

Optimization of Anthocyanin Extraction from Roselle (*Hibiscus sabdariffa*) Calyces: RSM, Kinetic Modelling, Mass Transfer and Thermodynamic Studies

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Abstract

Roselle calyces (*Hibiscus sabdariffa*) are becoming very important in the food and beverage industry, especially because of the presence of anthocyanin which is an antioxidant responsible for their red colour. The effect of processing parameters such as contact time, temperature and calyx-water ratio on the anthocyanin content of roselle calyces extract was studied and optimized along with evaluation of kinetic models, mass transfer and thermodynamic parameters. Extraction kinetics for anthocyanin were obtained at different time (5, 10 and 15 min), temperature (30, 50, 75 and 100 °C) and calyx-water mass ratio (1:50, 1:20 and 1:10). The maximum anthocyanin yield was obtained at 15 min; 100 °C and ratio of 1:10. The data obtained were fitted to 6 different extraction models and the ones that best suited the data were Weibull type, Peleg and Pseudo-second-order with Adj. R² of 0.98, 0.99 and 0.99 respectively. The data obtained were used to calculate the kinetic, mass transfer and thermodynamic parameters. The kinetic variables were also related to the fractional extraction or conversion model. The fractional extraction increased with increased temperature and calyx-water. The effective diffusion coefficient ranged between 1.04×10^{-11} to 1.48×10^{-11} m²/s. The mass transfer coefficient calculated ranged between 1.62×10^{-8} and 11.02×10^{-8} (m/s), Biot number ranges from 25 to 168. The thermodynamic properties: Activation energy ranged from 15.7 to 16.4 kJmol⁻¹; the enthalpy from 36.60 to 58.30 kJmol⁻¹; the entropy from 88 to 147 JK⁻¹mol⁻¹, and the Gibbs free energy from -5.80 to -11 kJmol⁻¹. The extraction process was observed to be endothermic, feasible and spontaneous.

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Keywords

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Introduction

Roselle plant (*Hibiscus sabdariffa*) known as *zobo* in Nigeria is usually cultivated for its calyces, though the seeds and leaves are

also useful (Omobuwajo *et al.*, 2000). Roselle calyces usually found in West Africa, Asia and South America is an underutilized food material that has a lot of

potentials (Meftahizadeh *et al.*, 2022). The calyces are known for its nutritional benefits due to the presence of antioxidants, organic acids, vitamins and minerals (Abdelhameed *et al.*, 2021; Abidoye *et al.*, 2022; Da-Costa-Rocha *et al.*, 2014). From literature, it has numerous health benefits and these include reduction of risks of coronary heart disease, cancer, and stroke (Lee *et al.*, 2009; Liu *et al.*, 2006; Wrolstad, 2004). There are ongoing studies on its potential in the production of fruit drinks (Ai *et al.*, 2021). The export value is very high. Its extract can be used as a raw material in the beverage and food industry (Cisse *et al.*, 2011).

Anthocyanin which is responsible for the red coloring of the calyces is an antioxidant that is very beneficial to humans. They occur in the calyces mainly in the form of delphinidin-3-sambubioside and cyanidin-3-sambubioside (Cissé *et al.*, 2012; Juliani *et al.*, 2009) and occur in minor compounds in the form of delphinidin-3-glucoside and cyanidin-3-glucoside (Ali *et al.*, 2005). They are water soluble pigments that are also found in fruits such as grapes, tomatoes, raspberry, cranberry etc. (Alara & Abdurahman, 2019; Basu *et al.*, 2010).

Solid-liquid extraction is the ideal means for the separation of bioactive components with antioxidant ability from plant resources (Ochoa-Velasco & Ruiz, 2019). Numerous factors influence the extraction technique, and the outcome should be determined experimentally and a number of models, including empirical and theoretical models, have been defined to investigate extraction data. Empirical models predominantly comprise response surface equations, which are used to define the influence of processing variables such as contact time, process temperature and solvent-to-solute ratio on particular parameters of the extraction procedure, such as the quantity or outcome of the precise constituents under study (Alara & Abdurahman, 2019; Xu *et al.*, 2017), though, these equations do not offer facts about their dynamic or mass transfer properties.

Mathematical modelling of solid-liquid extraction processes is an essential engineering technique in the design process which aims to reduce energy, time, and materials consumption (Jurinjak Tušek *et al.*, 2016; Matešić *et al.*, 2021). Kinetic modelling is of great importance for understanding complex diffusion, mass transfer and thermodynamic parameters affecting the extraction. Some of the models used include Peleg (Peleg, 1988), Second order (Jo & Kim, 2019; Park & Kim, 2018) and Weibull (Sant'Anna *et al.*, 2012). Effective diffusion coefficients, extraction rates and thermodynamic parameters of the extraction process can be calculated using the mentioned models (Janković *et al.*, 2021; Jurinjak Tušek *et al.*, 2016). Gibbs free energy signifies the useful work obtainable from a thermodynamic system at constant temperature and pressure, and the free energies of the various components of the extraction must be established in order to estimate Gibbs energies during chemical transformations (Janković *et al.*, 2021).

