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Mathematical Modeling Of Solid-Liquid Extraction Of Total Phenolic Compounds In Hibiscus Sabdariffa. An Application Of Peleg's Equation

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ABSTRACT
The objective of the research was to evaluate the applicability of the Peleg equation to model the solid-liquid extraction of total phenolic compounds in Hibiscus sabdariffa flowers. The content of these compounds was determined by the Folin-Ciocalteu method, and the extraction kinetics were estimated using the two-parameter Peleg equation. The agreement between the experimental results and the model predictions was assessed by calculating the Pearson correlation. The results revealed significant effects of temperature and extraction time on the content of phenolic compounds, with concentrations ranging from 0.08 to 0.84 g gallic acid equivalents per 100 g under different extraction conditions. The Peleg equation effectively modeled the extraction kinetics of phenolic compounds, with high Pearson correlation coefficients (0.99898 and 0.99943) confirming its suitability to predict the concentration of these compounds. These findings underline the importance of understanding extraction kinetics to optimize phenolic compound extraction processes, providing valuable information for industries that rely on Hibiscus sabdariffa extracts.

Keywords: Folin-Ciucalteu, Jamaica, Mathematical modeling

Introduction

Hibiscus sabdariffa, commonly known as rosella, roselle or jamaica, is an herbaceous subshrub of the Malvaceae family, cultivated in tropical and subtropical regions of the world. (García Muñoz et al., 2023). This plant is grown mainly during the rainy season and produces vibrant red flowers. The calyxes of H. sabdariffa are processed by freezing or drying for preservation. In the food industry, these calyxes are widely used in the production of beverages such as tea and wine, as well as jams, ice cream, sauces and syrups (Romero Hernández, Gutiérrez, Tovar, & Bello Pérez, 2023). Traditionally, calyxes are also used in the treatment of various ailments such as colds, coughs, hypercholesterolemia, hypertension and indigestion.

Research on foods containing bioactive compounds, especially antioxidants, has increased significantly. Antioxidants are molecules that can interact with reactive oxygen species (ROS), including oxygen ions, free radicals and peroxides, which are highly reactive due to the presence of unpaired electrons in their outer shell (Gómez Bellot, Guerrero, Yuste, Vallejo, & Sánchez Blanco, 2024).

Despite the numerous studies that report the total polyphenol content in H. sabdariffa flowers, the bibliographic information on the extraction kinetics of the solid-liquid process is limited. This lack highlights the need to develop mathematical models that facilitate the simulation, design and control of extraction processes, thus optimizing the use of resources such as energy, time and solvents.

In the context of solid-liquid extraction, this model can provide an accurate description of the equilibrium and extraction rate of phenolic compounds, allowing to optimize process parameters and improve process efficiency.

The objective of this study was to evaluate the applicability of the Peleg equation for modeling the solid-liquid extraction of total phenolic compounds in H. sabdariffa flowers.

Literature Review

H. sabdariffa calyxes from different regions contain between 29.53% and 87% carbohydrates, 7.4% and 12.3% ash, 5.5% and 9.14% protein, and 0.47% and 1.32% fat on a dry weight basis (Lavinia Stan et al., 2023).

In addition, they are rich in dietary fibers, organic acids and bioactive compounds. Recently, total phenolic compounds, flavonoids and anthocyanins have been quantified in dried calyxes in this plant, finding between 10.44 and 19.75 mg gallic acid equivalents/g, 5.8 and 42.57 mg catechin equivalents/g, and 4.45 and 5.39 mg cyanidin-3-glucoside/g, respectively (Lavinia Stan et al., 2023).

The elevated presence of free radicals in cells can damage proteins, cell membrane lipids and nucleic acids, and is closely associated with diseases such as cancer, diabetes, hypertension, obesity and other metabolic disorders. Antioxidants neutralize free radicals by donating electrons, thus stabilizing these compounds and preventing cell damage. H. sabdariffa flower is rich in antioxidants such as vitamins E and C, phenolic compounds, polyphenolic acids, flavonoids and

