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Optimization of antioxidant compounds extraction from feijoa (*Acca sellowiana berg*) residues

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Abstract

Feijoa residues has a high content of bioactive compounds with effective antioxidant activity compared with the content of other fruits. However, these wastes are disposed of without generating added value. Therefore, in order to determine the maximum time to extract the polyphenols (TPC) and flavonoids (TFC) present in the residue, solid-liquid extraction kinetics was performed. Next, in order to find the best conditions for maximizing the simultaneous extraction of TPC and TFC, a central composite design and response surface analysis were proposed. Three independent factors, temperature (ranging from 13.18 to 46.82 °C), solid:liquid ratio (ranging from 1:10 to 1:74 g:mL) and ethanol-water mixture (ranging from 16,36 to 83,64%v/v) were evaluated. The kinetics indicated a maximum extraction time of 90 minutes for TPC with 3,990.02 ± 22.403 mgGAE/100 g FW, and 45 minutes for TFC with 1,219.38 ± 19.24 mgCE/100 g FW. These values are within the range reported by other studies using advanced technology. The optimized extraction conditions were a temperature of 34.5 °C, 1:45 solid-liquid ratio and 39.43% for the solvent. Finally, extract from feijoa residues presented an effective antioxidant activity greater than 50%. Therefore, this residue can be used as a promising source of bioactive compounds with various agro-industrial applications.

Keywords: feijoa; extraction; polyphenols; flavonoids; antioxidant.

Practical Application: Feijoa residues can be used to produce extracts with a high content of bioactive compounds with effective antioxidant activity.

1 Introduction

Feijoa is a native fruit of South America that is grown in Brazil, Paraguay, Colombia, as well as New Zealand, and the United States due to its attractive organoleptic attributes (Sánchez-Riaño et al., 2020; Zhu, 2018). During the commercialization and processing of this fruit in supply centers and processing industries a large amounts of residue is generated. The main residue generated is the peel of feijoa because it represents around 50% of the fruit's weight (Santos et al., 2019; Tuncel & Yılmaz, 2015). Feijoa peel has a high content of bioactive compounds with effective antioxidant activity, such as polyphenols, flavonoids, and vitamins compared with other tropical fruits (Amarante et al., 2017; Sun-Waterhouse et al., 2013; Weston, 2010). This residue could be used to obtain bioactive compounds of interest for food, pharmaceutic, and nutraceutical industries (Liakou et al., 2018; Pasquariello et al., 2015; Sánchez-Riaño et al., 2020). These compounds include polyphenols such as ellagic, ferulic, gallic and caffeic acid derivatives, and flavonoids, for instance, isoquercetin, catechin, and rutin, which have been proposed to have antioxidant, anticancer and anti-inflammatory properties (Almeida et al., 2020; Peng et al., 2019; Schmidt et al., 2020). In order to obtain bioactive compounds, different traditional extraction techniques are used, such as Soxhlet extraction technology, which presents difficulties associated with long extraction time and high solvent and energy consumption (Ameer et al., 2017; Azmir et al., 2013; Vallejo-Castillo et al., 2020a). Conversely, other extraction techniques can reduce time and solvent consumption, such as ultrasonic, microwave, or supercritical fluid-assisted extraction (Aliakbarian et al., 2012; Chan et al., 2011; Vallejo-Castillo et al., 2020b), however, assisted extractions have the disadvantages of high initial investment and the difficulty of scaling up (Chemat et al., 2017; Watson, 2014). Orbital shaking extractions with green solvents such as water and ethanol, have been used to obtain bioactive compounds from different vegetables residues to achieve high levels of polyphenols and flavonoids with effective antioxidant activity. In addition, this type of extraction is cost-effective, scalable, and economically feasible (Castillo-Santos et al., 2017; Ilaiyaraja et al., 2015; Ochoa-Velasco et al., 2019). The techniques used to extract these compounds are affected by parameters related to solvent polarity, temperature, time, and solid-solvent ratio. Consequently, optimization strategies are used in order to reduce time, energy consumption, and increase the recovery of bioactive compounds with high antioxidant activity. The most used methodologies for this optimization are the experimental design of a response surface and a multiple response optimization (Bezerra et al., 2019; Gomes et al., 2013; Rajha et al., 2014).

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Therefore, the main objective of this work was to determine the optimum extraction conditions to obtain an extract with high content of bioactive compounds and effective antioxidant activity from feijoa residues. In this respect, the maximum extraction time of total polyphenol and flavonoid content with effective antioxidant activity was initially determined by means of solid-liquid extraction kinetics. Subsequently, a central composite design was carried out to maximize total polyphenol and flavonoid content using a response surface and a multiple response optimization. Finally, the effective antioxidant activity of the feijoa residue extract was determined.

2 Materials and methods

2.1 Chemical reagents

Folin-Ciocalteu reagent, sodium nitrite, sodium carbonate, DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), standard catechin, and gallic acid were purchased from Sigma-Aldrich (St. Louis, USA). Aluminum chloride, sodium hydroxide, ethanol, and methanol were obtained from Merck (Darmstad, Germany). All chemical reagents and solvents were analytical grade.