The mass transfer properties of anthocyanins from roselle calyces have been examined by several researchers (Cisse *et al.*, 2011; Ochoa-Velasco & Ruiz, 2019). However, those properties and thermodynamic characteristics of whole calyces were not reported. The aim of this study was optimization of anthocyanin extraction from roselle (*Hibiscus sabdariffa*) calyces, and for this purpose, the objectives were (i) determine the response surface models of anthocyanin content in the extract as a function of processing variables (contact time, process temperature and calyx-water ratio); (ii) to perform curve-fitting of the experimental data to extraction models and finally (iii) determine the kinetic and thermodynamic parameters of the extraction process.

Materials and methods

Sample preparation

Dried roselle calyces were purchased from Ogbete main market in Enugu, Nigeria. They were portioned into 5, 12.50, and 25 g and immersed in a conical flask with 250

mL distilled water. Distilled water was used as the solvent because it was established to be the most suitable for the extraction of bioactive materials from the calyces (Sindi et al., 2014). The calyx water mass ratio was 1:50, 1:20 and 1:10. The extraction temperatures were 30, 50, 75 and 100 °C and the extraction times were 5, 10 and 15 min. A water bath system with temperature control was set up and used to prepare the samples as shown in Fig. (1). The heater (1000W 220V, FP-234, China) raises the temperature of the water in the bath, while the thermocouple (K-type) senses the temperature of the calyx-water mixture. It then sends a signal to the temperature controller (REX C900, Thincol, China) which sends to the contactor (32 amps 220V). When the temperature gets to the set point, the circuit breaker trips off the current from the power source till the temperature go 1 °C below the set point. After the set time for the extraction, the extract was separated from the raffinate using a filter paper (Grade 1 Whatman 125 mm). A total of 36 samples were collected and labeled accordingly and stored at 4 °C for further analysis.

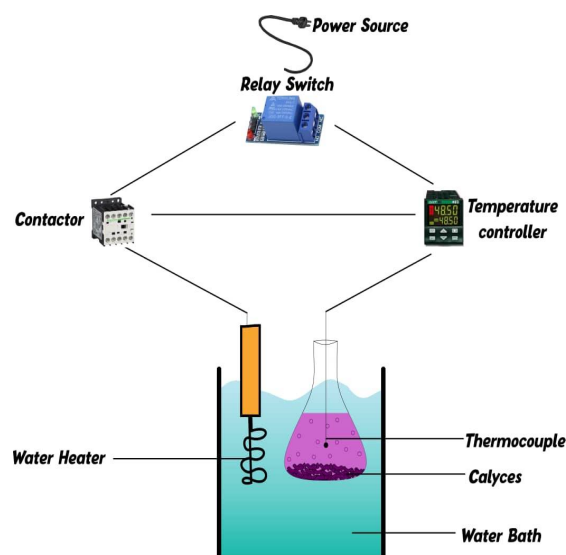


Fig. 1. Experimental setup for aqueous extraction of roselle calyces

Anthocyanin tests

Total anthocyanins content of Roselle extract was determined colorimetrically according to the procedure described by

Aly et al. (2019) and Du & Francis (1973). 5 mL of the sample was diluted with distilled water to 50 mL. Acidified methanol (1% HCl) was used to extract anthocyanin from the solution. A UV Vis spectrophotometer (UV-1200, Kyoto, Japan) was used to measure the absorbance of the clear filtered pigment solution at 535 nm, where the molecular weight of cyanidin 3-O-glucoside was 449.2 gmol⁻¹ and the molar extinction coefficient was 26.900 L.mol/cm. The total anthocyanins content referred to cyaniding -3-sambubioside was calculated using the Eq. (1). The tests were carried out in triplicates.

$$An(mg/100g) = \frac{a \times df \times 100}{m \times 55.9} \quad (1)$$

Where, An is total anthocyanin, a is absorbance, df is dilution factor and m is sample weight.

Response surface methodology

The analyzed results were subjected to statistical analysis using Design expert 11. A 3×4×3 factorial composite design was conducted in triplicates of central point to determine the effect of extraction contact time, process temperature and calyx-water solvent on the anthocyanin content of the extract.

The response surface can be represented in this second order polynomial in Eq. (2).

$$A = A_0 + A_1X + A_2Y + A_3Z + A_{12}XY + A_{13}XZ + A_{23}YZ + A_{11}X^2 + A_{22}Y^2 + A_{33}Z^2 \quad (2)$$

Where, A is the anthocyanin content, X , Y and Z are the coded values of extraction time, temperature and calyx-water ratio respectively, A_0 , A_1 , A_2 , A_3 , A_{12} , A_{13} , A_{23} , A_{11} , A_{22} and A_{33} are regression coefficients.

Extraction yields

The extraction yield is as shown in Eq. (3).

$$Y = \frac{m_e}{m_c} \quad (3)$$

Where Y is extraction yield, m_e is mass of anthocyanin in extract and m_c is mass of anthocyanin in calyces.

