Kinetic study of solid-liquid extraction of caffeine in *Ilex guayusa* Loes

Estudio cinético de la extracción sólido-líquido de cafeína en *Ilex guayusa* Loes

Estudo cinético da extração sólido-líquido de cafeína em *Ilex guayusa* Loes

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Abstract

The kinetic study of the solid-liquid extraction of caffeine in *Ilex guayusa* Loes addresses a critical stage in the isolation of alkaloids such as caffeine. Solid-liquid extraction, a widely used technique, plays a fundamental role in obtaining these compounds. The study aimed to evaluate the applicability of the Peleg equation to model the solid-liquid extraction of caffeine in *Ilex guayusa* Loes leaves. Caffeine content was determined by UV-visible absorption spectroscopy. Extraction kinetics were estimated using the two-parameter Peleg's equation. The correspondence between the experimental results and those predicted by the model was established by calculating Pearson's correlation. The results indicated significant extraction temperature and time effects on caffeine content, with concentrations ranging from 0.24 to 1.52 $g.100\ g^{-1}$ at different extraction temperatures (30, 40, and 50 °C). The Peleg equation effectively modeled caffeine extraction kinetics, with high Pearson correlation coefficients (0.96895 to 0.99685) confirming its suitability for predicting caffeine concentration. These results highlight the importance of understanding extraction kinetics to optimize caffeine extraction processes, offering valuable insights for industries using *Ilex guayusa* Loes extracts.

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Resumen

El estudio cinético de la extracción sólido-líquido de cafeína en *Ilex guayusa* Loes aborda una etapa crítica en el aislamiento de alcaloides como la cafeína. La extracción sólido-líquido, una técnica ampliamente utilizada, desempeña un papel fundamental en la obtención de estos compuestos. El estudio tuvo como objetivo evaluar la aplicabilidad de la ecuación de Peleg para modelar la extracción sólido-líquido de cafeína en hojas de *Ilex guayusa* Loes. El contenido de cafeína se determinó por espectroscopía de absorción UV-visible. La cinética de extracción se estimó utilizando la ecuación de Peleg de dos parámetros. La correspondencia de los resultados experimentales y los predichos por el modelo se estableció mediante el cálculo de correlación de Pearson. Los resultados indicaron efectos significativos de la temperatura y el tiempo de extracción en el contenido de cafeína, con concentraciones que variaron desde 0,24 hasta 1,52 g.100 g^{-1} a diferentes temperaturas de extracción (30, 40 y 50 °C). La ecuación de Peleg modeló eficazmente la cinética de extracción de cafeína, con altos coeficientes de correlación de Pearson (0,96895 a 0,99685) que confirmaron su idoneidad para predecir la concentración de cafeína. Estos resultados resaltan la importancia de comprender la cinética de extracción para optimizar los procesos de extracción de cafeína, ofreciendo ideas valiosas para las industrias que utilizan extractos de *Ilex guayusa* Loes.

Palabras clave: cinética, ecuación de Peleg, extracto acuoso, modelo matemático.

Resumo

O estudo cinético da extracção sólido-líquido da cafeína em Ilex guayusa Loes aborda uma etapa crítica no isolamento de alcalóides como a cafeína. A extração sólido-líquido, técnica muito utilizada, desempenha um papel fundamental na obtenção destes compostos. O estudo teve como objetivo avaliar a aplicabilidade da equação de Peleg para modelar a extração sólido-líquido de cafeína em folhas de *Ilex guayusa* Loes. O teor de cafeína foi determinado por espectroscopia de absorção UV-visível. A cinética de extração foi estimada através da equação de Peleg de dois parâmetros. A correspondência entre os resultados experimentais e os previstos pelo modelo foi estabelecida pelo cálculo da correlação de Pearson. Os resultados indicaram efeitos significativos da temperatura e do tempo de extração no teor de cafeína, com concentrações que variaram entre 0,24 a 1,52 g.100 g^{-1} a diferentes temperaturas de extração (30, 40 e 50 °C). A equação de Peleg modelou eficazmente a cinética de extração de cafeína, com elevados coeficientes de correlação de Pearson (0,96895 a 0,99685) confirmando a sua adequação para prever a concentração de cafeína. Estes resultados realçam a importância de compreender a cinética de extração para otimizar os processos de extração de cafeína, oferecendo informações valiosas para as indústrias que utilizam extratos de *Ilex guayusa* Loes.

Palavras-chave: cinética, equação de Peleg, extrato aquoso, modelo matemático.