2.2 Raw material preparation

About 10 kg of feijoa epicarp was obtained from both a local open market and feijoa canning processing companies. First, the epicarp was subsequently washed with water twice and rinsed with deionized water in order to remove dirt (Maisarah et al., 2013). Then, it was disinfected with 200 ppm sodium hypochlorite for five minutes to remove the initial microbial load (Sánchez-Riaño et al., 2020; Santos et al., 2014). Next, the raw material was cut into approximately 2 cm squares, which were dehydrated in a tray dryer at 50°C for approximately 24 hours until reaching a 10% constant moisture content (% wet basis) (Santos et al., 2019). Finally, dehydrated samples were subjected to size reduction using a laboratory mill (IKA A11 basic, Germany) and the particle size was adjusted to 180 μm with a vertical sieve (Ro-Tap, WS Tyler, USA) and stored at 4 °C until needed.

2.3 Extraction kinetics of the total content of polyphenols and flavonoids with effective antioxidant activity

The analysis of the solid-liquid extraction kinetics was performed in an orbital shaker at 120 rpm (Talboys, 1000-3, USA) for 160 minutes at 30 °C. 80 mL of 50% (v/v) aqueous ethanol were mixed with a quantity of powdered feijoa epicarp to achieve a solid-to-liquid ratio of 1:40 (g:mL). These solvents are Generally Recognized as Safe (GRAS) (Santos et al., 2019; Vallejo-Castillo, 2020a). Sampling was carried out at specified time periods with the purpose of establishing the time in which the total polyphenol content (TPC) and total flavonoid content (TFC) reach a maximum. Collected samples were subjected to centrifugation (SIGMA, Laborzentrifugen 2-16P, Germany) at 10,000 g for 15 minutes, the resulting supernatant was stored at 4 °C and used to determine the total content of polyphenols, flavonoids, and the antioxidant activity.

2.4 Optimization of the extraction of the total content of polyphenols and flavonoids

A central composite design of a response surface was applied to determine the optimal conditions for the extraction of TPC and TFC, using the independent variables temperature (°C), solid:liquid ratio (g:mL), and solvent (ethanol-water % v/v) (Katsampa et al., 2015; Kazemi et al., 2016; Vallejo-Castillo et al., 2020a). The central points of the experimental design correspond to the values of the independent variables used in the extraction kinetics, as shown in Table 1. In the response surface experimental design, an adequate amount of powdered feijoa epicarp was mixed with 80 mL of aqueous ethanol of defined concentration in order to achieve the solid-to-liquid ratio (g:mL) shown in Table 1. This mixture was subjected to extraction on an orbital shaker under a controlled temperature (Table 1) for the time established in the extraction kinetics. The supernatant obtained in each experimental test was prepared as described above and used for further analysis. Responses (TPC, TFC) at each design point were estimated according to the methodology described in section 2.5.

The data obtained were subjected to analysis using the general equation (Equation 1) for the response surface quadratic model, including three different factors as follows:

$$Y_i = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{1,1} A^2 + \beta_{2,2} B^2 + \beta_{3,3} C^2 + \beta_{1,2} AB + \beta_{1,3} AC + \beta_{2,3} BC \quad (1)$$

In Equation 1, Y_i indicates the response variable; A, B and C display the independent variables including temperature, solid:liquid ratio, and solvent, respectively. β_0 is a constant coefficient that fixed the response at the central point of the experiment; β_1, β_2 and β_3 are the coefficients of main effects, $\beta_{1,1}$, $\beta_{2,2}$ and $\beta_{3,3}$ are the quadratic effects coefficients, and $\beta_{1,2}$, $\beta_{1,3}$ and $\beta_{2,3}$ are the interactions coefficients (Bouras et al., 2015; Kazemi et al., 2016; Xynos et al., 2014). To select the mathematical model that adequately describes the behavior of each response, the highest percentage of coefficient of determination (R^2) was considered. In order to find a combination of factors that maximize the response variables, the results were analyzed according to the multiple response optimization methodology, finding a set of operating conditions that optimize all responses, according to the use of the desirability functions. These functions are a method that assigns a score always between 0 and 1 to a set of responses variables

Table 1. Experimental design for the optimization of the total content of polyphenols and flavonoids.

Factor	Levels				
	-1.68	- 1	0	+1	+1.68
A= Temperature (°C)	13.18	20	30	40	46.82
B= Solid:liquid ratio (g:mL)	1:10	1:20	1:40	1:60	1:74
C= Solvent (% v/v)	16.36	30	50	70	83.64

and chooses factors that maximize that score (1 represents a completely desirable value) (Felix et al., 2018; Montgomery, 2003; Candioti et al., 2014). Finally, an experimental validation was carried out with the optimal conditions for each factor.

2.5 Analytical methods

Total polyphenol content (TPC).

The total polyphenol content of the different extracts was determined by using the Folin–Ciocalteu assay (Koşar et al., 2005; Singleton et al., 1999; Vallejo-Castillo et al., 2020a). A standard curve was obtained using gallic acid standard at concentrations between 20 and 1000 mg/L. Results were expressed as mg of gallic acid equivalents/100 g of fresh weight of each sample (mg GAE/100 g FW).

Total flavonoid content (TFC).

