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CROP PRODUCTION

Mathematical modeling and effect of thin-layer drying and lyophilization on antioxidant compounds from ultrasonic-assisted extracted *Muntingia calabura* peels

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ABSTRACT. *Muntingia calabura* fruits are rich in bioactive compounds such as antioxidants, and the consumption of these compounds is associated with cancer prevention and aging. In this study, mathematical models were used to fit the experimental data of the *Muntingia calabura* peel drying kinetics, and the effective diffusion coefficient, activation energy and thermodynamic properties of the process were determined. Then, the effect of the drying temperature on the antioxidant activity and phenolic compounds of fruit peels was examined using conventional extraction and ultrasonication. Among the analyzed models, the logarithmic model was selected to represent the drying phenomenon of the calabura peel kinetics. The effective diffusion coefficient decreased by 74% as the temperature increased from 40 to 60°C, and the activation energy for liquid diffusion during drying was 23.96 kJ mol^{-1}. The enthalpy and entropy decreased with increasing temperature, while the Gibbs free energy increased by 5% for each 10°C increase in temperature. Regarding the content of phenolic compounds and the antioxidant activity of the calabura peel, it was observed that an increase in the drying temperature had a positive effect on the conservation of the bioactive compounds, making it possible to conclude that drying at 60°C and ultrasound extraction are the most suitable approach to conduct the process.

Keywords: effective diffusion coefficient; lyophilized; bioactive compounds.

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Introduction

Muntingia calabura L. is a fruit grown in Southeast Asia and Central and South America (Buhian, Rubio, & Martin-Puzon, 2017). Known popularly as both calabura or cherry jamaica, the plant belongs to the family Elaeocarpaceae, the only species in the genus *Muntingiacea* (Gomathi, Anusuya, & Manian, 2013).

Scientific studies on calabura have shown that stems, flowers, roots and fruits of the plant have antinociceptive properties (Amiruddin et al., 2016), anticarcinogenic activity (Nasir et al., 2017), antidiabetic activity (Sridhar, Thirupathi, Chaitanya, Kumar, & Mohan, 2011), antimicrobial activity (Patrick et al., 2017; Sufian, Ramasamy, Ahmat, Zakaria, & Yusof, 2013) and quinone reductase activity (Su et al., 2003).

Natural antioxidants, found in fruits and vegetables, cooperate in endogenous elimination of free radicals, and this effect is helpful to prevent diseases such as cancer and to aid in delaying cellular aging. In fruits, the retention rates of bioactive compounds in peels and seeds are higher than those in pulps (Morais et al., 2015)

The use of drying for fruits, seeds, peels and other foods is the oldest conservation technique that exists. In this way, the kinetics of thin-layer drying have been applied in plant material, such as pears (Silva, Figueiredo, Costa, & Guiné, 2014), apples (Vega-Galvez et al., 2012), passion fruit (Nascimento, Mulet, Ascheri, Carvalho, & Cárcel, 2016), rosemary leaves (Mghazli et al., 2017), tomatoes (Azeez, Adebisi, Oyedeji, Adetoro, & Tijani, 2019; Workneh & Oke, 2013), avocado (Avhad & Marchetti, 2016), hawthorn fruits (Aral & Bese, 2016), and grape seed (Roberts, Kidd, & Padilla-Zakour, 2008).

From the literature, there are no reports on the drying kinetics of *Muntingia calabura* or how drying affects the bioactive compounds of the fruit peel. The objective of the study was to select the best mathematical model to describe the drying curves, calculate the effective diffusion and thermodynamic properties, and evaluate the effects of forced convection drying and lyophilization on the antioxidant compounds from the peel using the ultrasound extraction method.

