Food Research 8 (3) : 487 - 493 (June 2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Kinetics studies on the ascorbic acid extraction from roselle (*Hibiscus sabdariffa)* calyces

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Article history:
Received: 20 June 2022
Received in revised form: 29 July 2022
Accepted: 21 May 2023
Available Online: 30 June 2024

Keywords:
Activation energy,
Biot number,
Effective diffusivity,
Mass transfer,
Pseudo first order,
Roselle

DOI:
https://doi.org/10.26656/fr.2017.8(3).330

Abstract

The effect of processing conditions of roselle (*Hibiscus sabdariffa*) calyces on the vitamin C content of the extracts was studied to develop models that best describe the process. The extraction parameters are the processing time (5, 10, 15 mins), process temperature (30°C, 50°C, 75°C and 100°C), and calyx-water mass ratio (1:50, 1:20 and 1:10). These samples were tested for ascorbic acid using the spectrophotometric method. Statistical analysis was conducted on the experimental results and regression models were developed. A total of six mathematical models were selected for the description of vitamin C extraction from the calyces. The results showed that time, temperature and calyx-water mass ratio have a linear significant effect ($p \leq 0.01$); while time-temperature and temperature-ratio had a significant ($p \leq 0.05$) interaction effect; time and temperature had a significant ($p \leq 0.05$) quadratic effect on the vitamin C content of the extract. Pseudo-first-order gave the highest overall coefficient of determination R^2 and lowest overall root mean square error (RMSE). The activation energy of vitamin C ranged from 3 to 8.4 kJ; the effective diffusion coefficient ranged from 1.5×10^{-9} to 5.0×10^{-7} m^2s^{-1}; while mass diffusivity ranged from 3.11×10^{-5} to 8.6×10^{-4} ms^{-1}. The Biot number calculated ranged between 33.93 and 541.94 which showed that there is an insignificant external mass transfer resistance.

1. Introduction

Roselle (*Hibiscus sabdariffa*) calyx usually found in Africa, Asia and South America (Cisse *et al*., 2009) is known to have some nutritional and health benefits. It contains a lot of minerals and vitamins and is also used for colorings and flavorings. The colour ranges from green to red to purple though the most popular one is dark red (Aregbesola *et al.,* 2016). The calyces are known to contain anti-oxidants such as anthocyanin, polyphenols, flavonoids (Cid-Ortega and Guerrero-Beltrán, 2015). Processing conditions often affect the properties of the extract (Cissé *et al*., 2012). The extract is consumed in the form of drinks, wines, pastries and yoghurts (Cisse *et al*., 2011). It is known for its health benefits such as the prevention of diabetes, hypertension, and cholesterol diseases amongst others (Da-Costa *et al*., 2014). The byproduct of the extraction which is fiber has the potential to be milled into flour and used as an additive in confectionery and also as a source of animal feed (Ahmed and Safaa, 2015).

Vitamin C (Ascorbic acid) is known to be a very important factor in the food and pharmaceutical industry. Vitamin C is a very important anti-oxidant. Its presence in roselle calyces makes it a very important raw material in the food industry. The presence of vitamins and minerals makes the extract a good base ingredient for the production of soft drinks which is a better alternative to the commercially available soft drinks (Pettigrew *et al*., 2015).

Extraction procedures are necessary to get the maximum possible quantity of a bioactive compound from plant material. These include maceration (Sant'Anna *et al*., 2012), distillation, ultrasound-assisted extraction (Ali *et al*., 2018), microwave extraction (Neelesh *et al*., 2016), and supercritical CO_2 extraction. Solid-liquid extraction is an important aspect of food processing in that it enables one to separate the solute which is the important aspect of the product. Water being a universal solvent is used in this study. Other studies carried out using other solvents such as hexane, ethyl acetate, methanol showed that water is the most suitable for the extraction of the calyces (Sindi *et al*., 2014). Solid-liquid extraction is the ideal system for the separation of bioactive compounds with antioxidant

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eISSN: 2550-2166 /
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ability from plant materials (Alara and Abdurahman, 2019). The extraction process is affected by numerous variables whose influence is experimentally established and several models have been expressed to study extraction data, comprising empirical and theoretical ones (Ali *et al.*, 2018). Empirical models mostly comprise response surface equations, which are used to designate the effect of processing variables (temperature, time, particle size, solvent-to-solute ratio) on particular performance indices of the extraction operation, such as the quantity or yield of the remote constituents (Jo and Kim, 2019). Extraction curves are very often displayed by an unsteady-state diffusion model based on Fick's second law (Neelesh *et al.*, 2016), this forms a basis for the characterization of dynamic and equilibrium periods for mass transfer during solid-liquid extraction.

