaboticaba peel when 40 mL solvent and 75 min of stirring time were used. However, they report a Tph and Ta of 32.9 and 7.21 mg g⁻¹, respectively, lower than the results obtained using the eight assays in the present study. Herein, it should be noted that these authors used a mixture of the toxic solvents methanol/formic acid (9:1).

Color index and tone

The color index and tone values were 0.893 μ a. and 0.833, respectively, obtained when the FJP was

treated with 64 mL solvent volume and 75 min stirring time (Table 3).

Although there were interactions of factors $\theta_t \times V_t$ for Ton, but not for color index (Table 4), these color properties are closely related to the concentrations of phenolic compounds contained in the extracts. Table 3 shows that the color intensity varied from 0.257 to 1.125 u.a., and the Ton, from 0.760 to 1.133. The minimum values occurred in assay 5, while the maximum values were detected in different assays.

Extraction process optimization

An important aspect in the preparation of plant extracts is to obtain the maximum concentration of its constituents with less degradation. In this sense, the desirable function was applied aiming to establish the

best conditions of stirring time and solvent volume to obtain FJP extracts with more Tph, Ta and TMA and, consequently, a higher color index and tone. Figure 2 shows the conditions of the desirable function applied for each dependent variable and the results of the global desirable and predicted values.

The vertical dashed lines in Figure 2 signal maximum conditions overall. Desirability reached 0.731 (it can be seen in the last chart line on the left), corresponding to a time and solvent volume equal to 75 min and 40 mL, respectively. Under these conditions, the simulated extract shows 27.2, 6.6 and 5.1 mg g⁻¹ Tph, Ta and TMA, with color and tone indexes of 0.9 μ a. and 1.0, respectively.

By applying the optimization parameters (75 min and 40 mL solvent volume) in the extraction of the FJP phenolic compounds from each of the samples of the studied soils, it was observed that the results (Table 5) did not

differ from the respective values calculated in optimization according to the t-test (Table 6).

These comparative results indicate that the extraction process optimization was successful. Phenolic compounds of 1.0 g dry and FJP planted in any type of soil studied in the present study can be extracted with a 40 mL solution of EtOH/HCl (9: 1, v/v) while stirring for 75 min, yielding good Tph, T, TMA results of 27.24 \pm 2.11; 5.83 \pm 0.13 and 4.61 \pm 0.71 g g⁻¹, respectively, and color and tone indexes of 0.9 \pm 0.01 u.a. and 0.73 \pm 0.30, respectively.

Soil effect on the phenolic compounds

Soil type had an effect on the content of phenolic compounds, with significant differences among them according to the ANOVA (Table 6). The extracts obtained from the jaboticaba peels of the soils S2 and S5 presented the highest levels of Tph, T, TMA and CI and the lowest