Material and methods

Plant material

Calabura (*Muntingia calabura*) mature fruits were harvested at the headquarters campus of the State University of Maringá (UEM) in the city of Maringá, Paraná State, Brazil (latitude: 23° 25' 31" S; Longitude: 51° 56' 19" W). The fruits were selected and sanitized with sodium hypochlorite solution at 200 ppm for 15 minutes at 25°C. The peels were divided into two parts: one part frozen at -18 ± 1°C to be used in the lyophilization process and the other part used to conduct the drying kinetics analysis.

Forced convection drying process and lyophilization

The forced convection drying process of the calabura peel was determined in triplicate at temperatures of 40, 50, and 60 ± 2°C in a greenhouse with recirculation of air at a constant speed of 1 m s^{-1}. The reading intervals were 15 minutes in the first hour, 30 minutes during the first six hours and then every 60 minutes until the fruit peels reached a constant mass with a final moisture content lower than 0.10 ± 0.01 g water g^{-1} peel (Aral & Bese, 2016). The dry samples in the drying kinetics experiment were coded as follows: dry sample at 40°C = CD40, at 50°C = CD50, and at 60°C = CD60. The samples of frozen peel at -18 ± 1°C were subjected to lyophilization with the aid of equipment (CHRIST Mark - Model Alpha 1-2 LDplus) for 24 hours.

Mathematical Models

To determine the values of the moisture content of the calabura (MR) fruit peel at any time, Equation 1 was used. Nonlinear regression analysis was performed by using the Gauss-Newton method to obtain the constants of the mathematical models selected. To evaluate each model, the coefficient of determination (R^2) (Equation 2), Chi-square test (X^2) (Equation 3), and square root mean error (RMSE) (Equation 4) were calculated.

$$MR = \frac{(M_X - M_{X0})}{(M_{Xi} - M_{X0})} \quad (1)$$

$$R^2 = \sum_{i=1}^{N} \frac{MR_{est} - \bar{M}R_{obs}}{(MR_{obs} - \bar{M}R_{obs})^2} \quad (2)$$

$$\mathrm{X}^2 = \sum_{i=1}^{n} \frac{(\mathrm{MR_{obs}} - \mathrm{MR_{est}})}{\mathrm{GLR}} \quad (3)$$

$$\mathrm{RMSE} = \sqrt{\left(\frac{\sum_{i=1}^{n}(\mathrm{MR_{obs}} - \mathrm{MR_{est}})^2}{\mathrm{GLR}}\right)} \quad (4)$$

where: MR is the water content ratio (dimensionless value), Mx is the water content of the product represented on a dry basis (b.s), Mx0 is the equilibrium water content of the product (b.s.), and Mxi is the initial water content of the product (b.s). MRobs is the value observed experimentally; MRest is the value calculated by the model; and GLR is the degrees of freedom of the model (observations minus the number of parameters of the model).

To adjust the experimental data collected during the drying process of the *Muntingia calabura* peel, the most commonly used mathematical models for plant products were selected (Table 1).

Determination of the effective diffusion coefficient and Thermodynamic Properties

To obtain the thickness (L) of the calabura peel, a digital micrometer was used; the average thickness was calculated based on 90 repetitions, 30 fruit peels were evaluated, and in each peel, the thickness was evaluated at three different points. The average value found for the thickness of the peel was 0.176 mm. The shape of the calabura peel was geometrically similar to a flat plate, and the geometric form of the product is considered to approximate eight terms based on Fick's second law (Equation 5) (Darvishi et al., 2014). The thickness of the shell was considered the value for the thin layer. The activation energy was calculated by the Arrhenius equation (Equation 6).

$$MR = \frac{(Mx - Mxo)}{(Mxi - Mxo)} = \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)} \exp\left[(2n+1)^2 \pi^2 Di \left(\frac{t}{4L}\right)^2\right] \quad (5)$$

$$D_{eff} = Do\ exp \frac{-Ea}{RT} \quad (6)$$

where: MR is the ratio of the water content (dimensionless value); "n" is the number of terms in the model; Di denotes the effective diffusion coefficient (m^2 s^{-1}); “t” is the drying time (s); and L is the thickness of the product (m). Do is the pre-exponential factor; Ea is the activation energy (kJ mol^{-1}); R is the universal gas constant (8,134 kJ $kmol^{-1}$ K^{-1}); and T is the absolute temperature (K).