anthocyanins, giving it anticancer, cardioprotective, diuretic, anti-inflammatory and antimicrobial properties (Bello Perez, Santana Galeana, Flores Silva, & Tovar, 2024).
In Ecuador, H. sabdariffa flower production has grown in the Amazon region, where the temperature conditions, between 15°C and 38°C, are optimal for its cultivation. This expansion represents a sustainable economic development alternative for the local population. The extraction of bioactive compounds is a crucial step in the isolation and identification process, and there is no single method that guarantees maximum efficiency. The solid-liquid extraction technique is widely used to quantify phenolic compounds, playing a fundamental role in their extraction. Fick's second diffusion law is commonly used to describe the mass transfer phenomena involved in this process (Deng, Wang, & Liu, 2024).
Mathematical models are also essential in the description of sorption processes, such as dehydration and rehydration of food materials. The non-exponential Peleg model, which consists of two parameters, has proven to be particularly useful in these cases (Pezo et al., 2024). This equation is used to describe both the kinetics of water absorption and desorption in food materials, providing a mathematical framework for predicting the behavior of these processes under various conditions (Kaleta, Górnicki, Obranović, & Kosiorek, 2024).

Methodology

Sample treatment

Fresh flowers of H. sabdariffa were purchased at the local market in the city of Puyo-Ecuador, located at coordinates 1.4837° S 78.0026° W. The flowers were washed with distilled water and dried in the shade. They were then placed in an oven (brand: memmert, model: SFE700) at 45°C for 48 h and the moisture content was calculated by weight difference according to equation (1). This result was used to express the initial weight on a dry matter basis. The dried flowers were crushed in a grinder (KitchenAid brand, model BCG111OB and nominal frequency of 60 Hz) and the resulting powder was sieved to a particle size of less than 0.5 mm.

$$\%H = \frac{m-ms}{m} \cdot 100 \quad (1)$$

Where: m represents the mass of the fresh sample (g) and ms indicates the mass of the dry sample (g).

Preparation of aqueous extracts

The procedure used by Peñafiel Bonilla, Luna Fox, García Quintana, & Arteaga Crespo (2023). Ultrasonic extraction was used, for which an ultrasonic bath equipment (Wisd.23 brand, model WUC-DO6H) was used. To 2±0.1 g of ground H. sabdariffa flowers, 100 mL of distilled water were added in glass beads and placed inside the equipment. Each extraction was performed in triplicate at 35, 45 and 55°C in time periods between 5 and 60 min with 5 min increments per interval for each temperature. The liquid extracts were filtered using Whatman No. 4 paper and the analyses of total phenolic compounds were carried out immediately.

Spectrophotometric quantification of total phenolic compounds (TPC)

The Folin-Ciucalteu colorimetric technique was applied (Luna Fox et al., 2023). To 1 mL of aqueous extract, 0.5 mL of Folin-Ciucalteu's reagent diluted by half with distilled water was added, this mixture was allowed to stand at room temperature for 10 min. Then, 0.5 mL of 20% Na2CO3 was added and the total mixture was made up to 10 mL with distilled water and allowed to stand for 2 h at room temperature. The absorbance of the samples was read at 765 nm in a UV-visible spectrophotometer (Perkin Elmer brand). The TPC concentration was calculated using the mathematical model of the calibration curve (Eq. 2) prepared with gallic acid and the results were expressed in g gallic acid equivalents per 100 g of dry matter (g GAE/100 g).

$$A = 0{,}0734C - 0{,}0028 \quad (2)$$

Where: A is the absorbance of the sample and C is the TPC concentration (mg/L).

Extraction kinetics using the Peleg equation

The solid-liquid extraction process of TPC can be described according to the mathematical model (3) proposed by Peleg (1988).

$$C(t) = c_o + \frac{t}{k_1 + k_2 \cdot t} \quad (3)$$

Where C(t) indicates the TPC concentration as a function of time (g GAE/100g), t is the extraction time (min), C_o shows the initial TPC concentration at t=0 (g GAE/100g), k_1 represents the Peleg rate constant (min-100g/g) and k_2 is the Peleg capacity constant (100g/g).
Since the initial TPC concentration (C_0) was zero, equation (3) can be rewritten as follows:

$$C(t) = \frac{t}{k_1 + k_2 \cdot t} \quad (4)$$

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The value of the constants k_1 and k_2 were obtained by plotting the linearized equation (4):

$$\frac{t}{C(t)} = k_1 + k_2 \cdot t \qquad (5)$$

Where k_1 represents the intercept and k_2 the slope of the line.