Total flavonoid content of the different extracts was determined by the aluminum chloride colorimetric method (Aliakbarian et al., 2012; Yang et al., 2009). A calibration curve made with catechin standards (15 to 1200 mg/L) was used. Results were presented as mg of catechin equivalents/100 g of fresh weight of each sample (mg CE/100 g FW).

Antioxidant activity.

A 50 µL sample was mixed with 1950 µL of a methanolic solution of DPPH radical and incubated at room temperature in the dark for 30 minutes. Next, the absorbance was measured at 517 nm in a spectrophotometer (Thermo Fisher Scientific Inc, Genesys 10 Bio UV-VIS, USA). Equation 2 was used to calculate the percentage of antioxidant activity (% AA):

$$\%AA = \frac{\left(A_{t0} - A_{tf}\right)}{A_{t0}} * 100 \quad (2)$$

Where, A_{t0} = Initial absorbance of the DPPH radical solution, and A_{tf} = Absorbance of the sample.

The standard solution was prepared with a Trolox standard. The results were expressed as a percentage of antioxidant activity (% AA) and µmol of Trolox equivalents (TE)/100 g of fresh weight of each sample (µmol TE/100 g FW) (Matsusaka & Kawabata, 2010; Singh et al., 2016). The results of the % AA were presented with an EC50 value, which is defined as the effective extract concentration that decreased a 50% the initial absorbance value of the DPPH radical solution. The EC50 was estimated from the extract obtained under optimal conditions, for which a non-linear regression with an four-parameter equation was used (Equation 3) (Chen et al., 2013; Sridhar & Charles, 2019).

$$Y = A_1 + \frac{A_2 - A_1}{1 + 10^{\left[\left(\log(EC_{50}) - \log(x)\right)p\right]}} \quad (3)$$

Where, Y = Percentage of antioxidant activity (% AA), A_1 = Minimum response (% AA), A_2 = Maximum response (% AA), p = Slope of the curve, and x = Total polyphenol content (mg GAE/100 g FW). The estimation of the parameters of the Equation 3 was performed with the Matlab® R2014a software (The Mathworks Inc., Natick, MA, USA).

2.6 Statistical analysis

An analysis of variance (ANOVA) and a Tukey´s multiple comparison test were used to find significant differences between each time during the extraction kinetics of total polyphenols and flavonoids, through the IBM SPSS Statistics® 21.0 software (SPSS, Inc., USA). The results of the response surface experimental design and multiple response optimization were analyzed with the Statgraphics 16.1.11 statistical package (StatPoint Technologies, Inc., USA). Pearson correlation coefficients were calculated to establish the correlation between the antioxidant activity and the contribution of total phenols and flavonoids to the antioxidant activity. All analyses were performed at a 95% confidence level and all assessments of response variables were carried out in triplicate, expressing the data as mean ± standard deviation.

3 Results and discussion

3.1 Extraction kinetics of the total content of polyphenols and flavonoids with effective antioxidant activity.

A high mass transfer rate was observed during the first 20 minutes of the extraction kinetics of the total polyphenols and flavonoids (Figures 1A and 1B). This behavior could be due to the fact that initially the driving force of the bioactive compounds concentration gradient between the solvent and the solid sample is high. As the extraction progresses, the mass transfer is reduced until the concentration difference between the bulk solvent and the sample is minimal (Silva et al., 2018; Oreopoulou et al., 2020). The statistical analysis of the extraction kinetics using the Tukey test showed that there were no statistically significant differences ($p < 0.05$) after 90 and 45 minutes of extraction of total polyphenols and flavonoids, respectively. The concentration of polyphenols after a 90-minute extraction was 3990.02 ± 22.403 mg GAE/100 g FW, while the concentration of flavonoids after 45 minutes was 1219.38 ± 19.24 mg CE/100 g FW (Figure 1). The values obtained for total polyphenols in the extraction kinetics are higher than those reported by Sánchez-Riaño et al. (2020) for feijoa epicarp. They reported a value of 1614.1 mg GAE/100 g FW through an extraction using orbital agitation and acetone as solvent. In addition, the polyphenol concentration values obtained in our study are within the range reported by Santos et al. (2019), who achieved total polyphenol values between 800, 1500 and 6000 mg GAE/100 g FW via supercritical fluid extraction, ultrasound assisted extraction and pressurized liquid extraction (PLE), respectively. Regarding the total flavonoid content, the value obtained by kinetics extraction was higher than 1146.7 mg QE/100 g FW, which was reported by Sánchez-Riaño et al. (2020). However, this value can only be used as a reference for a high content of total flavonoids because they used quercetin as standard reagent for the calibration curve and flavonoid assessment whereas catechin was employed in our study.