In order to enhance the extraction conditions, experimental data is often used for mathematical modelling. Mathematical modelling of solid-liquid extraction processes is an important engineering tool in the design process in order to minimize the use of energy, time and materials (Jo and Kim, 2019). Kinetic modelling is of great significance for the comprehension of complex diffusion, mass transfer and thermodynamic parameters affecting extraction. Effective diffusion coefficients, extraction rates and thermodynamic parameters of the extraction process can be calculated using these models.

The objectives of this study were to determine the effect of contact time, process temperature and calyx-water ratio on the vitamin C content of roselle extract. To employ six different extraction models from literature to describe the extraction characteristics. To calculate the kinetic parameters such as coefficient effective diffusion, mass diffusivity, Biot number and activation energy.

2. Materials and methods

2.1 Sample preparation

Dried roselle calyces were obtained from the local market in Enugu, Nigeria. The calyces were sorted and cleaned. The calyces were portioned into 20, 50 and 100 g. The extraction times were 5, 10 and 15 mins and the extraction temperatures were 30°C, 50°C, 75°C and 100°C. The mass of water used for each extraction cycle was 1 kg. After extraction time had elapsed, the spent calyces were separated from the extract using a muslin cloth. The extracts were labelled accordingly and stored at 4°C pending further analysis.

2.2 Experimental design

A 3×4×3 full factorial design was used to study the effect of extraction time (t), temperature (T) and calyx-water mass ratio (R) on the extraction of vitamin C from roselle calyces. Three levels of time (5, 10 and 15 mins), 4 levels of temperature (30°C, 50°C, 75°C and 100°C) and 3 levels of calyx-water mass ratio (1:50, 1:20, and 1:10) were used. Mass ratio beyond 1:10 would not have the calyces fully immersed in the water (Cissé *et al.*, 2012).

2.3 Vitamin C determination

The vitamin C content of the samples was determined by the method used by Hernández *et al.* (2006). Approximately 20 mL of the sample solution was diluted with 100 mL of distilled water and homogenized. A few drops of bromine water were added until the solution became colored to ensure that oxidation of the ascorbic acid to dehydroascorbic acid was complete. Then a few drops of thiourea solution were added to the solution to remove the excess bromine and hence a clear solution was attained. A 2, 4-Dinitrophenyl hydrazine solution was added methodically with all standards and also with the oxidized ascorbic acid. Total vitamin C was determined by the reaction of 2, 4-Dinitrophenyl hydrazine dye with vitamin C. The absorbance was measured using a Shimadzu spectrophotometer (model UV-1601) at a wavelength of 500 nm as described by Pathy (2018).

2.4 Modelling the ascorbic acid extraction

A quadratic first-order regression model was assumed to connect the response with the extraction process factors and their interactions (Bezerra *et al.*, 2008) as shown in Eq. (1)

$$y = \beta_0 + \sum_{i=1}^{k}\beta_i x_i + \sum_{i=1}^{k}\beta_{ii} x_i^2 + \sum_{1\leq i\leq j}^{k}\beta_{ij} x_i x_j + \varepsilon \quad (1)$$

Where y is the dependent variable in this case vitamin C concentration, β_0 is the constant regression coefficient, β_i, β_{ii}, β_{ij} are the linear, quadratic and interaction regression coefficients respectively, x_i, x_j are the independent variables (contact time, process temperature and calyx-water ratio respectively), ε is the residuals or errors associated with the experiment. This was done using Design Expert version 11.