Data Analysis

All analyses were performed in triplicate to minimize experimental errors. The results were expressed as mean values ± standard deviation (SD) for three measurements (n=3). Experimental data were analyzed using Design Expert version 10 software. Analysis of variance (ANOVA) and F-test were used to evaluate the influence of temperature and extraction time on TPC concentration. Graphs were generated with Origin 2021 software, and agreement between experimental results and those predicted by Peleg's model was determined by calculating Pearson's correlation coefficient, according to equation (6).

$$r = \frac{\sum(x_i-\bar{x})\cdot(y_i-\bar{y})}{\sqrt{\sum(x_i-\bar{x})^2\cdot\sum(y_i-\bar{y})^2}} \qquad (6)$$

Where:

x_i and y_i represent the individual values of the experimental results and those predicted by the Peleg model.

$\bar{x}$ and $\bar{y}$ are the mean values of x_i and y_i respectively.

Results

Effect of temperature and extraction time on TPC

Analysis of variance (Table 1) revealed that both temperature and extraction time presented significant differences ($p<0.05$) in TPC extraction. These results indicate that both factors have a notable impact on the efficiency of the extraction process. Furthermore, the lack of model fit did not show statistical significance, with a p-value of 0.985. This result is positive, as it suggests that the experimental data adequately fit the Peleg equation.

Table 1. ANOVA for the effect of temperature and time on TPC extraction.

Source	Sum of squares	df	Mean square	F-value	p-value	
TPC	1,43	2	0,7165	1096,39	< 0.0001	Significant
A-Temperature	1,26	1	1,26	1927,42	< 0.0001	
B-Time	0,1734	1	0,1734	265,35	< 0.0001	
Residual	0,8638	33	0,0007			
Lack of adjustment	0,0887	31	0,0089	0,857	0,985	Not Significant
Pure error	0,5859	17	0,0345			

Figure 1 illustrates that TPC extraction was proportional to both temperature and extraction time. The results varied significantly as a function of these variables, with values ranging from 0.12 to 0.63 g GAE/100g at 35°C, 0.17 to 0.79 g GAE/100g at 45°C, and 0.23 to 0.92 g GAE/100g at 55°C. These data indicate a clear trend of increasing extraction efficiency with increasing temperature.

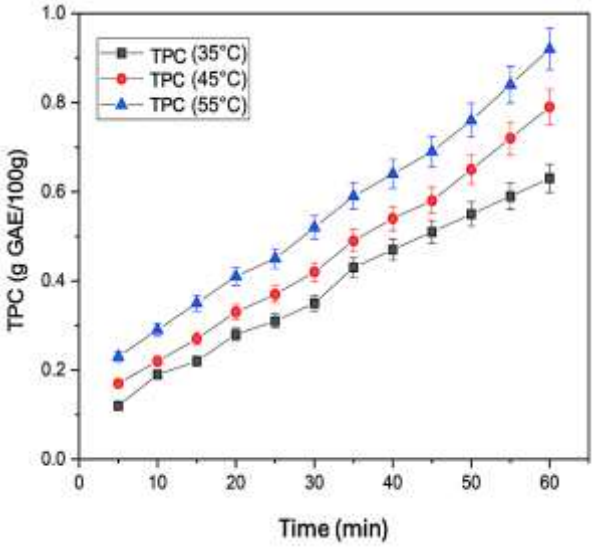

Figure 1. TPC extraction curves at 35, 45 and 55°C.

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Solid-liquid extraction kinetics of TPC

The TPC experimental results were fitted to the Peleg equation. To calculate the constants k_1 and k_2 corresponding to the TPC extracted at 35, 45 and 55 °C were plotted $\frac{t}{c(t)}$ against t, using the linearized Peleg equation (5). The results obtained are presented in Table 1. The mathematical models generated were as follows:

$$C(t)_{35°C} = \frac{t}{60{,}549 + 0{,}6038t}$$

$$C(t)_{45°C} = \frac{t}{49{,}480 + 0{,}5718t}$$

$$C(t)_{55°C} = \frac{t}{38{,}638 + 0{,}5594t}$$

Table 2. Peleg's constants (k_1 and k_2) for solid-liquid extraction of TPC and coefficients of determination.