Table 1. Mathematical models used in *Muntingia calabura* drying kinetics.

Name of model	Mathematical expression.	References
Difussion Approach	MR = a exp(-kt) + (1-a) exp(-kbt)	(Akpinar, 2006)
Two Term	MR = a exp(-k_1t) + b exp(-k_2t)	(Sharaf-Eldeen, Blaisdell, & Hamdy, 1980)
Henderson & Pabis	MR = a exp(-kt)	(Henderson, 1974)
Logaritmic	MR = a exp(-kt) + c	(Kingsly, Goyal, Manikantan, & Ilyas, 2007)
Midilli et.al. (2002)	MR = a exp(-kt^n) + bt	(Midilli et al., 2002)
Newton	MR = exp(-kt)	(Lewis, 1921)
Page	MR = exp(-kt^n)	(Page, 1949)
Thompson	MR = exp(-a-(a^2+4bt)^{0,5}/(2b))	(Thompson, Peart, & Foster, 1968)
Valcam	MR = a + bt + $ct^{1,5}$ + dt^2	(Madamba, Driscoll, & Buckle, 1996)
Verma	MR = a exp(-k_1t)) + (1-a) exp(-k_2t)	(Lalit, Verma, Bucklin, Endan, & Wratten, 1985)

The thermodynamic properties associated with the drying process were determined according to the method proposed by Jideani and Mpotokwana (2009). Equations 7, 8, and 9 were used to calculate the specific enthalpy, specific entropy and Gibbs free energy, respectively.

$$\Delta H = E_a - RT_a \quad (7)$$

$$\Delta S = R\left(ln\, D_0 - ln\frac{K_B}{h_p} - ln\, T_a\right) \quad (8)$$

$$\Delta G = \Delta H - T_a\Delta S \quad (9)$$

where: ΔH is the specific enthalpy (J mol^{-1}); ΔS is the specific entropy (J mol^{-1} K^{-1}); ΔG is the Gibbs free energy (J mol^{-1}); KB is the Boltzmann constant (1.38 x 10^{-23} J K^{-1}); and hp is Planck's constant (6.626 x 10^{-34} m^2 J^{-1} s^{-1}).

Ultrasonic and conventional assisted extraction

The extraction of the bioactive compounds from the calabura peel was performed in an ultrasonic bath in indirect contact (Model USC - 1400) with an ultrasonic frequency of 40 kHz and ultrasonic power of 135 watts RMS and by the method of extraction by maceration (conventional). The extraction conditions (time 45 minutes, temperature 40°C and 25% aqueous ethanol) for both methodologies were identical for comparison purposes. The extracts were obtained with modifications of the method of Singh, Chidambara Murthy, and Jayaprakasha (2002); samples of peel at a ratio of 1:25 mg mL^{-1} (peel/solvent) were placed in an Erlenmeyer flask and homogenized for 5 minutes in a homogenizer at a temperature of 25°C.

Analyses to quantify bioactive compounds

The ABTS radical assay was performed according to Rufino et al. (2007), and the results are expressed in µM Trolox g^{-1} sample. The iron reduction power (FRAP) method was determined according to Benzie and Strain (1996), and the antioxidant potential of the extracts was determined in triplicate based on the calibration curve drawn using ferrous sulfate ($FeSO_4$) (2 mmol L^{-1}), expressed in µM $FeSO_4$ mg^{-1} sample. The content of total phenolic compounds (TPC) was determined by the Folin-Ciocalteu (FC) spectrophotometric method, according to Singleton and Rossi (1965), expressed in mg gallic acid 100 g^{-1} of sample, using the standard curve of gallic acid.