2.4.1 Fitting to models

The experimental data obtained were fitted into some extraction kinetic models obtained from literature to determine which one best fits vitamin C extraction are shown in Table 1. The non-linear regression methods were used using Curve Expert Professional 2.7.3.

2.5 Kinetics studies

2.5.1 Activation energy estimation

The activation energy was calculated by plotting -lnK is against 1/T from Arrhenius equation as shown in

https://doi.org/10.26656/fr.2017.8(3).330

Table 1. Kinetic extraction models.

Model	Equation	Source
Nth order	$C = kt^{-n}$	Sant'Anna *et al*. (2012)
Weibull-type	$C = C_o exp(kt^n)$	Amendola *et al*. (2010)
Two-rates	$C = A[1 - exp(-Bt)] + C[1 - exp(-D)t]$	Cacace and Mazza, 2003)
Sorption/Desorption	$C = \frac{t}{k_1 + k_2 t}$	Bucić-Kojić *et al*. (2007)
Pseudo-First order	$C = C_\infty - \frac{C_\infty}{exp(kt + a)}$	Amendola *et al*. (2010)
Minchev and Minkov	$C = A - Bexp(-kt)$	Simeonov *et al*. (1999)

Eq. (2). This was done using Curve Expert Professional 2.7.3 and Scilab 6.0.1

$$\ln(k) = \ln(k_o) - \frac{E_a}{RT} \tag{2}$$

2.5.2 Determination of effective diffusion coefficient and mass transfer coefficient of vitamin C

A pseudo-first-order model was used for kinetic analysis. The effective diffusivity (D_e) and mass transfer coefficient (M_T) were calculated according to Fick's second law as shown in Eqs. (3 and 4). Biot number (Bi) was used to measure the relative magnitude of the external and internal resistances to mass transport.

$$\frac{\partial C_s(x,t)}{\partial x} = D_e \left[\frac{\partial^2 C_s}{\partial x^2} + \frac{P}{x}\frac{\partial C_s}{\partial x}\right] \tag{3}$$

Where, C_s is the concentration of vitamin C in solid phase (kgkg^{-1} calyx), x is coordinate (m), t is time (s), D_e is the effective diffusion coefficient (m^{2}s^{-1}), P is the shape factor.

$$\frac{C_s}{C_i} = \Sigma_1^\infty \frac{8}{\pi^2(2n-1)^2} exp\left[\left(-\frac{\pi^2}{4}\right)(2n-1)^2 \frac{D_e t}{r^2}\right] \tag{4}$$

Where C_i is the initial concentration of vitamin C in the calyx (kgkg^{-1} calyx), r is particle size (m)

At n=1; Eq. (5) is reduced to Eq. (6)

$$\frac{C_s}{C_i} = \Sigma_1^\infty \frac{8}{\pi^2} exp\left[\left(-\frac{\pi^2}{4}\right)\frac{D_e t}{r^2}\right] \tag{5}$$

$$log\frac{C_t}{C_i} = \log A - 2.468\left(\frac{D_e t}{r^2}\right) \tag{6}$$

The graph of $log\frac{C_t}{C_i}$ was plotted against t. The slope was used to calculate the effective diffusion coefficient.

Eq. (7) as described by Yedhu and Rajan (2016) and Neelesh *et al*. (2016) was used to evaluate the mass transfer coefficient of vitamin C in the extract.

$$ln\frac{C_{ss}}{C_{ss} - C_{st}} = \frac{M_T A}{V_s} t \tag{7}$$

Where C_{ss} is the saturation concentration of vitamin C in the solution, C_{st} is the concentration of vitamin C at time t, M_T is the mass transfer coefficient, A is the total surface area of the calyces, V_s is the volume of the solution.

The graph of $ln\frac{C_{ss}}{C_{ss}-C_{st}}$ was plotted against time. The slope was used to determine the mass transfer coefficient.

2.5.3 Biot number evaluation

Biot number (*Bi*) was used to measure the relative magnitude of the external and internal resistances to mass transport (Jo and Kim, 2019). It was calculated using Eq. (8). Calculation of the Biot number makes it possible to determine whether the limiting factor is internal or external. If the internal transfer is limiting, the intensification of the surface exchanges will not have any effect and vice versa (Rakotondramasy-Rabesiaka *et al*., 2010).

$$Bi = \frac{M * r}{D_{eff}} \tag{8}$$

Where r is the particle size (Aregbesola *et al*., 2016).