Table 1. Extraction models used for this study

Model name	Equation	Source
Weibull type	$C = C_o \exp(kt^n)$	(Sant'Anna <i>et al.</i> , 2012)
Two rates	$C = A[1 - \exp(-Bt)] + C[1 - \exp(-D)t]$	(Amendola <i>et al.</i> , 2010)
Sorption/Desorption (Peleg)	$C = \frac{1}{k_1 + k_2 t}$	(Corradini & Peleg, 2007; Matešić <i>et al.</i> , 2021)
Minchev and Minkor	$C = A - B \exp(-kt)$	(Amendola <i>et al.</i> , 2010; Simeonov <i>et al.</i> , 1999)
Pseudo first order	$C = C_\infty - \frac{C_\infty}{\exp(kt + a)}$	(Jo & Kim, 2019)
Pseudo 2 nd order	$C = \frac{C_\infty^2 kt}{1 + C_\infty kt}$	(Jo & Kim, 2019; Park & Kim, 2018)

Kinetic modeling

Mathematical modelling was employed to investigate the kinetics of the solid-liquid extraction process. Experimental data were fitted to different extraction kinetic models namely Weibull type, two rates, Sorption/Desorption (Peleg), Minchev and Minkor, pseudo first order and pseudo 2nd order (Table 1) using non-linear regression, minimizing the squared errors by using Gauss-Newton method from Curve Expert Professional. Adjusted R² and root mean square error (RMSE) were used to estimate how well these models represent the experimental data.

According to (Sabbaghi, Ziaifar, *et al.*, 2018b), the following equations Eq. (4) and Eq. (5) are used for calculating Adj R² and RMSE, respectively. In these equations, *o* and *P* are observed value and predicted value, respectively, *n* is the number of observations and *p* is the number of model parameters.

$$Adj R^2 = R^2 - \frac{p-1}{n-p} (1 - R^2) \tag{4}$$

$$RMSE = \sqrt{\frac{(o-p)^2}{n-p}} \tag{5}$$

Fractional extraction

Fractional extraction follows fractional conversion models. The fractional conversion model indicates the amount of reaction required at a given time to complete a phenomenon (Sabbaghi, Ziaifar, *et al.*, 2018b). Fractional extraction is calculated using Eq. (6).

$$E = \frac{C_i - C_f}{C_i - C_e} = \exp[-kt] \tag{6}$$

Where *E* is fractional extraction, *C_i* is initial concentration, *C_f* is final concentration, *C_e* is equilibrium concentration, *k* is rate constant and *t* is time.

Effective diffusion coefficient

Fick's second law (Eq. 7) was used to describe anthocyanin diffusion from inside the spherical biomass to the surface during the extraction process (Yedhu Krishnan *et al.*, 2016).

$$\frac{Y_t}{Y_s} = 1 - \frac{6}{\pi^2} \exp\left(-\frac{D_e \pi^2 t}{r^2}\right) \tag{7}$$

Taking the natural logarithm of the equation, Eq. (8) is obtained.

$$\ln\left(\frac{Y_t}{Y_s}\right) = \ln\frac{6}{\pi^2} - \frac{D_e \pi^2 t}{r^2} \tag{8}$$

Where, *Y_t* is yield of anthocyanin at time *t* (kg dry matter/kg solvent), *Y_s* is the yield at saturation state (kg dry matter/kg solvent), *D_e* is effective diffusion coefficient (m²/s), *t* is time (s) and *r* is cell radius (m); *D_e* is calculated from each slope by plotting $\ln\left(\frac{Y_t}{Y_s}\right)$ versus *t*.

Mass transfer coefficient

Analytical expression of diffusive mass flux based on Fick's first law was used to describe the mass transfer of anthocyanin from the surface to the solvent (Yedhu Krishnan *et al.*, 2016).

$$\ln \frac{C_s}{C_s - C_t} = \frac{M_T A}{V_l} t \tag{9}$$

C_s , is concentration at saturation (g/L), C_t concentration at time t (g/L), M_T mass transfer coefficient (m/s), A total surface area of particles (m^2) and v_l volume of solution (m^3). M_T is calculated from the slope of $\ln \frac{C_s - C_t}{C_s - C_0}$ versus t .

Biot number

To analyse the behaviour of mass transfer, dimensionless Biot numbers were used to characterize the diffusion parameters during extraction. The Biot number is a ratio of internal and external diffusion resistance that connects relative transport resistances for solid extraction in liquid phase. (Jo & Kim, 2019). Eq. (10) was used to obtain the Biot number (Bi) for the solid-liquid extraction process. Where P_s is particle size (m).

$$Bi = \frac{P_s M_T}{D_e} \quad (10)$$

Thermodynamic study

Activation Energy

Using the Arrhenius equation (Eq. 11) and its linear form as (Eq. 12) according to Sabbaghi, Ziaifar, *et al.* (2018a) and Sabbaghi, Ziaifar and Kashaninejad (2018), the activation energy was determined, which was used to illustrate the temperature-extraction rate constant relationship.

$$k = A e^{-\frac{E_a}{RT}} \quad (11)$$

$$\ln(k) = \ln(A) - \left(\frac{E_a}{R}\right) \frac{1}{T} \quad (12)$$

Where, k is the extraction coefficient (min^{-1}), A is the pre-exponential factor (min^{-1}), E_a is the activation energy (kJmol^{-1}), R is the gas constant ($8.314 \text{ Jmol}^{-1}\text{K}^{-1}$), and T is the absolute temperature (K).