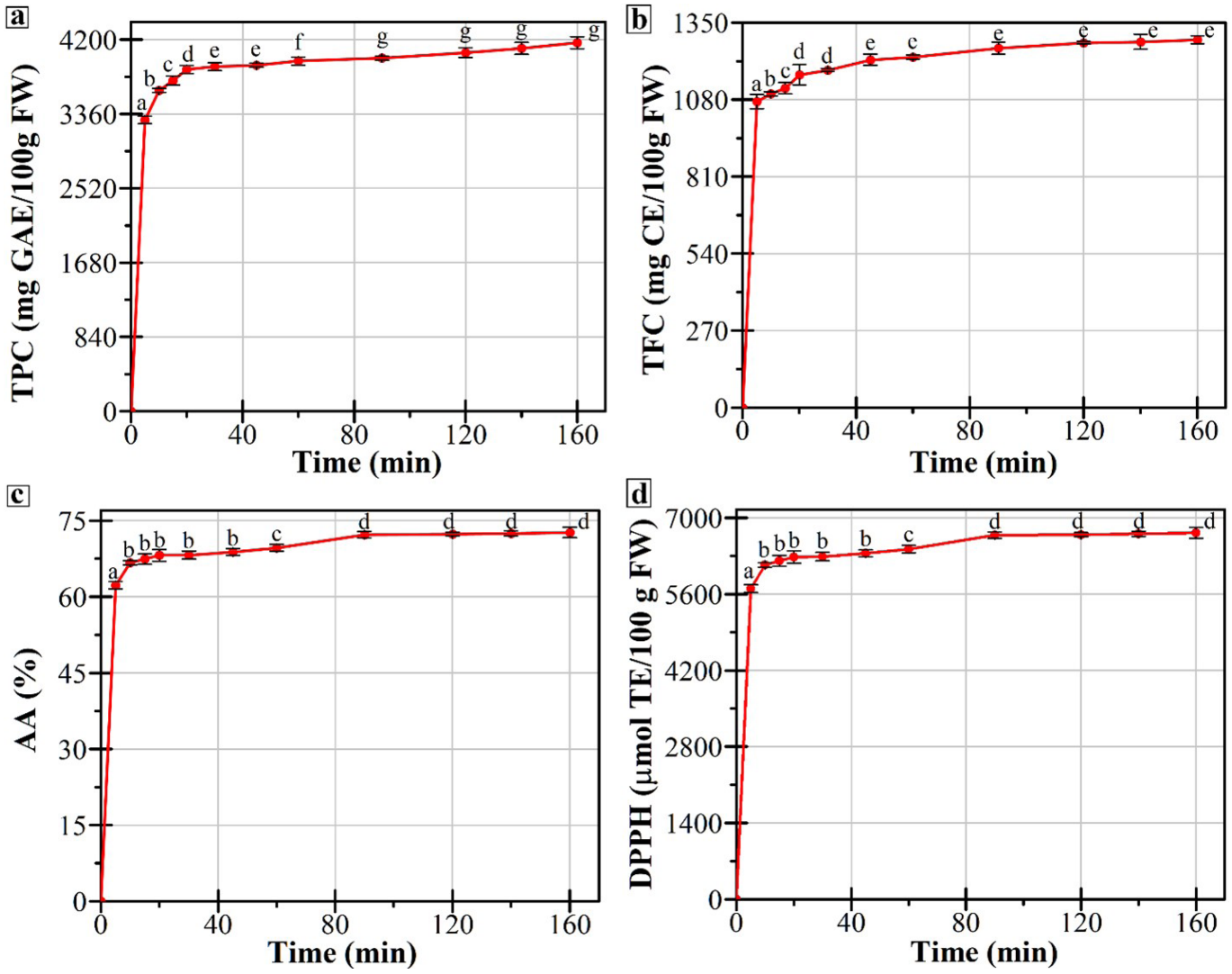

Figure 1. Extraction kinetics of feijoa residues: **a)** Total polyphenols; **b)** Total flavonoids; **c)** %AA; and **d)** DPPH (µmol TE/100 g FW). *Different letters indicate significant differences (Tukey, $p \leq 0.05$), (Values correspond to media ± standard deviation, n = 3).

Therefore, it is not possible to carry out a direct comparison because of the differences in molecular structure and weight between quercetin and catechin. It is also important to highlight that our TFC value was higher than that obtained by Pasquariello et al. (2015) (33.17 mg CE/100 g FW) for feijoa pulp, thus indicating a high flavonoid content in feijoa epicarp. The results for total polyphenols and flavonoids are higher to those reported in other studies possibly because the extraction methodology used in this study is less aggressive compared to other methods, which use high temperatures with non-green solvents such as methanol and acetone (Capello et al., 2007; Amarante et al., 2017; Nanda et al., 2021). Furthermore, the differences in polyphenol and flavonoid values between published reports may be due to factors associated with crops, varieties, harvest time, and ripeness state (Pasquariello et al., 2015; Zhu, 2018). The percentage of antioxidant activity and DPPH values (µmol TE/100 g FW) increase as the extraction progresses (Figure 1C and 1D). The % AA after 90 minutes of extraction was greater than 50%, which indicates that the extracts have effective antioxidant activity (Chen et al., 2013; Amarante et al., 2017; Sridhar & Charles, 2019). In terms of antioxidant activity expressed as Trolox equivalents, the value was 6680.34 ± 58.38 µmol TE/100 g FW. This antioxidant activity figure was higher than that obtained by Amarante et al. (2017) with feijoa epicarp. They reached antioxidant activity values of 1309 µmol TE/100 g FW for epicarp extracts obtained by conventional extraction using methanol and acetone as solvents. On the other hand, our value was lower than the activity observed in epicarp extracts obtained by PLE (values higher than 8000 µmol TE/100 g FW). However, the DPPH values were higher compared to other fruit residues, with activities from 510 µmol TE/100 g FW for passion fruit to 4710 µmol TE/100 g FW for mango (Martínez et al., 2012). As mentioned above, the difference between the antioxidant activity results obtained in this study and those presented by other authors may be due to various factors associated with the extraction methodology, and those related to the agricultural and physiological aspects of feijoa. Figure 2A shows the correlation between the total polyphenol content and antioxidant activity