Temperature (°C)	k_1 (min·100g/g)	k_2 (100g/g)	R^2
35	60,549	0,6038	0,9850
45	49,480	0,5718	0,9819
55	38,638	0,5594	0,9913

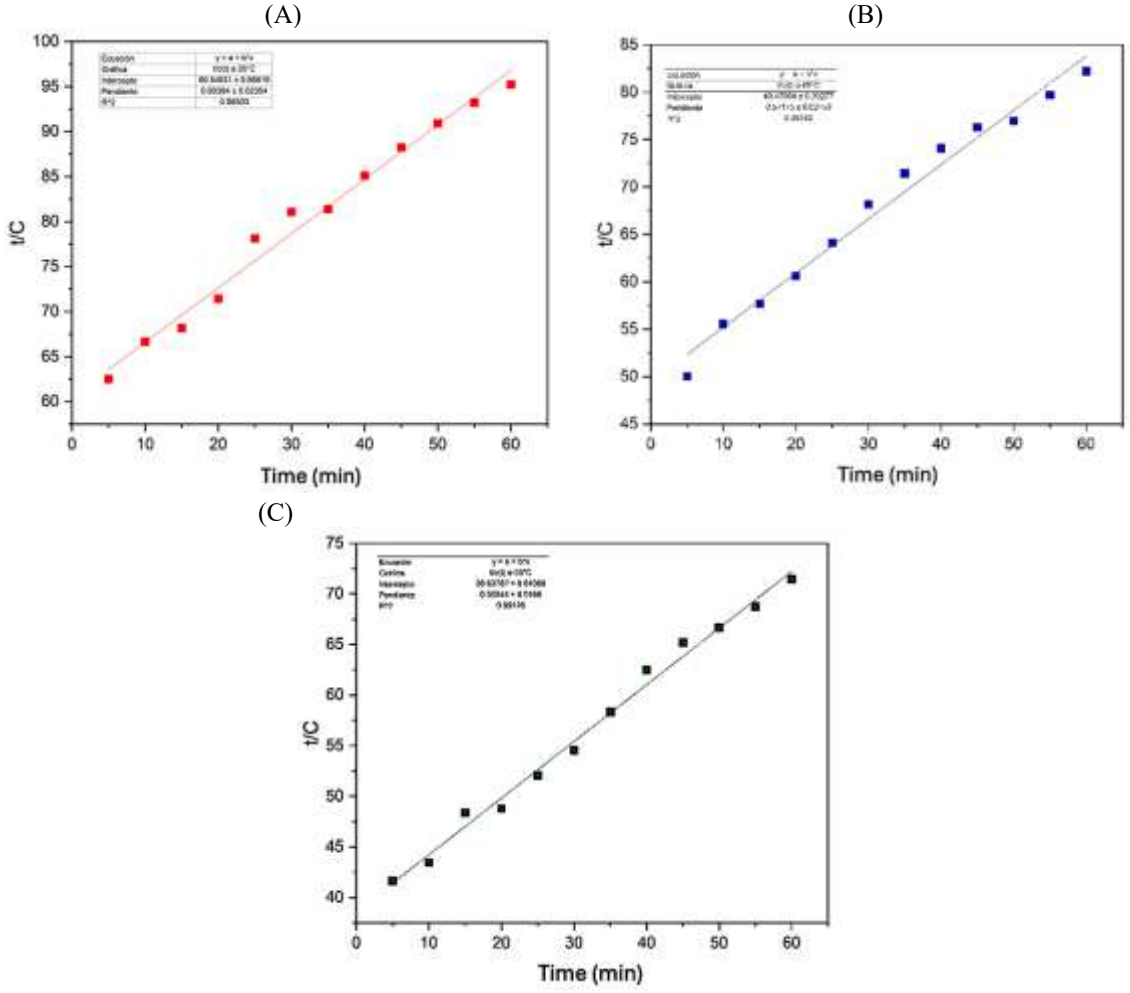

Figure 2. Calculation of constants k_1 and k_2 in the extractions performed at 35°C (A), 45°C (B) and 55°C (C).

Correlation between TPC experimental data and those predicted by Peleg's model.

The experimental TPC results were compared with the predictions obtained from the Peleg models. The details of this comparison are presented in Table 3 and illustrated graphically in Figure 3.

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Table 3. Comparison between experimental values and those predicted by Peleg's models of TPC expressed in g GAE/100g

Time (min)	Experimental (35°C)	Predicted (35°C)	Experimental (45°C)	Predicted (45°C)	Experimental (55°C)	Predicted (55°C)
5	0,08±0,01	0,08	0,10±0,0	0,10	0,12±0,01	0,12
10	0,15±0,03	0,15	0,18±0,02	0,18	0,23±0,06	0,23
15	0,22±0,10	0,22	0,26±0,01	0,26	0,31±0,02	0,32
20	0,28±0,02	0,28	0,33±0,04	0,33	0,41±0,01	0,40
25	0,32±0,01	0,33	0,39±0,02	0,39	0,48±0,03	0,48
30	0,37±0,01	0,38	0,44±0,03	0,45	0,55±0,02	0,54
35	0,43±0,0	0,43	0,49±0,01	0,50	0,6±0,01	0,60
40	0,47±0,12	0,47	0,54±0,01	0,55	0,64±0,03	0,66
45	0,51±0,07	0,51	0,59±0,02	0,60	0,69±0,01	0,71
50	0,55±0,01	0,55	0,65±0,03	0,64	0,75±0,01	0,75
55	0,59±0,09	0,59	0,69±0,11	0,68	0,8±0,02	0,79
60	0,63±0,01	0,62	0,73±0,01	0,72	0,84±0,07	0,83