A plot of $\ln(k)$ versus $\frac{1}{T}$ gives a straight slope where $\left(-\frac{E_a}{R}\right)$ represents the activation energy of the extraction process and the intercept is the Arrhenius constant (or pre-exponential factor).

Equilibrium constant

The equilibrium constant is calculated using Eq. (13).

$$k_e = \frac{C_L}{C_s} \quad (13)$$

Where, k_e is the equilibrium constant, C_L is the concentration of anthocyanin in the extract and C_s is the concentration of anthocyanin in the calyces.

Enthalpy change, entropy change and Gibbs free energy change. The major driving factor of the solid liquid extraction process was diffusion of the solute from the solid into the liquid solvent. The standard enthalpy of formation and the standard entropy of the compound can be used to calculate the standard free energy of formation (Janković *et al.*, 2021), as indicated in Eq. (14).

$$\Delta G = -RT \ln k_e \quad (14)$$

Where ΔG is standard Gibbs free energy change (kJmol^{-1}). The change in enthalpy and entropy was calculated using Van't Hoff equation (Jurinjak Tušek *et al.*, 2016) shown in Eq. (15).

$$\Delta G = \Delta H - T \Delta S \quad (15)$$

ΔH is standard enthalpy change (kJmol^{-1}), and ΔS is the standard entropy change ($\text{JK}^{-1}\text{mol}^{-1}$). Combining Eq. (14) and (15) we get Eq. (16).

$$\ln K_e = \frac{\Delta G}{RT} = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (16)$$

ΔH and ΔS was gotten by using the slope and y-intercept of the plot $\ln K_e$ against $\frac{1}{T}$, centered on Eq. (16).

Results and discussion

Effects of extraction time, temperature and calyx-water ratio

It was generally observed that elevated temperature increases the anthocyanin concentration of the extract; this is as a result of increased solubility which facilitates the diffusion of the anthocyanin

from the calyces to the solvent. This trend was reported by researchers for paclitaxel extraction from *Taxus chinensis* (Jo & Kim, 2019). The yield also increased with higher calyx water ratio except at 100 °C where the yield reduced from 1:20 to 1:10. This may be as a result of the increased saturation at high temperatures of the anthocyanin. Fig (2) show the 3D response surface of extraction, the effect of time temperature and calyx-water ratio on the extraction yield. A rise of the solvent volume in the system increased the extraction yield leading to a more efficient extraction. These results were consistent with the equilibrium and mass transfer principles. The driving force during mass transfer was the concentration gradient between solid and liquid which was greater for larger liquid-to-solid ratio, resulting in an increase of the diffusion rate (Figs. 2B and 2C).

The ANOVA shows that the extraction time, temperature and mass ratio of the calyx-solvent ratio had a significant linear

and interaction effect on the anthocyanin content of the extract. Only temperature and calyx-solvent ratio had a significant quadratic effect on the anthocyanin content.

$$An = 3.10 - 0.04t - 0.19T + 0.01m + 0.003tT + 0.002tm + 0.001Tm - 0.004t^2 + 0.001T^2 - 0.0005m^2$$

$$R^2 = 0.94$$

An increase of the extraction temperature resulted in higher yield of anthocyanin, This could be due to the softening of plant tissue as reported by other researchers (Ali *et al.*, 2018; Shi *et al.*, 2003).

A decline in anthocyanin content of the extract was not detected as reported by Cissé *et al.* (2012). This could be because the extraction time during the experiment was not extended beyond 15 min which can bring about destruction of the anthocyanin molecule.