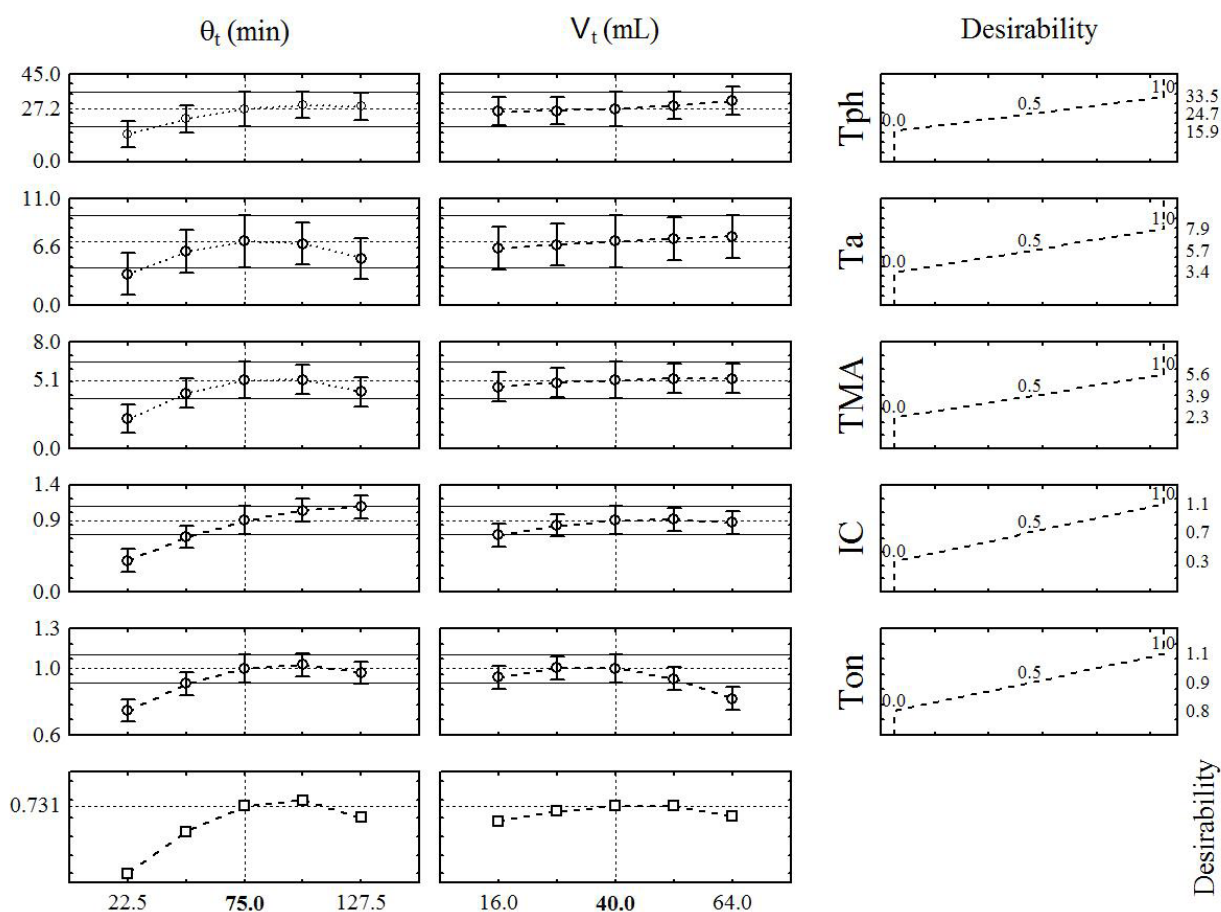


Figure 2: Optimization of Desirability function values of the phenolic compounds obtained as a function of the total stirring time (θ_t) and total solvent volume (V_t) for the freeze-dried jaboticaba peel extracts.

T content, while soil S3 presented the lowest levels of these phenolic compounds. The differences in results among these soil types are related to the availability of light and nutrients (Kraus; Zasoski; Dahlgren, 2004). Jorge et al. (2017) reported that a higher nitrogen content is associated with an increase in the antioxidant capacity and phenolic compounds. Ingersoll et al. (2010) reported a positive correlation between phenolic compounds and light availability.

This tendency was observed in the jaboticaba peel of S3, which presented low levels of phenolic compounds in the soil fertilized with low solar luminosity, whereas

the soils with a low balance of nutrients presented the highest levels of phenolic compounds. These results corroborate those found by Duarte et al. (2010) in a study of jaboticaba fruit coming from the same species and soils.

Due to the high phenolic content found in jaboticaba peel, it is suggested that it can be used as a potential source of natural pigments for food, pharmaceutical and chemical industries. Therefore, it is necessary to look for alternatives for the use of this fruit in order to make use of its antioxidant properties, as well as to increase the useful life of the fruit.

Table 5: Responses obtained from maximum desirability for total phenolic (Tph in mg g⁻¹), tannin (Ta in mg g⁻¹), total monomeric anthocyanin (TMA in mg g⁻¹), color index (CI) and tone (Ton) from freeze-dried jaboticaba peel.

Soil	Tph	Ta	TMA	CI	Ton
S ₁	26.28 ± 0.15d	5.33 ± 0.03d	4.25 ± 0.02c	0.81 ± 0.07d	0.93 ± 0.09b
S ₂	28.49 ± 0.20b	6.63 ± 0.08b	5.13 ± 0.03b	0.95 ± 0.15b	0.55 ± 0.12d
S ₃	24.28 ± 0.17e	4.27 ± 0.06e	3.84 ± 0.03d	0.63 ± 0.09e	1.07 ± 0.06a
S ₄	27.34 ± 0.06c	5.75 ± 0.06c	4.28 ± 0.02c	0.91 ± 0.22c	0.75 ± 0.03c
S ₅	29.81 ± 0.05a	7.17 ± 0.08a	5.57 ± 0.01a	1.20 ± 0.01a	0.33 ± 0.02e

Values are means ± SD; n = 3 samples. Means in columns without letters in common differ significantly (P<0.05).

Table 6: Summary of the analysis of variance applied to the total phenolic (Tph in mg g⁻¹), tannin (Ta in mg g⁻¹), total monomeric anthocyanin (TMA in mg g⁻¹), color index (CI) and tone (Ton) from freeze-dried jaboticaba peel, according to the type of soil in the Jaboticaba orchard.

	Tph	Ta	TMA	CI	Ton
Mean Square	13.4	3.8	1.5	0.13	0.26
F-test	288.0**	191.1**	54.1**	4.70*	56.30**
Means ± SD	27.2 ± 2.1	5.8 ± 1.1	4.6 ± 0.7	0.9 ± 0.2	0.7 ± 0.3
Reference data	27.2	6.6	5.1	0.9	1.0
t-test	0.042 ^{ns}	-1.521 ^{ns}	-1.528 ^{ns}	0.000 ^{ns}	-2.076 ^{ns}

*P<0.05); **P<0.01; ^{ns} Non-significant.

CONCLUSIONS

The interaction between the parameters of stirring time and solvent volume showed that the binary solvent mixture ethanol and hydrochloric acid 9/1 (v/v) was the most effective in extracting phenolic compounds from freeze-dried jaboticaba peel. A maximum quantity of phenolic compound was achieved with 64 mL and 75 min extraction, presenting the highest values of total phenols (33.5 mg g⁻¹), tannins (7.9 mg g⁻¹) and total monomeric anthocyanins (5.57 mg g⁻¹), with a color index and tone of 0.893 μ a. and 0.833, respectively. The desirable function calculated a stirring time of 75 min and a solvent volume of 40 ml. Estimated values of phenolic compounds were 27.2 mg g⁻¹, 6.6 mg g⁻¹, 5.1 mg g⁻¹, 0.9 μ a., and 1.0 for total phenols, tannins, total monomeric anthocyanins, color index, and tone, respectively. T-tests showed non-significant differences between estimated values and experimental data of phenolic compounds obtained with a stirring time of 75 min and solvent volume of 40 ml. According to the kind of soil, a different content of phenolic compounds was observed in freeze-dried jaboticaba peel extracts. In nutrient-poor soils, the highest levels of phenolic compounds, a high color index and a low tone were found.

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