(% AA, DPPH), displaying Pearson's correlation coefficient values higher than 0.9. Similar results have been obtained by Gayosso-García Sancho et al. (2010, 2011), Kelebek et al. (2015); Santos et al. (2019) in feijoa and other fruits. On the contrary, the correlation between total flavonoid content and antioxidant activity (% AA, DPPH) showed Pearson's correlation coefficient values were lower than 0.9 (Figure 2B). These results coincide with those published by Iqbal et al. (2012) y Singh et al. (2016) for different plants. Therefore, the high correlation between polyphenol content and antioxidant activity (R = 0.918, $p < 0.05$) demonstrates that polyphenols are the main bioactive compounds responsible for eliminating free radicals in feijoa extracts.

The contribution of total polyphenols to antioxidant activity is mainly due to the fact that these bioactive compounds neutralize free radicals and prevent the decomposition of hydroperoxide into free radicals (Santos et al., 2019; Vallejo-Castillo et al., 2020a; Watson, 2014). The extraction kinetics results for polyphenols, flavonoids, and antioxidant activity support the conclusion that a total extraction time of 90 minutes is sufficient to ensure the maximum extraction of polyphenols and flavonoids with an effective antioxidant activity. Therefore, the subsequent extraction optimization experimental assays were carried out using a total extraction time of 90 minutes.

3.2 Optimization of the extraction of the total content of polyphenols and flavonoids

Effect of extraction factors on the total polyphenol content.

The mathematical model of the response surface for the TPC (Equation 4) had a coefficient of determination (R^2) close to 1, a high F–value, and a *p*–value < 0.05 (Table 2), which shows that this model is adequate to describe the values obtained for the extraction of total polyphenols from feijoa epicarp. The *p*–value for lack of fit was not significant indicating that the response surface mathematical model can be used for optimization (Khajeh, 2011; Montgomery, 2003).

According to the Pareto diagram (Figure 3A), the solid:liquid ratio (B) and temperature (A) had a positive and direct effect on the TPC response variable ($p < 0.05$). The positive effect of the solid:liquid ratio may be due to the fact that a high amount of solvent increases the driving force of the concentration gradient

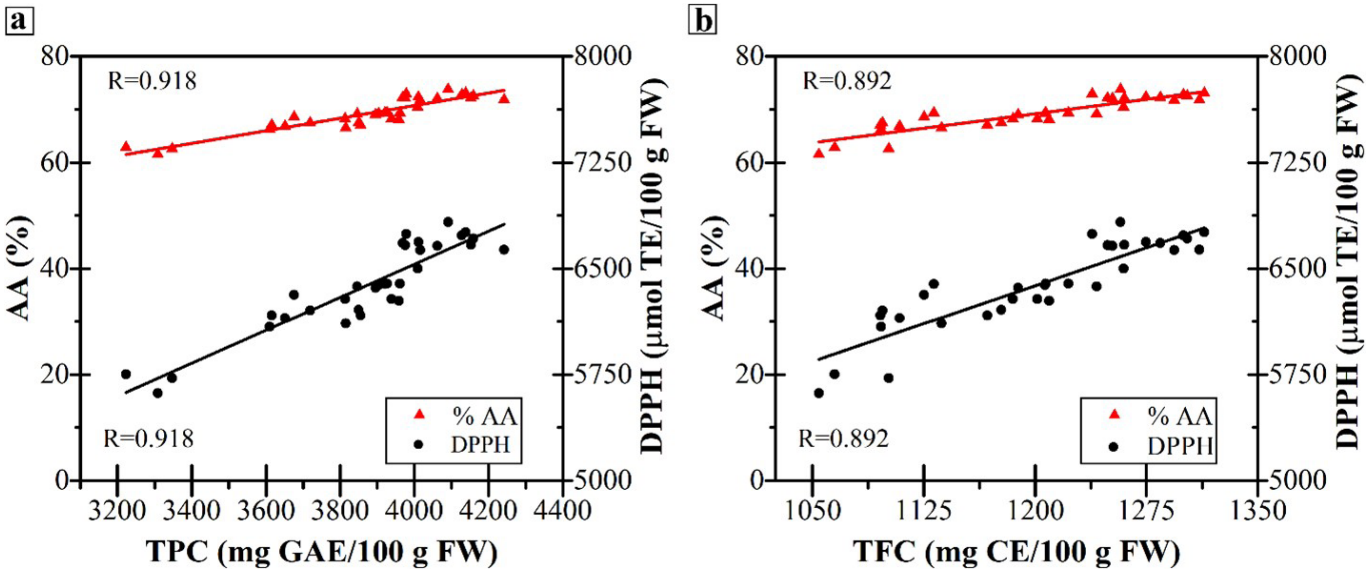

Figure 2. Correlation between antioxidant activity (% AA, DPPH) and: **a)** polyphenols, and **b)** flavonoids.