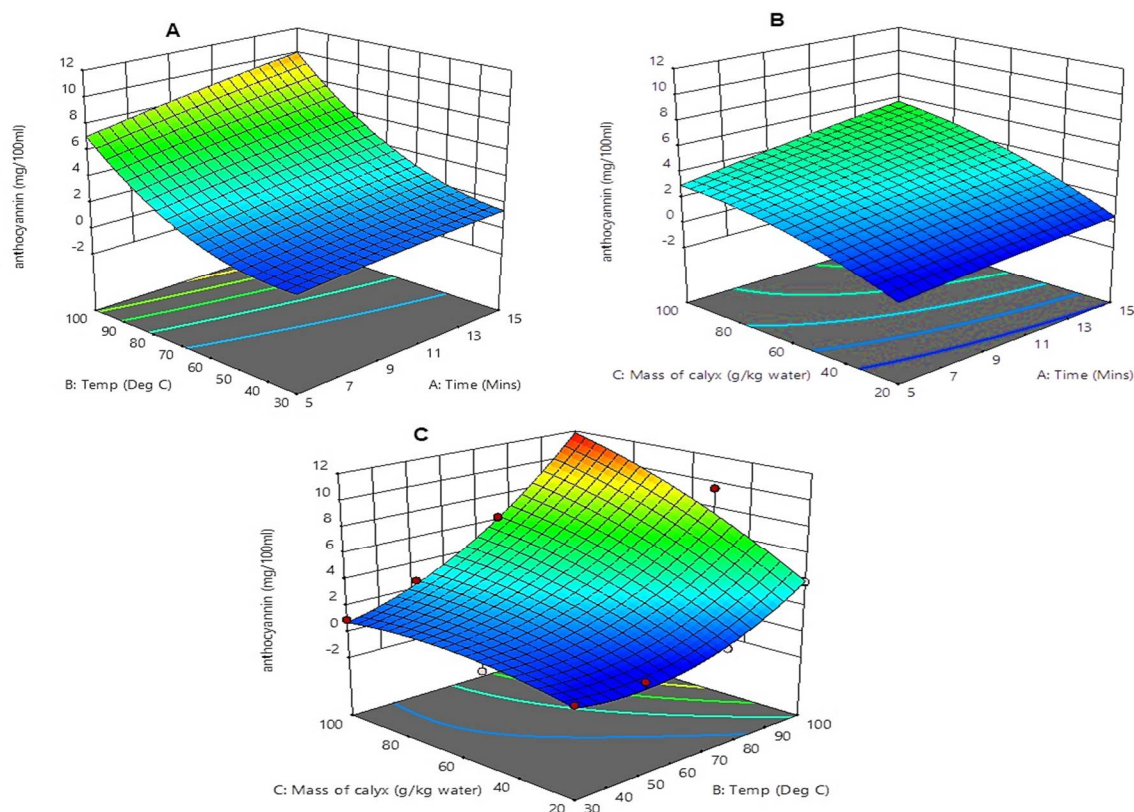


Fig. 2. 3D response surface of the extraction of anthocyanin (A) function of time and temperature, (B) function of time and calyx-solvent ratio and (C) function of temperature and calyx-solvent ratio

Table 2. Kinetic parameters for Pseudo-second-order model

Temperature	calyx-water ratio	$C_e \times 10^{-2}$ (g/L)	$k \times 10^{-3}$ (L/g.min)	E	Adj. R ²	RMSE
30	1:50	4.31	56.72	0.42	0.99	0.00
	1:20	16.60	36.70	0.57	0.99	0.02
	1:10	58.10	1.23	0.98	0.99	0.02
50	1:50	9.08	51.80	0.46	0.99	0.02
	1:20	18.90	29.60	0.64	0.99	0.02
	1:10	60.90	0.85	0.98	0.99	0.04
75	1:50	10.90	23.40	0.70	0.97	0.02
	1:20	24.20	14.70	0.80	0.97	0.08
	1:10	85.21	0.61	0.99	0.99	0.06
100	1:50	11.76	19.85	0.74	0.99	0.5
	1:20	34.52	12.80	0.82	1.00	0.03
	1:10	91.25	0.39	0.99	0.99	0.04

C_e is equilibrium concentration, k is rate constant, and E is fractional extraction

Table 3. Kinetic parameters for Weibull model

Temperature	Water-calyx ratio	$C_0 \times 10^{-5}$ (g/L)	k (min ⁻¹)	$n \times 10^{-2}$	Adj. R ²	RMSE
30	1:50	2.02	6.41	11.90	0.99	0.10
	1:20	3.50	6.52	13.20	1.00	0.13
	1:10	3.94	6.64	15.90	0.99	0.35
50	1:50	2.36	6.13	14.40	0.99	0.16
	1:20	4.23	6.68	17.50	0.99	0.09
	1:10	5.00	6.96	20.50	0.99	0.53
75	1:50	4.33	7.15	16.50	0.97	0.36
	1:20	6.16	7.32	19.00	0.99	0.36
	1:10	9.27	8.33	22.90	0.99	0.66
100	1:50	5.57	7.72	20.60	0.97	0.84
	1:20	7.30	8.74	24.80	0.99	0.64
	1:10	10.37	9.01	29.70	1.00	0.52

Kinetic modelling

Tables (2), (3) and (4) show the kinetic parameters for Pseudo-second-order, Weibull and Peleg respectively. These models were fitted to all sets of experimental data with the average adj. R² of 0.99, 0.99 and 0.98 respectively and were used to describe the kinetics of extraction of anthocyanin from roselle calyces. Two rates, Minchev and Mincor and Pseudo first order models could not fit all the sets of experimental data and so they were dropped from being used for further analysis. In Pseudo-second-order model, the equilibrium concentration (C_e) was seen to increase with increased temperature and increased calyx-water ratio while the rate constant (k) decreased with increased temperature and increased calyx-water ratio (Table 2). This trend was similar to what

was reported by Jo & Kim (2019). This enhanced extraction efficiency is due to the increase in anthocyanin's solubility with increasing temperature. The initial high extraction rate is due to the availability of a robust dynamic force of fresh solvent but later on the extraction rate lowers due to higher resistance of solute to move from the spent calyces to the liquid extract. The Peleg's constants reduced with increased temperature (Table 4), this trend was observed by Jurinjak Tušek *et al.* (2016). An increase in the mass ratio of the calyx to water also reduced the initial rate constant (k_1) and capacity constant (k_2) of the model. Fig. (3) show the anthocyanin content at the temperatures 30, 50, 75 and 100 °C respectively using the Pseudo-second-order equation.