Introduction

Caffeine, a methylxanthine naturally present in several plants, has been the subject of extensive research due to its stimulant effects on the human central nervous system (Mahoney *et al*., 2019). Among these plants, *Ilex guayusa* Loes, a shrubby species in the family Aquifoliaceae, has emerged as a promising source of caffeine, especially in the Amazon of South America (Kelebek *et al*., 2024). The solid-liquid extraction of caffeine in *I. guayusa* represents a fascinating and relevant field of study in the current scientific context.

The extraction of bioactive compounds represents a fundamental step in the isolation and identification of alkaloids such as caffeine (Rajput, 2022), and there is no single method that guarantees its efficiency. Among the most commonly used extraction techniques for alkaloid isolation is solid-liquid extraction (Vandeponseele *et al*., 2021), which plays a key role in obtaining these compounds. To describe the mechanism underlying this process, Fick's second law of diffusion is commonly employed, offering an in-depth understanding of the matter transfer processes involved (Li *et al*., 2020; Hashim *et al*., 2023).

Recent research has reported the caffeine content in *I. guayusa* (Paladines-Santacruz *et al*., 2021; Carvalho *et al*., 2021). However, bibliographic data on the extraction kinetics of the solid-liquid process are scarce. This lack of specific information highlights the need to develop mathematical models that facilitate the simulation, design, and control of extraction processes, thus contributing to the efficient use of resources such as energy, time, and solvent.

On the other hand, mathematical models have an important role in describing sorption processes, such as dehydration (Korniyenko and Ladieva, 2021) and rehydration of food products (Tepe, 2024). Among these models, the non-exponential Peleg model (Lalji *et al*., 2022), which consists of two parameters, has proven to be particularly useful. Given the similarity between extraction kinetics and sorption, the study aimed to evaluate the applicability of Peleg's equation to model the solid-liquid extraction of caffeine in the leaves of *Ilex guayusa* Loes.

Materials and methods

Experiment location and sample preparation

This study was carried out at the Bromatology Laboratory of the Amazonian State University, located at km 2 ½ on the road to Tena, in the canton and province of Pastaza, with an altitude of 940 meters above sea level, 00° 59´-1" latitude and 77° 49´0" west longitude. The leaves of *I. guayusa* were purchased in the market of the city of Puyo, Pastaza-Ecuador, at coordinates 1.4837° S 78.0026° W. The leaves were washed with deionized water and then dried under shade at room temperature. Subsequently, they were placed in a stove (Memmert brand, SFE700 model) at 40 °C for 72 h, and the humidity present in the leaves was calculated by mass difference (Yu *et al*., 2022). The result was used to express the initial mass of the leaves based on the dry matter. The plant material was crushed in a mill (KitchenAid brand, BCG111OB model), with a nominal frequency of 60 Hz. They were then sieved to obtain particles smaller than 0.5 mm.

Preparation of the extracts

For the solid-liquid extraction of caffeine, the ultrasound-assisted extraction technique (UAE) was performed using Wisd.23 equipment, WUC-DO6H model according to the procedure described by Peñafiel-Bonilla *et al.* (2023). In each experiment, 5 ± 0.1 g of ground *I. guayusa* was weighed into round-bottom flasks, and 100 mL of distilled water was added. Each extraction was performed in triplicate at 30, 40, and 50 °C for periods ranging from 10 to 90 minutes, with 5 intervals for each temperature. The obtained extracts were filtered using Whatman No. 4 filter paper and the caffeine analyses were carried out immediately.

Quantification of caffeine

The determination of caffeine was made according to Luna-Fox *et al.* (2023). Prior to quantification, a liquid-liquid extraction was carried out, using chloroform as an extraction solvent. For this, each aqueous extract was placed in a separating funnel, alkalized with 1 mL of sodium hydroxide (0.1 M), and the caffeine was extracted with two portions of chloroform of 15 mL each. The chloroformic extracts were joined in an Erlenmeyer and placed in a water bath for complete evaporation. The caffeine was then dissolved with 50 mL of hot distilled water (60-90 °C), then cooled and transferred to a 100 mL volumetric flask, and the volume was completed with distilled water. From the previous solution, 5 mL was taken and transferred to a 25 mL volumetric flask, then 1 mL of hydrochloric acid (0.01 M) was added and measured with distilled water. Finally, the absorbance of the sample was read at 275 nm in a visible ultraviolet spectrophotometer (Perkin Elmer brand). The concentration of caffeine was determined by a calibration curve according to equation (1), prepared with nine concentrations (1, 2, 3, 5, 10, 12, 16, 20, and 25 $mg.mL^{-1}$) and R^2=0.9991. The results were expressed in grams per 100 grams of dry matter ($g.100\ g^{-1}$ ms).

$$A = 0.006C + 0.0011 \quad (1)$$

Where, C: concentration of caffeine in the sample ($mg.mL^{-1}$) and A: absorbance of the sample.