3. Results and discussion

3.1 Extraction kinetics

3.1.1 Influence of extraction conditions on the vitamin C content of the extract

It was observed that an increase in time, temperature and calyx-water ratio increased the vitamin C content of the extract. The analysis of variance (ANOVA) shows that time, temperature and calyx solvent ratio each had a significant effect ($p < 0.01$) on the vitamin C content of the extract. An increase in temperature brought about an increase in the vitamin C extracted from the calyces. This is similar to what was reported by Ochoa-Velasco and Ruiz-López (2019). Likewise, an increase in the calyx-water ratio also increased the quantity of vitamin C extracted. Time-temperature interaction and temperature-ratio interaction had a significant effect ($p < 0.01$) on the vitamin C content of the extract. Time and temperature also had a quadratic significant effect ($p < 0.01$) on the vitamin C content of the extract. The regression equation

https://doi.org/10.26656/fr.2017.8(3).330

is as shown in Eq. (9). Figure 1(A, B and C) shows the interactive effect of time and temperature; time and calyx -water ratio; and temperature and calyx-water ratio on vitamin C content of the extract respectively. It was observed that at 15 mins and 100°C the concentration of vitamin C in the extract was 25 mg/100 mL.

$$y = 0.02712 - 0.002755x_1 - 0.00072x_2 + 0.00006x_3 + 0.00003x_1x_2 + 0.000002x_2x_3 + 0.0008x_i^2 + 0.000004x_2^2 \quad (9)$$

Where x_1 is time, x_2 is temperature and x_3 is calyx-water ratio.

$R^2 = 0.83$; adjusted $R^2 = 0.81$

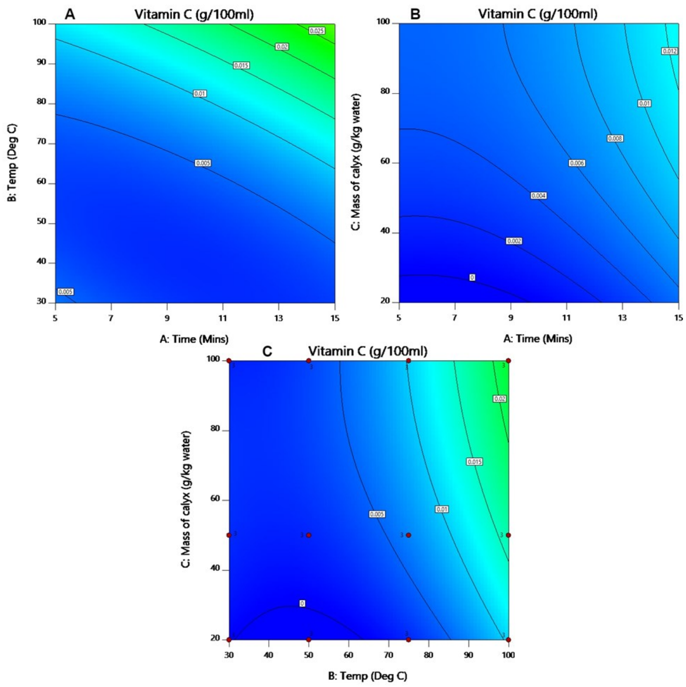

Figure 1. Interactive effect of the factors on the vitamin C content of the extract: A. Temperature vs Time, B. Mass of calyx vs Time, C. Mass of calyx vs Temperature.

3.1.2 Fitting to kinetic models

Table 2 shows the average error analysis of the different models used. All the models used reasonably described the experimental data except for Nth order which failed in all the tests. Pseudo-first order gave the highest (R^2) and lowest root mean square error (RMSE).

Table 2. Average fitness of mathematical models for vitamin C extraction from roselle.