Researchers relate many reactions and changes to fractional conversion models (Rose & Kintner, 1966; Sabbaghi, Ziaifar, *et al.*, 2018b). The rate constant obtained using the Pseudo-second order model was used to calculate the fractional extraction

and the results are presented in Table (2). The fractional extraction increased and tends towards zero as the temperature and calyx-water ratio increased. This trend is similar to what was obtained by Sabbaghi *et al.* (2017).

Table 4. Kinetic parameters for Peleg model

Temperature	Water-calyx ratio	$k_1 \times 10^2$ (min.L/g)	k_2 (L/g)	Adj. R ²	RMSE
30	1:50	41.10	27.80	0.99	0.07
	1:20	33.50	22.80	1.00	0.01
	1:10	9.40	18.10	0.99	0.26
50	1:50	21.40	15.10	0.99	0.13
	1:20	10.20	10.90	0.99	0.24
	1:10	3.50	5.30	0.99	0.40
75	1:50	20.40	12.50	0.97	0.29
	1:20	5.47	7.40	0.99	0.39
	1:10	1.34	3.30	0.99	0.67
100	1:50	2.20	8.90	0.97	0.66
	1:20	0.37	6.90	1.00	0.32
	1:10	0.04	2.50	0.99	0.48

k_1 is rate constant at the beginning of the extraction, k_2 is capacity constant which represents the maximum anthocyanin concentration in the entire extraction process.

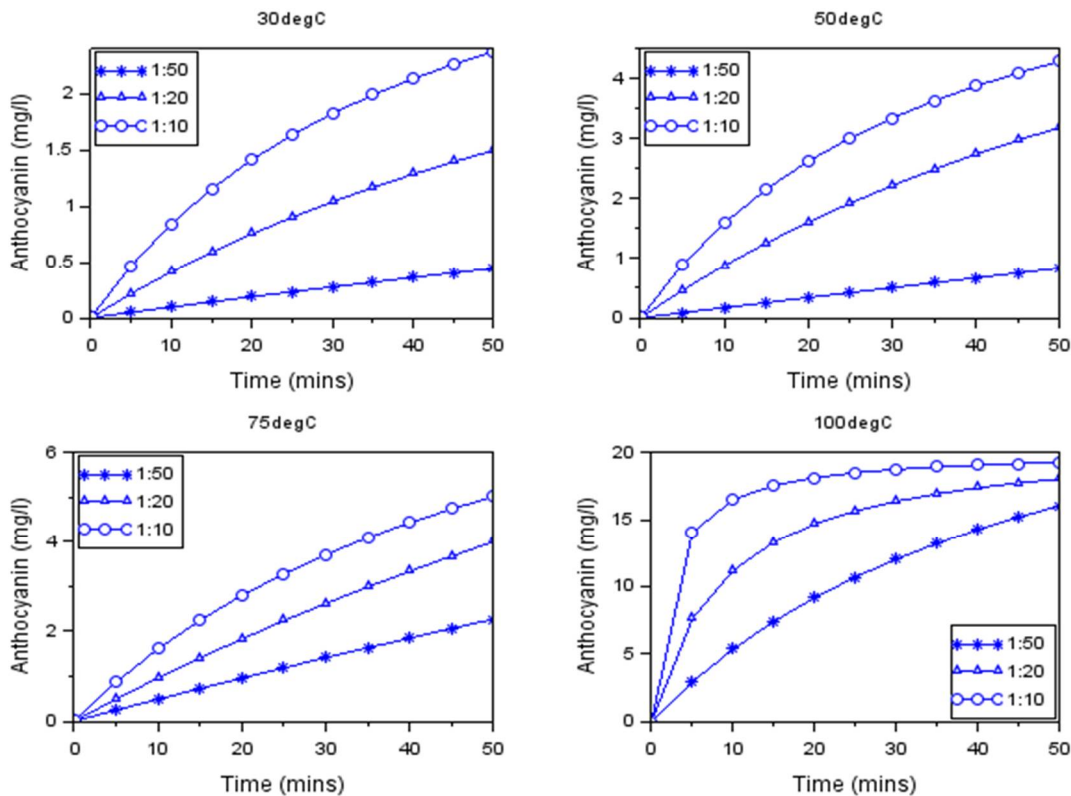


Fig. 3. Effect of calyx-water ratio on the anthocyanin content at 30 (A), 50 (B), 75 (C) and 100 °C (D)

Table 5. Coefficient of effective diffusion, Mass transfer coefficient and Biot number for the extraction of anthocyanin from roselle calyces at different temperatures and calyx-water mass ratio