Solid-liquid extraction kinetics

Since there is a similarity between the caffeine vs. time extraction curves and the humidity vs. time sorption curves, solid-liquid caffeine extraction in *I. guayusa* can be described by the model proposed by Peleg (1988). This equation can be written as follows:

$$C(t) = c_o + \frac{t}{k_1 + k_2 \cdot t} \quad (2)$$

where C(t) indicates the concentration of caffeine in relation to time ($g.100\ g^{-1}$), t denotes the extraction time (min), indicates the initial concentration of caffeine in ($g.100\ g^{-1}$), is Peleg's velocity constant ($min \cdot 100\ g.g^{-1}$) and is Peleg's capacity constant ($100\ g.g^{-1}$).

Since the initial concentration of caffeine was zero, equation (3) is represented as follows:

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

The values of the constants were obtained by plotting the linearized equation, according to:

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

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

Statistical analysis

The data obtained were processed using the Origin 2021 program (Orji *et al*., 2022). An analysis of variance (ANOVA) was performed with the F-test to determine how temperature and extraction time affected caffeine concentration. The validity of Peleg's model was evaluated by comparing the experimental results with the predicted values, using Pearson's correlation coefficient according to equation (5).

$$r = \frac{\sum(x_i - \bar{x}) \cdot (y_i - \bar{y})}{\sqrt{\sum(x_i - \bar{x})^2 \cdot \sum(y_i - \bar{y})^2}} \quad (5)$$

Where:

x_i and y_i are respectively the results of each experiment and those obtained by Peleg's model.

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

Results and discussion

Factors that affected caffeine extraction

ANOVA indicated that both temperature and extraction time were statistically significant ($p<0.05$) on caffeine content, with p values of 0.0001 for temperature and time. On the other hand, the lack of adjustment did not show statistical significance ($p>0.05$), which is good because the experimental data are intended to fit the Peleg model.

Figure 1 shows that temperature and extraction time had a proportional behavior for caffeine extraction, i.e., an increase in temperature and extraction time led to an increase in caffeine concentration. Extractions performed at 30 °C, 40 °C, and 50 °C indicated a caffeine concentration between 0.24 and 1.03 $g.100\ g^{-1}$, 0.37 and 1.27 $g.100\ g^{-1,}$ and 0.64 and 1.52 $g.100\ g^{-1}$, respectively.

The caffeine present in dried leaves of *I. guayusa* has been reported by different authors. In the research carried out by Cadena-Carrera, caffeine values of 2.27 % were obtained in aqueous extracts using supercritical fluids. On the other hand, Santana *et al*. (2018) reported caffeine concentrations of 2.98-3.02 % analyzed by high-efficiency liquid chromatography. These results are superior to those obtained in this study. The variation in caffeine concentration in *I. guayusa* can be due to different factors. According to Rai *et al*. (2021), the concentration of bioactive compounds can vary depending on the age of the plant. Toscano *et al*. (2019) have indicated that climatic conditions are a determining factor when collecting plant samples since the concentration of secondary metabolites can change significantly. Jha and Sit (2022) showed that the content of chemical compounds in plant samples can vary depending on the extraction method used, likewise, the type of solvent can significantly influence the extraction.

Temperature and extraction time were significant factors with a positive effect on caffeine extraction, this result is in agreement with what is indicated by Luna-Fox *et al*. (2023) when demonstrating that

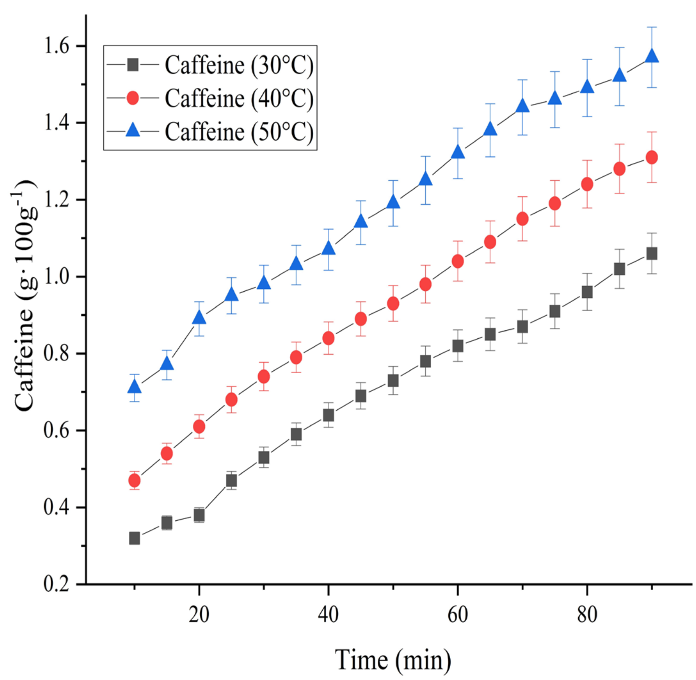

Figure 1. Caffeine extraction curves at 30°C, 40°C and 50°C.