Statistical analysis

The experimental data were recorded as the mean ± standard deviation, and three replicates were made for each sample. The results were analyzed by the analysis of variance (ANOVA) method, followed by the Tukey test with 95% confidence interval, using Statistic 8.0 software.

Results and discussion

Drying kinetics and thermodynamic properties

The peel of *Muntingia calabura* fruits submitted to drying at temperatures of 40 to 60°C presented an initial mean moisture content of 3.00 ± 0.495 g of water 100 g^{-1} of dry matter (decimal b.s.). The time required to dry the peels to equilibrium moisture content values of 0.0123 ± 0.001, 0.0139 ± 0.001, and 0.0135 ± 0.001 (b.s.) was 990, 690, and 510 minutes at 40, 50, and 60°C, respectively. The time needed to reach the equilibrium moisture of the sample decreased with increasing temperature; at 40°C, it took 30% longer than at 50°C, 26% longer at 50°C than at 60°C, and 48% longer at 40°C than at 60°C (48%).

This phenomenon was also observed by Kaleta and Górnicki (2010) in drying apple peels, by Oliveira, Nascimento, Borges, Ribeiro, and Ruback (2002) for pear slices, and by Zielinska and Michalska (2016) with blueberry fruits. It is also worth mentioning that during the initial 60 minutes of drying, a higher reduction in humidity was observed for the higher temperature, and at 60°C, the humidity was 22.8% lower than at the temperatures of 40°C and 14.03% lower than at 50°C.

According to Fratianni et al. (2017), the temperature used in drying is related to the internal diffusion of the sample, which is responsible for the loss of water in the process; as the internal resistance for the conduction of water in the liquid state increases, the speed of drying decreases.

To evaluate the quality of the adjustments, the highest values of R^2 and the lowest values of X^2 and RMSE were used as the criteria for comparison. Table 2 indicates all the values found for each model, and all models presented values greater than 0.99 for R^2 and less than 0.0004 for X^2, and 0.01 for RMSE.

Regarding the comparison criteria, the models that presented the best adjustments for the three drying temperatures (40, 50, and 60°C) were the Midilli models, followed by the Valcam and Logarithmic models. In food-drying studies, similar results were found in slices of tomato, strawberry, gooseberry, hawthorn, and apple (Aral & Bese, 2016; Azeez et al., 2019; Junqueira, Luiz, & Corr, 2017; Kaleta & Górnicki, 2010; Méndez-Lagunas, Rodríguez-Ramírez, Cruz-Gracida, Sandoval-Torres, & Barriada-Bernal, 2017).

The models of Midilli and Valcam present four coefficients (a, k, n, and b; a, b, c, and d, respectively), while the logarithmic model presents only three coefficients (a, c, and k). Thus, the logarithmic model was chosen as the best model to represent the drying phenomenon of *Muntingia calabura* peel considering its simplicity in relation to the other models.

Table 2. Coefficients of determination (R^2, decimal), Chi-square test (X^2, decimal) and square root mean error (RMSE, decimal) for the ten mathematical models used to describe the drying process of *Muntingia calabura* peel at 40, 50, and 60°C.

Model Name	Drying temperatures								
	40°C			50°C			60°C		
	R^2	χ^2	RMSE	R^2	χ^2	RMSE	R^2	χ^2	RMSE
Difussion Approach	0.9989	0.00023	0.01503	0.9995	0.00012	0.01117	0.9979	0.00046	0.02155
Two Term	0.9996	0.00010	0.00989	0.9995	0.00012	0.02203	0.9985	0.00036	0.01897
Henderson e Pabis	0.9996	0.00009	0.00950	0.9995	0.00011	0.01043	0.9985	0.00031	0.01774
Logaritmic	0.9996	0.00009	0.00938	0.9997	0.00007	0.00863	0.9989	0.00025	0.01588
Midilli, Kucuk, and Yapar (2002)	0.9999	0.00003	0.00542	0.9998	0.00005	0.00696	0.9993	0.00017	0.01314
Newton	0.9989	0.00021	0.01442	0.9994	0.00012	0.01075	0.9979	0.00041	0.02024
Page	0.9996	0.00009	0.00927	0.9995	0.00011	0.01067	0.9983	0.00035	0.01879
Thompson	0.9992	0.00017	0.01292	0.9995	0.00012	0.01099	0.9980	0.00043	0.02079
Valcam	0.9998	0.00004	0.00650	0.9998	0.00004	0.00628	0.9994	0.00015	0.01205
Verma	0.9989	0.00023	0.01501	0.9995	0.00012	0.01117	0.9979	0.00046	0.02155