Model	R^2	RMSE
Nth order	Failed	Failed
Weibull type	0.970	0.039
Two rates	0.984	0.038
Sorption/Desorption	0.969	0.033
Minchev and Minkor	0.953	0.047
Pseudo first order	0.992	0.017

RMSE: Root mean square error

Table 3 shows the kinetic parameters obtained using the Pseudo first-order model. The equilibrium concentration (C_∞) increased as temperature increased. This same trend was reported by Santana *et al*. (2012). As the calyx-water ratio increased, the equilibrium concentration increased as well. The rate constant (K) increased with increased calyx-water ratio and decreased with increased temperature this was also obtained by Sant'Anna *et al*. (2012). The K-values were used in the Arrhenius equation to determine the activation energy as shown in equation (2). The pseudo-first order model was used to plot the graphs of concentration of vitamin C against time for different calyx-water ratios and different temperatures as shown in Figure 2. Extraction occurs exponentially at the different temperatures represented until it reaches an equilibrium concentration in which the solvent is not able to remove vitamin C from the roselle calyces.

Table 3. Kinetic parameters for extraction of vitamin C from roselle calyces.

Temp	Ratio	C_∞ (mg/g)	K(min^{-1})	a
30	0.02	0.00188	0.19450	0.00365
	0.05	0.00337	0.22570	0.02230
	0.10	0.00387	0.37260	0.00323
50	0.02	0.00229	0.18080	0.02370
	0.05	0.00452	0.21700	0.00012
	0.10	0.00911	0.35500	0.00908
75	0.02	0.00492	0.18000	-0.00309
	0.05	0.00566	0.19100	0.90500
	0.10	0.00942	0.28700	-0.00185
100	0.02	0.00961	0.15220	0.95300
	0.05	4.66220	0.16840	-0.00014
	0.10	3.62000	0.20400	0.00018

C_∞: equilibrium concentration, K: rate constant.

3.1.3 Activation energy estimation

The regression Equations (10, 11 and 12) were obtained as:

For ratio of 1:50 $$\ln(k) = 0.626 + \frac{370}{T} \quad (10)$$

For ratio of 1:20 $$\ln(k) = 0.104 + \frac{503}{T} \quad (11)$$

For ratio of 1: 0 $$\ln(k) = -1.814 + \frac{1010}{T} \quad (12)$$

The Arrhenius plot is shown in Figure 3. The slopes of the equations were used to evaluate the activation energies for the extraction of vitamin C. The activation energies were obtained as 3.0, 4.2 and 8.4 kJ for the calyx-water ratio of 1:50, 1:20 and 1:10 respectively. This is similar to what was obtained by Janković *et al*. (2021). The activation energy for diffusion process is a good pointer of the mechanism category which prevails in the material. The low activation energies indicate that

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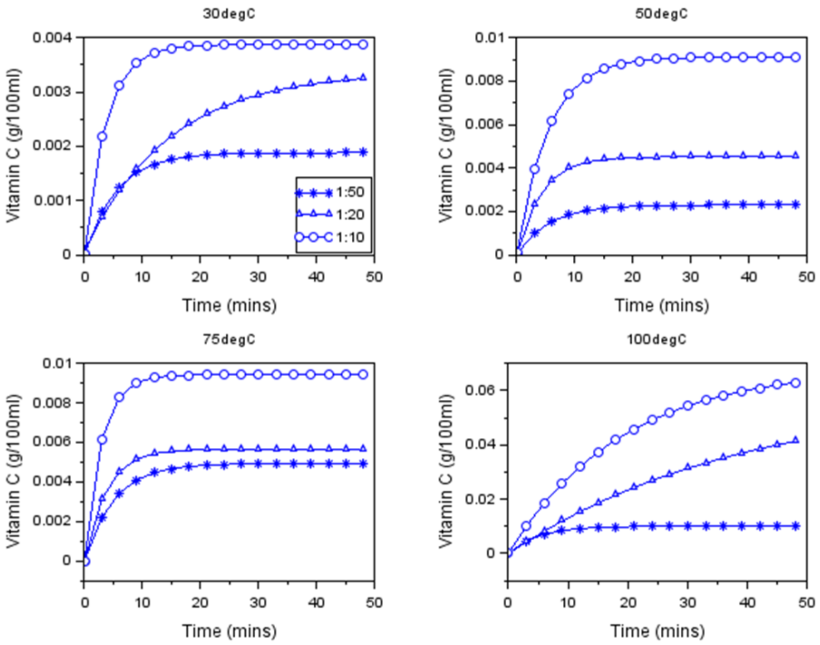

Figure 2. Extraction of vitamin C from roselle calyces at 30°C, 50°C, 75°C and 100°C at different calyx-water ratios.