the concentration of caffeine increased with increasing extraction time and temperature. The effect of temperature can be explained by the kinetic energy of caffeine molecules increasing at higher temperatures, which facilitates their release and dissolution in the solvent (Schaefer *et al.*, 2020). This phenomenon aligns with the law of mass action, in which an increase in temperature favors the reaction toward product equilibrium. On the other hand, by prolonging the contact time between the *I. guayusa* leaves and the solvent, further extraction of caffeine and other bioactive compounds present in the leaves is allowed. This extra time allows the solvent to penetrate the structure of the leaves, facilitating the release of caffeine more effectively.

It is important to highlight that the interaction between temperature and extraction time can have synergistic effects on the final concentration of caffeine obtained. For example, a higher temperature can initially accelerate the extraction rate (Bitwell *et al.*, 2023), while a long extraction time allows this process to reach an optimal balance, thus maximizing the amount of caffeine extracted from the leaves of *I. guayusa*.

Solid-liquid extraction kinetics of caffeine

The caffeine concentrations obtained experimentally were adapted to Peleg's equation (3). The values of the constants for caffeine extracted at 30, 40, and 50 °C were calculated by plotting (figure 2) the values vs t, according to Peleg's linearized equation (4).

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 located at 0.97933, 0.96567, and 0.97209 in the extractions carried out at 30, 40, and 50 °C respectively. These results demonstrated a good relationship between the variables involved. On the other hand, the Peleg velocity constants (k_1) and the Peleg capacity constants decreased with increasing time and temperature. These results coincide with those reported by Segovia-Gómez *et al*. (2013) and Bucić-Kojić *et al.* (2007).

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

The experimental values of caffeine and those estimated by the Peleg models were compared and are indicated in table 2 and figure 3.

The Pearson coefficients indicated in Figure 3 varied between 0.96895 and 0.99685. These results close to the unity indicate that Peleg's model has a good fit for the experimental data. A high correlation coefficient shows that the values predicted by the model are in close agreement with the experimental values, suggesting that mathematical models are suitable to represent the behavior of the experimental data obtained in this study.

The successful adaptation of the experimental values to the Peleg equation for predicting the concentration of caffeine in leaves of *I. guayusa* has important implications because it confirms the suitability of the Peleg model to represent the relationship between the relevant

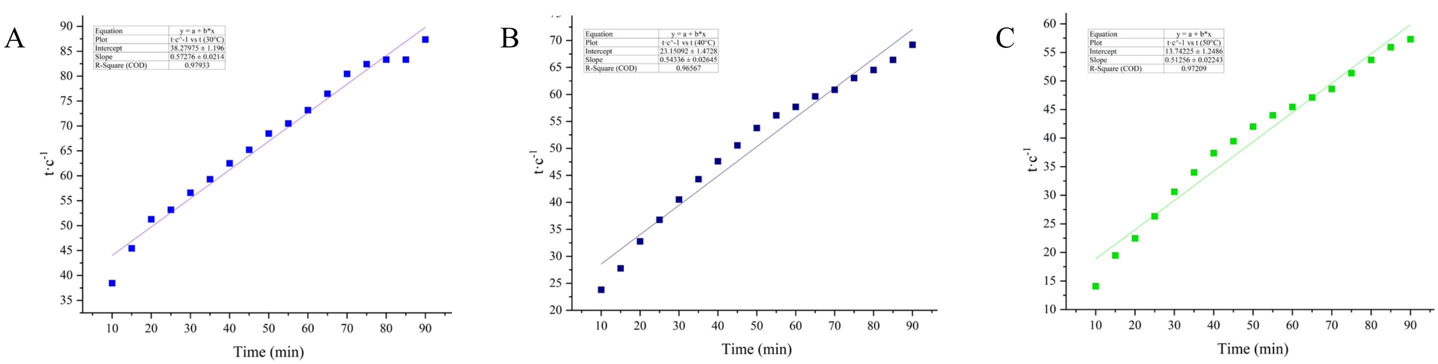

Figure 2. Calculation of the constants k_1 and k_2 in extractions made at 30°C (A), 40°C (B) and 50°C (C).