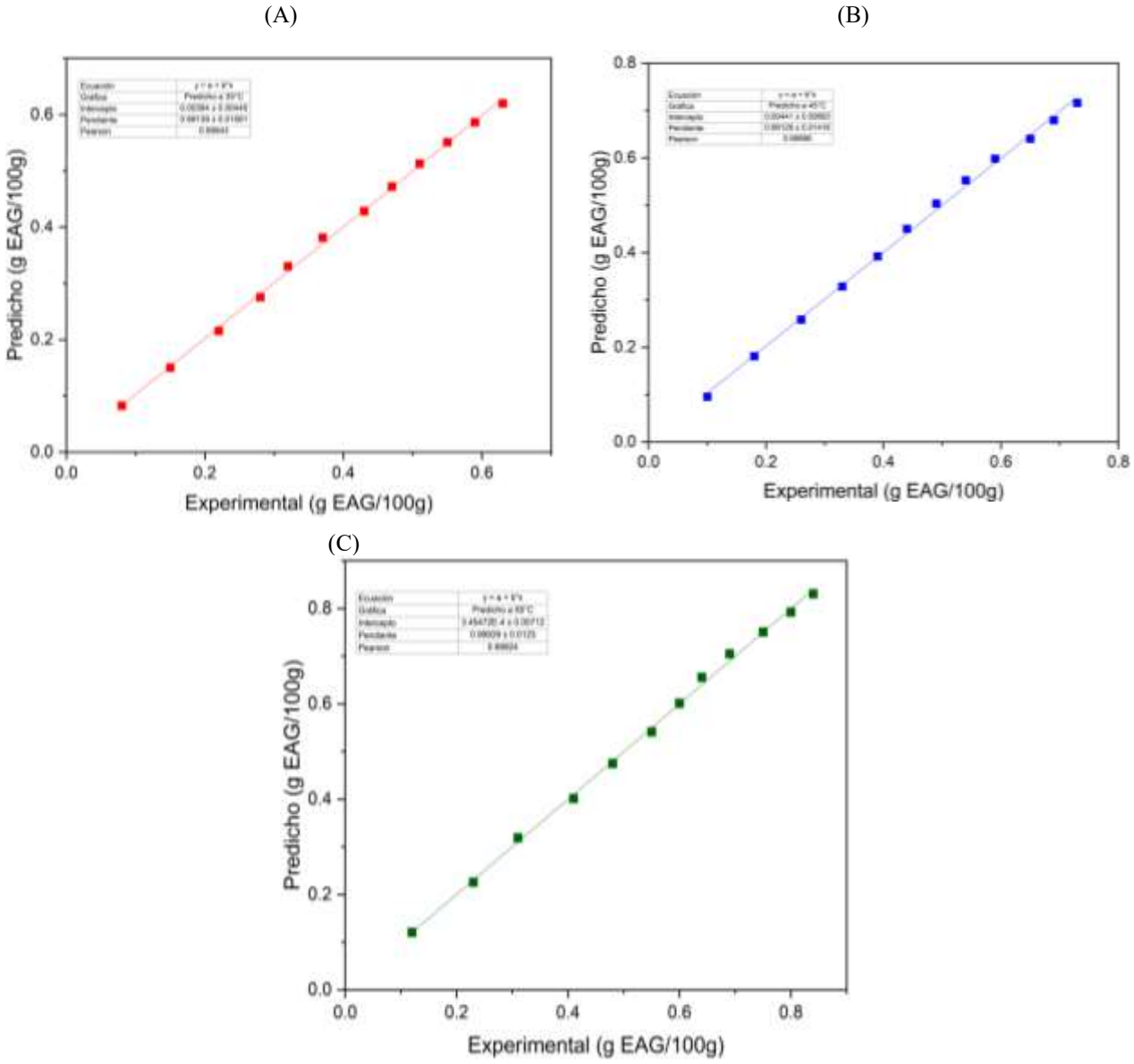

Figure 3. Experimental and predicted values of TPC at 35°C (A), 45°C (B) and 55°C (C).