The increase in temperature directly affected the effective diffusion of the sample since as observed in Table 3, there was a decrease of 74% in Def compared to that at temperatures of 40 and 60°C. Similar values were found in the literature for fruits and vegetables under similar temperature conditions, with Def values of 15.30 x 10^{-9} m^2 s^{-1} for apple (Vega-Galvez et al., 2012). In the drying procedures, the lower the activation energy is, the greater the water diffusivity is in the product.

The activation energy was calculated through the Arrhenius plot, and the average value for the three drying temperatures (40, 50, and 60°C) employed in the calabura peel was 23.96 kJ mol^{-1}. Galaz et al. (2017) found a value of 58.9 kJ mol^{-1} for pomegranate peel, and Nascimento et al. (2016) found a value of 43.86 kJ mol^{-1} for passion fruit peel; regarding the activation energy found for the calabura peel, it is stated that the structure of the material interferes directly with the barrier that must be broken so that effective diffusion occurs in the sample.

When analyzing the values of the thermodynamic properties shown in Table 3, it can be seen that the specific enthalpy (ΔH) decreased as the temperature used in the drying kinetics experiments increased (40, 50, and 60°C), confirming that the higher the temperature used, the less energy that will be spent during the drying process, thus explaining why the time to reach RX at 60°C is half that used at 40°C. On the other hand, the specific entropy (ΔS) and Gibbs free energy had a reverse behavior to that of the enthalpy, with values increasing as the temperatures increased. The small entropy change from -0.3399 to -0.3404 is related to the low variation of the temperatures used (10°C), and the negative values are usually related to the changes in the structure of the material.

Table 3. Thermodynamic properties of *Muntingia calabura* peel: specific enthalpy (ΔH), specific entropy (ΔS), Gibbs free energy (ΔG), and effective diffusion (Def).

Propriedades termodinâmicas				
Temperature (K)	ΔH (kJ mol^{-1})	ΔS (kJ mol^{-1} K^{-1})	ΔG (kJ mol^{-1})	Def (m^2 s^{-1})
313.16	21.3607	-0.3399	127.4250	1.9164 x 10^{-11}
323.16	21.2775	-0.3402	131.1610	2.5447 x 10^{-11}
333.16	21.1944	-0.3404	134.8996	3.3302 x 10^{-11}

Effects of drying temperatures on antioxidant compounds

Quantification of the phenolic compounds and antioxidant activity are presented in Table 4. Of the four samples analyzed (CD40, CD50, CD60, and lyophilized), those with the best bioactive compound values were the CD60 and lyophilized samples, and the type of extraction used also influenced these results.

With the dry samples that underwent the forced convection drying process, the phenolic content was not significantly different between the CD40, CD50, and CD60 samples when compared to that from conventional extraction. However, in the extraction method performed with ultrasonication, the phenolic content of the CD60 sample showed a significant difference in relation to that of samples CD40 and CD50. Chen et al. (2015) explain that higher temperatures favor the extraction process since they increase both the diffusion coefficient and the solubility of the sample; however, the maximum temperature that the sample can be subjected to must be considered because very high temperatures end up denaturing the phenolic compounds.