Mass ratio	Temp	$D_{\text{eff}} \times 10^{-11}$ (m ² /s)	$M_T \times 10^{-8}$ (m/s)	Biot number
1:50	30	1.24	9.22	164.47
	50	1.37	10.20	133.50
	75	1.43	10.70	148.15
	100	1.48	11.02	147.81
1:20	30	1.04	3.93	74.88
	50	1.06	4.09	76.49
	75	1.11	3.95	70.52
	100	1.39	3.98	56.69
1:10	30	1.04	1.87	35.65
	50	1.08	1.84	33.67
	75	1.22	1.73	28.03
	100	1.24	1.62	25.81

Mass transfer parameters

The coefficient of effective diffusion (D_{eff}) increased with increasing temperature as seen in Table (5). While the increase in diffusion coefficient may be credited to increased thermal energy at higher temperatures, the increase in mass transfer coefficient could be attributed to both increase in diffusion coefficient and decrease in viscosity. This could probably explain the higher influence of temperature on mass transfer coefficient than that on diffusion coefficient. Hence, the mass transfer Biot number increased with temperature. The higher values of Biot number (>50) which occurred at mass ratio of 1:50 and 1:20, indicates the external resistances for mass transfer is insignificant confirming efficient mixing between solute and solvent at those ratios (Tao *et al.*, 2014) and therefore, internal transfer is rate-limiting (Rakotondramasy-Rabesiaka *et al.*, 2010).

Thermodynamic study

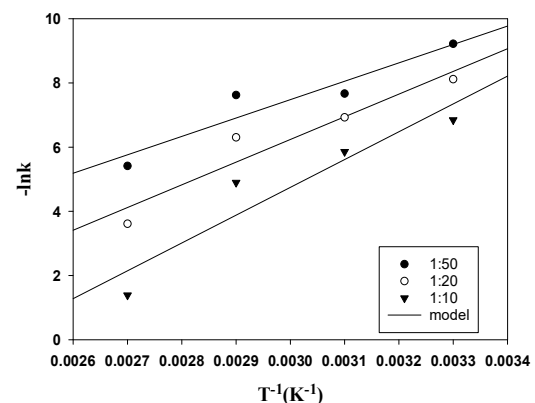
Activation energy

As one of the best performing models, the extraction constant obtained from the Peleg model was used to determine the activation energy of anthocyanin during extraction. The activation energy was evaluated and shown in Table (6). It ranged from 51.40 to 62.10 kJmol⁻¹ and this is similar to the range obtained for anthocyanin in roselle extract which ranged between 47 and 61 kJmol⁻¹ as reported by Cisse *et al.* (2009). The activation energy decreased as the

calyx-water ratio increased. The Arrhenius plot is as shown in Fig. (4).

Table 6 Activation energies at different calyx-water ratios

Ratio	E_a (kJmol ⁻¹)	Adj. R ²	RMSE
1:50	62.10	0.94	0.78
1:20	58.80	0.95	0.82
1:10	51.40	0.94	0.99

**Fig. 4.** Arrhenius plot using constants from Peleg sorption-desorption model

Gibbs, Free enthalpy and Entropy

The values of the thermodynamic parameters obtained are shown in Table (7). The enthalpy change was seen to be positive indicating that the process is endothermic which means that the process requires an external source of energy to speed it up. The value is in the range of 36 to 58 kJmol⁻¹ and this is similar to what was obtained by Jurinjak Tušek *et al.* (2016) which was within the range of 32 to 67 kJmol⁻¹ for total phenol from *Asteraceae* plants.

Table 7. Thermodynamic Parameters

Time (min)	Mass ratio	ΔH (kJmol ⁻¹)	ΔS (JK ⁻¹ mol ⁻¹)	ΔG (kJmol ⁻¹)	Adj. R ²	RMSE
5	1:50	57.90	138.00	-11.30	0.86	1.09
	1:20	53.30	130.90	-7.50	0.96	0.73
	1:10	48.50	118.50	-7.00	1.00	0.22
10	1:50	58.30	137.60	-8.80	0.92	0.94
	1:20	50.10	128.20	-7.10	0.99	0.46
	1:10	37.00	88.70	-5.80	0.99	0.39
15	1:50	48.60	122.00	-7.70	0.97	0.64
	1:20	46.90	112.30	-5.90	0.98	0.52
	1:10	36.40	88.90	-6.60	0.96	0.61

ΔH is enthalpy, ΔS is entropy, ΔG is Gibbs free energy

During extraction, the anthocyanin molecules diffuse into the solvent thereby increasing the entropy of the mixture. The entropy, which ranged between 88 and 138 JK⁻¹mol⁻¹ is positive and this indicates that the reaction is irreversible and spontaneous due to the increased degree of randomness of the anthocyanin molecules dissociated from the calyces into the solvent. The values of ΔS obtained were higher than what was obtained by Yedhu Krishnan *et al.* (2016) for flavonoids from *Terminalia bellerica* which was 54 JK⁻¹mol⁻¹. The entropy decreased as calyx-water ratio increased and as contact time increased. The Gibbs free energy change is negative which indicates that the reaction is feasible and spontaneous. It ranged between -5 and -11 kJmol⁻¹ which is a little less than what was obtained by Yedhu Krishnan *et al.* (2016) which ranged between -1 and -4 kJ.mol.