Discussions

The dry basis content of TPC in H. sabdariffa flowers has been reported in previous studies. In the research conducted by Sanou et al., (2022) reported a TPC content of 5.64 g GAE/100 g using water as solvent and ultrasound extraction. On the other hand Vargas León et al., (2018) reported values between 1.16 and 1.76 g GAE/100 g in alcoholic extracts. Luna Fox, et al., (2024) concentrations in aqueous extracts ranged from 0.26 to 2.84 GAE/100g. These results are higher than those reported in the present investigation. In several studies Villalobos Vega, et al., (2023), Khalil et al., (2024), Mansinhos, Gonçalves, & Romano, (2024) and Gómez Bellot et al., (2024) it has been indicated that the variability in the concentration of bioactive compounds may be due to a number of factors, including, plant GAE, climatic and soil conditions, time of sample collection, method of analysis, solvent used and particle size.

Temperature and extraction time were found to be significant factors, showing a proportional relationship with the amount of TPC extracted. These findings are consistent with those reported by Arteaga Crespo, et al., (2020), Anila & Mohammed, (2022) and Al-Hatim et al., (2022), who demonstrated that higher temperatures and prolonged times during the extraction process increase the yields of bioactive compounds. The observed behavior shows that, at higher temperatures, the solubility of the TPCs improves, facilitating their release and extraction from the plant material. At 35°C, the extraction is lower compared to temperatures of 45°C and 55°C, showing the importance of a suitable thermal environment to maximize the efficiency of the process. The proportional relationship between temperature and extraction efficiency can be attributed to the higher kinetic energy available at elevated temperatures, which favors the breakdown of chemical and physical interactions that maintain phenolic compounds in the plant matrix.

Likewise, extraction time plays a crucial role in the amount of TPC extracted. A prolonged extraction time allows greater contact between the solvent and the bioactive compounds, facilitating a more effective mass transfer. The variability in the results also suggests that there is an optimal point at which both temperature and extraction time are balanced to maximize the release of phenolic compounds without causing thermal degradation of the phenolic compounds.

The coefficients of determination (R^2) in the equations presented in Figure 2 for the calculation of the constants k_1 and k_2 were high with values at 0.9850, 0.9819, and 0.9913 for extractions performed at 35°C, 45°C, and 55°C, respectively. These results demonstrate a good relationship between the variables involved. As for the Peleg velocity constants (k_1) and Peleg's capacity constants (k_2), a clear trend of decreasing with increasing time and temperature was observed. This implies that, as the experimental conditions change, the rate and capacity constants, which are fundamental to describe the behavior of the system, tend to decrease. This decrease may reflect a modification in the reaction mechanism or in the dynamics of the process under the more extreme conditions of time and temperature evaluated. These results agree with those reported by Segovia Gómez, Corral, & Almajano, (2013) and Bucić Kojić, Planinić, Tomas, Bilić, & Velić, (2007).

The Pearson correlation coefficients shown in Figure 3 ranged from 0.99898 to 0.99943, indicating a strong and positive relationship between the variables analyzed. These high values suggest a high correlation between the experimental data and the predictions generated by the Peleg models.

A correlation coefficient close to 1 reflects a high accuracy in the fit of the model to the experimental data. That is, the Peleg model is effectively reflecting the trends and patterns in the TPC data. This is particularly relevant because it indicates that the model is reliable in predicting behavior under the experimental conditions evaluated. These results show the ability of the Peleg model to provide accurate and consistent predictions, which is crucial for process planning and optimization in practical applications. The high correlation also suggests that the model could be applicable to a variety of similar experimental conditions, increasing its usefulness in different scenarios. To further strengthen the validity of the model, it would be beneficial to consider further evaluation under different experimental conditions and with larger data sets. This would ensure that the high correlation is not specific to a particular subset of data but reflects a general ability of the model to accurately predict TPC extraction.

Conclusion

Temperature and extraction time were identified as significant factors in the extraction of total phenolic compounds from Hibiscus sabdariffa flowers. The Peleg model proved to be effective in describing the solid-liquid extraction kinetics of these compounds under the conditions studied. The findings of this research may be useful for the simulation and optimization of the extraction process of phenolic compounds in Hibiscus sabdariffa flowers.

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