Méndez-Lagunas et al. (2017) studied the drying effects with conventional extraction of bioactive compounds from strawberry residues and reported values ranging from 1,230.1 to 848 mg of gallic acid 100 g^{-1} for dry samples at 50 and 60°C. Siqueira, Rosa, Fustinoni, Sant'Ana, and Arruda (2013) extracted conventional antioxidants from Brazilian Cerrado fruits and reported values of 580 ± 143 and 1,095 ± 159 mg of gallic acid 100 g^{-1} in ethanoic and aqueous solvents, respectively, from *Annona crassiflora* Mart. and 651 ± 61 and 1,015 ± 62 mg of gallic acid 100 g^{-1} from *Genipa americana* L. under extraction conditions of 30°C for 60 minutes. It is possible to observe that the results reported in the literature are similar to those found for calabura peel.

According to Hani, Torkamani, Zainul Abidin, Mahmood, and Juliano (2017), ultrasonic waves facilitate the penetration of solvents into the cellular matrix because they cause amplification of diffusion into the medium and allow the use of milder temperatures in the extraction process, resulting in higher yields.

Regarding the antioxidant activity, the values from the FRAP and ABTS methods showed that when comparing the type of extraction (ultrasound or conventional), there was a significant difference among all the dry samples by forced convection. When comparing the drying kinetics samples with each other, CD60 obtained the best results, and when the drying methods were compared, there was, in large part, no significant difference between the CD60 and lyophilized samples.

Chen et al. (2015) explained that the higher the temperature is, the higher the diffusion coefficient and the better the solubility of the sample. However, caution must be taken because very high temperatures will denature the phenolic compounds to be studied. In general, with increasing drying temperature, the phenolic compound and antioxidant activity indexes increased significantly, positive impacting these values.

Table 4. Effect of drying temperatures on phenolic compounds and antioxidant activity of *Muntingia calabura* peels. Mean values for TPC (mg gallic acid 100 g^{-1}), antioxidant activity ABTS (µM Trolox g^{-1}), and FRAP (µM $FeSO_4$ mg^{-1}).

Extraction		Sample			
		CD40	CD50	CD60	Lyophilized
Conventional	TPC	1,102.83 ± 87.37^{bB}	1,086.17 ± 5.77^{bA}	1,092.83 ± 81.29^{bB}	1,544.5 ± 207.85^{aA}
	ABTS	22.56 ± 2.07^{bB}	29.94 ± 1.99^{bB}	34.9 ± 0.94^{abB}	45.31 ± 1.5^{aA}
	FRAP	482.32 ± 39.81^{cB}	582.46 ± 12.96^{cB}	782.74 ± 45.4^{bB}	857.6 ± 95.34^{aA}
Ultrassound	TPC	1,322.83 ± 135.31^{bcA}	1,246.17 ± 60.48^{cA}	1,579.5 ± 43.3^{aA}	1,531.17 ± 87.8^{abA}
	ABTS	48.61 ± 1.1^{bA}	48.51 ± 9.6^{bA}	62.71 ± 10.22^{aA}	51.43 ± 4.39^{abA}
	FRAP	587.81 ± 27.53^{cA}	739.96 ± 65.74^{bA}	888.22 ± 33.71^{aA}	1,159.96 ± 6.68^{aB}

Expressed results with mean ± standard deviation (triplicate). ^{abcd} The values with lowercase letters on the same line are not significantly different (Tukey test, p < 0.05). ^{ABCD} Values with uppercase letters in the same column and of the same chemical analysis are not significantly different (Tukey test, p < 0.05).

Conclusion

The logarithmic model best fit the data, and it can be used to represent the kinetics of drying for the peels. The lyophilization method can be replaced by convective drying at 60°C since the former is an expensive method. In relation to conventional extraction and ultrasonication, ultrasound-assisted techniques presented better results for phenolic compound amounts and antioxidant activity values. This study may provide useful information for future work as it provides data on the drying behavior and thermodynamic properties of calabura peel and the results of unconventional extraction methods.

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