Conclusions

Extraction time, temperature and calyx-water ratio were observed to have linear, interaction and quadratic significant ($P < 0.01$) effects on the anthocyanin Content of the extract. Pseudo-second-order, Weibull and Peleg models reasonably described the extraction data. The equilibrium concentration increased with increased temperature and calyx-water mass ratio. The kinetic variables were also related to the fractional extraction or conversion model. The fractional extraction increased with increased temperature and calyx-water ratio. The effective diffusion coefficient and mass transfer coefficient

increased with increased temperature and reduced with increased calyx-water ratio. While the Biot number reduced with increased temperature and calyx-water ratio. The enthalpy and entropy reduced with increased time and vice versa. The extraction process was seen to be endothermic, feasible and spontaneous.

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Author contributions

Nneoma Nkem Aneke: Data collection, Data analysis, Writing the draft of the manuscript, Data analysis and interpretation, Presenting the research idea and study design, Approval of the final version; **Wilfred Ifeanyi Chukwu Okonkwo:** Writing the draft of the manuscript, Revising and editing the manuscript, Supervising the study, Approval of final the version; **Sunday Louis Ezeoha:** Writing the draft of the manuscript, Supervising the study, Approval of the final version; **Gabriel Ifeanyi Okafor:** Data analysis, Revising and editing the manuscript, approval of the final version; **Cosmas Ngozichukwu Anyanwu:** Data analysis and interpretation, Presenting the research idea and study design, Approval of the final version.

Conflict of Interests

There is no conflict of interest based on the writers.

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بهینه‌سازی استخراج آنتوسیانین از گل چای ترش (*Hibiscus sabdariffa*) با استفاده از روش‌های روش‌شناسی سطح پاسخ (RSM)، مدل‌سازی جنبشی، انتقال جرم و مطالعه‌های ترمودینامیکی

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چکیده

گل چای ترش (*Hibiscus sabdariffa*) در صنایع غذایی و نوشیدنی بسیار در حال اهمیت یافتن است، به‌ویژه به دلیل وجود آنتوسیانین که آنتی‌اکسیدان است که به این گل رنگ قرمز می‌بخشد. تأثیر پارامترهای فراوری مانند زمان تماس، دما و نسبت این گل به آب بر میزان آنتوسیانین عصاره گل، همراه با ارزیابی مدل‌های جنبشی، انتقال جرم و پارامترهای ترمودینامیکی، مورد مطالعه و بهینه‌سازی قرار گرفت. استخراج جنبشی برای آنتوسیانین در زمان‌های مختلف (۵، ۱۰ و ۱۵ دقیقه)، دما (۳۰، ۵۰، ۷۵ و ۱۰۰ درجه سانتی‌گراد) و نسبت جرم چای ترش به آب (۱:۵۰، ۱:۲۰ و ۱:۱۰) صورت گرفت. حداکثر عملکرد آنتوسیانین در مدت زمان ۱۵ دقیقه دمای ۱۰۰ درجه سانتی‌گراد و نسبت ۱:۱۰ به دست آمد. داده‌های به دست آمده در ۶ مدل استخراج مختلف برازش داده شد و مدلی که به بهترین وجه برای داده‌ها مناسب‌تر بودند، نوع وایبول، پلگ و شبه مرتبه دوم (Pseudo-second-order) همراه Adj بودند. R^2 به ترتیب ۰/۹۸، ۰/۹۹ و ۰/۹۹ بود. داده‌های به دست آمده برای محاسبه پارامترهای جنبشی، انتقال جرم و ترمودینامیکی مورد استفاده قرار گرفت. متغیرهای جنبشی نیز به مدل استخراج کسری یا تبدیل مرتبط بودند. استخراج کسری با افزایش دما و گل چای ترش افزایش یافت. ضریب نفوذ مؤثر بین ۱۰- 1.04×10^{-11} تا 1.04×10^{-11} مترمربع بر ثانیه بود. ضریب انتقال جرم محاسبه شده بین 1.04×10^{-11} و 1.04×10^{-11} (متر بر ثانیه) بود، تعداد Biot از ۲۵ تا ۱۶۸ متغیر است. آنتالپی از ۳۶/۶۰ تا ۵۸/۳۰ بر کیلوژول مول، آنتروپی از ۸۸ تا ۱۴۷ بر ژول کیلوگرم بر مول و انرژی آزاد گیبس از ۵/۸۰- تا ۱۱- بر کیلوژول مول. براساس مشاهده‌های این تحقیق، فرایند استخراج به صورت گرماگیر، امکان‌پذیر و خود در نظر گرفته شد.

واژه‌های کلیدی: آنتوسیانین، استخراج کسری، انتقال جرم، روش سطح پاسخ (RSM)، مدل‌سازی جنبشی