Table 2. Response surface mathematical models and experimental validation of the optimization of the total polyphenols and flavonoids extraction.

Response variables	F-value	p-value	R^2	Lack of fit (p-value)	Quadratic Equation[c]		Predicted value	Experimental validation[d]
TPC[a]	16.59	<0.0006	0.955	0.0533	$-8230.21+17.31A+13149.96B$ $+60.15C-0.59A^2-4452.33B^2-0.73C^2+20.54AB$ $-9.09*10^{-2}AC+9.60BC$	(4)	4050	4004.79 ± 28.22
TFC[b]	8.79	<0.0045	0.919	0.0575	$-3926.12+38.81A+5655.75B$ $+13.41C-0.39A^2-1674.26B^2$ $-4.22*10^{-2}C^2-6.95AB+$ $1.60*10^{-2}AC-7.89BC$	(5)	1311	1378.65 ± 12.78

[a] mg GAE/100 g FW. [b] mg CE/100 g FW. [c] Factors: A = Temperature (°C); B = Solid:liquid ratio (g:mL); C = Solvent (% v/v). [d] Mean ± standard deviation, n = 3.

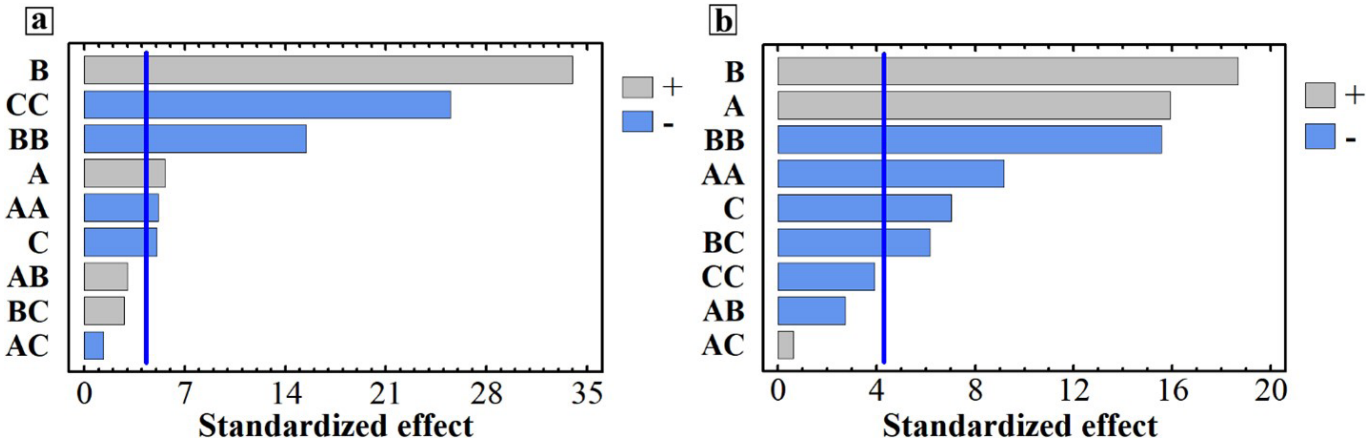

Figure 3. Pareto diagram for response variables: **a)** Total polyphenol content; and **b)** Total flavonoid content. Factors: A = Temperature (°C), B = Solid:liquid ratio (g:mL), and C = Solvent (% v/v). Bars exceeding the vertical line on the diagram indicate that the corresponding factor terms are statistically significant ($p < 0.05$).

between the polyphenols of the interior plant cell and the solvent, therefore, the solute diffuses quickly into the solvent leading to an enhanced mass transfer rate and increased extraction yield. On the other hand, a smaller amount of solvent decreases the driving force of the concentration gradient (Kislik, 2012; Oreopoulou et al., 2020; Pinelo et al., 2005). Nevertheless, excessive solvent will limit mass transfer and reduce the extraction rate, as evidenced by the negative influence of the quadratic effect (BB) on the response variable (Gao et al., 2021; Maran et al., 2017). The positive effect of temperature may be due to the fact that heating leads to a rupture of the cell wall, improving the diffusion of bioactive compounds from the solid matrix into the surrounding solvent (Castillo-Santos et al., 2017; Tuncel & Yılmaz, 2015). In addition, high temperature will increase the solubility of polyphenols in the solvent, thus increasing mass transfer. As the temperature increases, the bonds between phenolic compounds and other compounds (proteins, polysaccharides, etc.) could be broken, which would facilitate the process of diffusion of these compounds through the membranes of plant cells (Jovanović et al., 2017; Kazemi et al., 2016). However, increasing temperature above certain values may promoting possible simultaneous degradation of phenolic compounds which were previously mobilized at lower temperature or even the decomposition of residual phenolics remaining in the plant cell (Gomes et al., 2013; Mokrani & Madani, 2016). As evidenced by the negative influence of the quadratic effect (AA) on the response variable.