The results of the constants are presented in table 1. The mathematical models generated were the following:

$$C(t)_{30°C} = \frac{t}{38.2797 + 0.5728t}$$

$$C(t)_{40°C} = \frac{t}{23.1509 + 0.5434t}$$

$$C(t)_{50°C} = \frac{t}{13.7422 + 0.5126t}$$

Table 1. Peleg constants (k_1 and k_2) for solid-liquid extraction of caffeine and coefficients of determination.

Temperature (°C)	K_1 (min.100 g.g^{-1})	K_2 (100 g.g^{-1})	R^2
30	38.2797	0.5728	0.97933
40	23.1509	0.5434	0.96567
50	13.7422	0.5126	0.97209

variables in this particular context. This validation is essential, as it provides a reliable tool to predict the concentration of caffeine in leaves of I. *guayusa*, which can be crucial for the energy drink industry.

Conclusions

Temperature and extraction time were determining factors in obtaining caffeine from *Ilex guayusa* leaves. The Peleg equation proved to be adequate for modeling the solid-liquid extraction kinetics of caffeine under the studied conditions. The results of this study could contribute to the simulation and optimization of caffeine extraction kinetics in dried leaves of *I. guayusa*.

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Table 2. Comparison between the experimental values and those predicted by the Peleg model, expressed in g.100 g^{-1}.

Time (min)	Experimental (30°C)	Predicted (30°C)	Experimental (40°C)	Predicted (40°C)	Experimental (50°C)	Predicted (50°C)
10	0.24	0.23	0.37	0.35	0.64	0.53
15	0.33	0.32	0.50	0.48	0.74	0.70
20	0.39	0.40	0.61	0.59	0.85	0.83
25	0.47	0.48	0.68	0.68	0.95	0.94
30	0.53	0.54	0.74	0.76	0.99	1.03
35	0.59	0.60	0.81	0.83	1.08	1.10
40	0.64	0.65	0.87	0.89	1.15	1.17
45	0.69	0.70	0.91	0.95	1.23	1.22
50	0.74	0.75	0.98	0.99	1.24	1.27
55	0.78	0.79	1.02	1.04	1.29	1.31
60	0.82	0.83	1.09	1.08	1.32	1.35
65	0.85	0.86	1.09	1.11	1.38	1.38
70	0.87	0.89	1.15	1.14	1.44	1.41
75	0.91	0.92	1.19	1.17	1.46	1.44
80	0.96	0.95	1.22	1.20	1.49	1.46
85	1.01	0.98	1.24	1.23	1.51	1.48
90	1.03	1.00	1.27	1.25	1.52	1.50

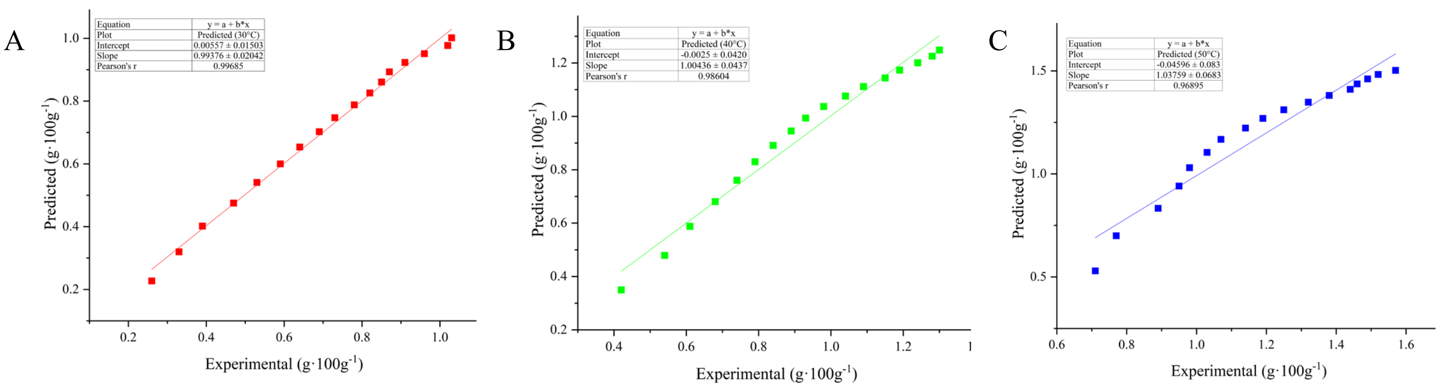

Figure 3. Experimental and predicted values of caffeine obtained at 30 °C (A), 40 °C (B) and 50 °C (C).

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