In contrast, the percentage of solvent (C) had a negative and inverse effect on the TPC variable response ($p < 0.05$) and this could be due to the fact that high concentrations of ethanol can cause protein denaturation, reducing the solubilization of bioactive compounds in the solvent. This is also evidenced by the negative influence of the quadratic effect (CC) on the response variable. Taking into account that soluble phenolic compounds and flavonoids are distributed in the cell vacuoles and walls, respectively, the use of water and low concentrations of ethanol would lead to the easy entry of the solvent into cells due to the high permeability of water through the pores of the plant material (Galvan D'Alessandro et al., 2012; Jovanović et al., 2021). In addition, the use of organic solvents mixed with water, contributes to the creation of a moderately polar solvent that enhance the extraction of polyphenols (Do et al., 2014; Mokrani & Madani, 2016; Vallejo-Castillo et al., 2020a). Regarding interactions between factors, it was observed that they did not show significance ($p < 0.05$) with respect to the TPC response variable. This result demonstrates that this response variable depends to a greater extent on the individual variation of each factor than on the interactions between factors. On the other hand, the significant effect of the quadratic factors A, B, and C confirms a non-linear relationship between these factors and the response variable. The response surfaces plot of TPC and TFC are shown in Figure 4A and 4B with solvent percentage constant at 39.43% (v/v) according to the optimization as presented below. Figure 4A shows that when increasing the solid:liquid ratio the value of the TPC response variable also increases regardless of the extraction temperature used, until reaching a maximum for this response variable. Nevertheless, high values of the solid:liquid ratio have a negative effect on the total polyphenol content, which is supported by the inversely proportional relationship of the quadratic effect of this factor on this response variable.

Effect of the extraction factors on the total flavonoid content.

According to the response surface optimization design, the experimental data of the total flavonoid extraction showed an appropriate fit to the proposed mathematical model (Equation 5) because the mathematical model had a R^2 coefficient close to 1, a high F–value, and a p–value < 0.05 (Table 2). These figures confirm that this quadratic model is adequate to describe the values obtained from the extraction of total flavonoids from feijoa epicarp. Based on the Pareto diagram (Figure 3B), the significant factors ($p < 0.05$) that define the behavior of the TFC response variable were, solid:liquid ratio (B), temperature (A), solvent (C), interaction between factors B-C, and quadratic effects of the factors A and B. The significant effects of factors A, B and C are similar to the behavior recorded for the TPC extraction.

The quadratic effects A and B show a non-linear relationship between each effect and the TFC response variable. As seen in Figure 4B, increments in both temperature and solid:liquid ratio lead to higher values of the TFC response variable until a maximum is reached, which indicates the effect of the interaction between factors on the curvature of the response surface. In order to reach optimal extraction conditions to simultaneously maximize the total content of polyphenols and flavonoids an optimization of multiple responses was performed. Optimal conditions corresponding to a value of 0.985 for desirability function were, temperature of 34.55 °C, 1:45 for solid:liquid ratio (g:mL), and 39.43% (v/v) of solvent. Figure 5A displays an overlaid contour plot, in which the yellow area represents the appropriate range of factors that leads to optimal response variables. Using optimal conditions for each factor, an experimental validation was conducted as shown in Table 2. The relative absolute error between the experimental and predicted values was lower than 6% and 2% for TPC and TFC, respectively. Therefore, the optimization of multiple responses for the extraction of total polyphenols and flavonoids was adequate and reproducible. Finally, the total content of polyphenols (mg GAE/100 g FW) corresponding to a 50% antioxidant activity (EC50) was determined with extracts

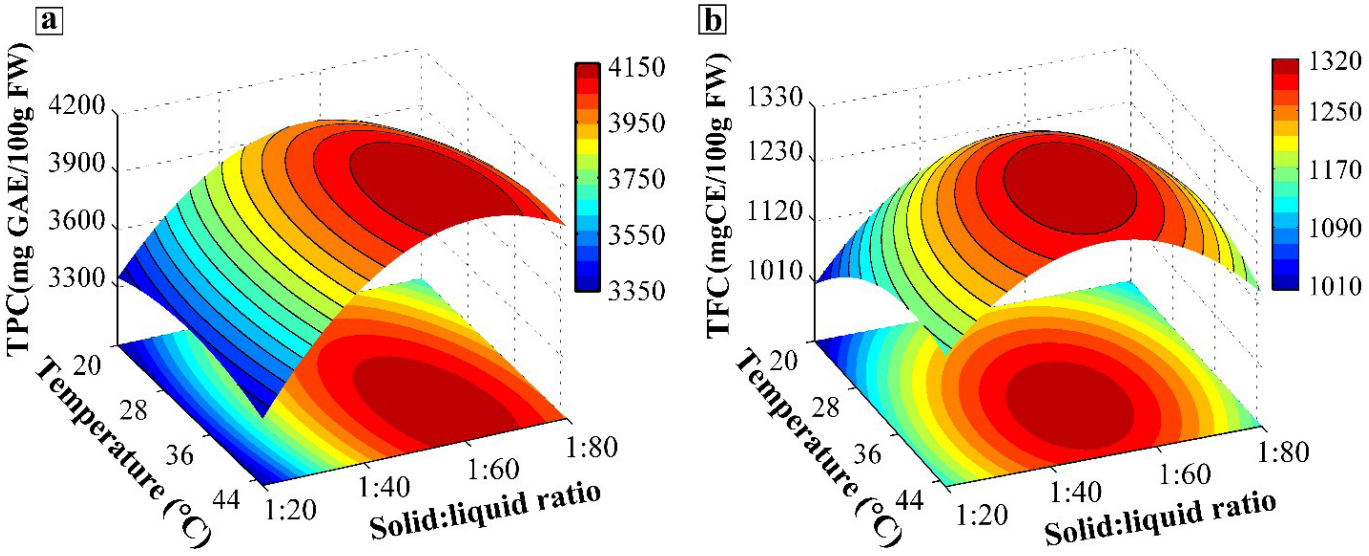

Figure 4. Response surface and contour plots of: **a)** Total polyphenol content; **b)** Total flavonoid content. Solvent percentage was constant at 39.43% (v/v).

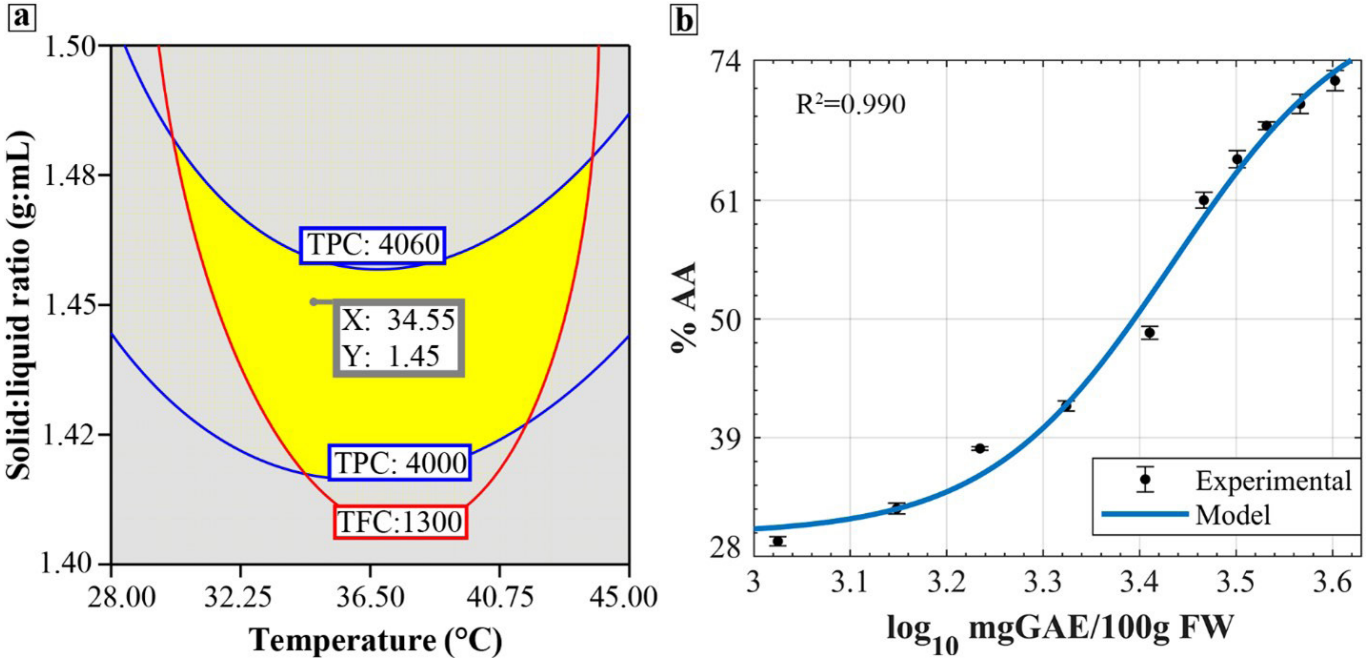

Figure 5. a) Overlaid contour plot for extraction of total polyphenol and flavonoid content. The solvent percentage was constant at 39.43% (v/v). **b)** Effective antioxidant activity (EC50) estimation under the optimum extraction conditions.

obtained through optimal conditions. The four-parameter model based on Equation 3 had a high fit to the experimental data with a value of 0.990 for the R^2 coefficient (Figure 5B). Using this model, a value of 3.434 was obtained for log(EC50), which corresponds to 2716.44 mg GAE/100 g FW. Therefore, in order to reach an efficient antioxidant activity, it is necessary to use an extract of feijoa residues with a total polyphenol content higher than EC50.

4 Conclusions

The extraction kinetics of polyphenols and flavonoids from feijoa residues showed that as the extraction progresses the mass transfer rate stabilizes until achieving the highest content of flavonoids and polyphenols after 45 and 90 minutes, respectively. All extracts obtained by the extraction kinetics showed an antioxidant activity greater than 50%. The optimized factors using multiple response optimization were 34,55 °C, 1:45 solvent:liquid ratio (g/mL) and 39,43% (v/v) of solvent. These factors had a significant effect on the extraction of total polyphenols and flavonoids, registering an increase in the recovery of these compounds at higher temperatures and solid:liquid ratios. In contrast, the concentration of ethanol in the solvent decreases the polyphenol and flavonoid content. Predicted values for TPC and TFC were confirmed by experimental validation with a low estimation error. Thus, feijoa residues could be used at a minimal purchase cost by the nutraceutical industry. Finally, orbital shaking extraction is a feasible and economical technique to recover large amounts of bioactive compounds. Considering that advanced technology is not required, this extraction methodology can be scaled up to industrial processes.

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