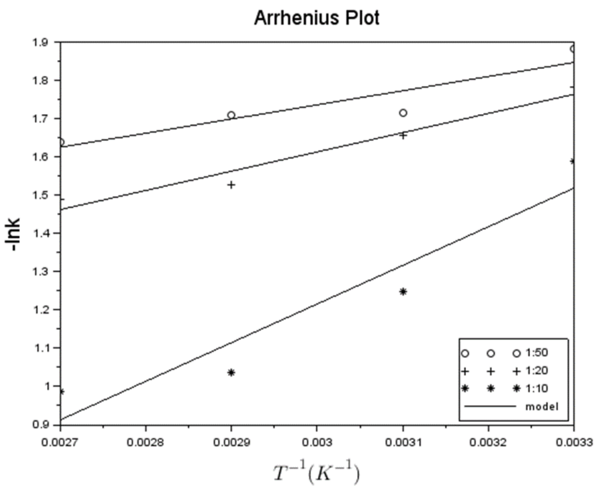

Figure 3. Arrhenius plot of –lnk versus T^{-1}.

the diffusion of vitamin C into the extract from the calyces did not require so much effort (Pedreschi *et al*., 2009).

Table 4 shows the estimated values of the effective diffusion coefficient, mass transfer coefficient and Biot number of the vitamin C in the extract. The effective diffusion coefficient of vitamin C in the extract ranged between 4.99×10^{-7} and 1.52×10^{-9} m^2s^{-1}. This is similar to what was reported for vitamin C from blanching peas by Abdel-Kader (1991) which ranged from 9.4×10^{-7} to 1.94×10^{-8} m^2s^{-1}. An increase in temperature increases the diffusivity of vitamin C in the extract; this can be attributed to the increased thermal energy that occurs at higher temperatures (Tao *et al*., 2014). This is similar to what was observed by other researchers (Pedreschi *et al*., 2009; Jo and Kim, 2019) in the extraction of paclitaxel and reducing sugar respectively. An increase in the calyx -water ratio reduced the effective diffusion coefficient. The mass transfer coefficient increased with an increase in temperature and reduced with an increase in the calyx-water ratio. This could be a result of the increase in diffusion coefficient and decrease in the viscosity of the extract (Tao *et al*., 2014). K_T ranged between 3.22×10^{-5} ms^{-1} at 30°C and 8.6×10^{-4} ms^{-1} at 100°C. From Figure 4, it is observed that high temperatures between 75°C and 100°C increased the diffusion coefficient of vitamin C for a higher calyx-water ratio.

Table 4. Effective diffusion coefficient, mass transfer and Biot number for the extraction of Vitamin C at different temperatures and calyx-solvent ratios.

Temp	Calyx-water ratio	De×10^{-9} (m^2s^{-1})	M_T×10^{-5} (ms^{-1})	Bi
30	1:50	46.9	11.4	47.79
	1:20	22.2	5.07	44.89
	1:10	1.52	3.11	403.24
50	1:50	57.7	14.0	47.76
	1:20	46.3	11.7	49.77
	1:10	4.61	11.2	476.32
75	1:50	133	29.6	43.89
	1:20	94.0	19.2	40.24
	1:10	4.65	12.8	541.94
100	1:50	499	86.0	33.93
	1:20	428	73.8	33.95
	1:10	18.6	39.1	413.97

De: Effective diffusion coefficient, MT: mass transfer coefficient, Bi: Biot number.

Biot number was seen to have a sharp increase between the mass ratio of 1:20 and 1:10, This indicates that the external resistances of mass transfer are negligible confirming efficient mixing between solute and solvent and therefore internal transfer is rate-limiting. Biot number greater than 50 signifies that there is sufficient mixing between the feed and solvent phases

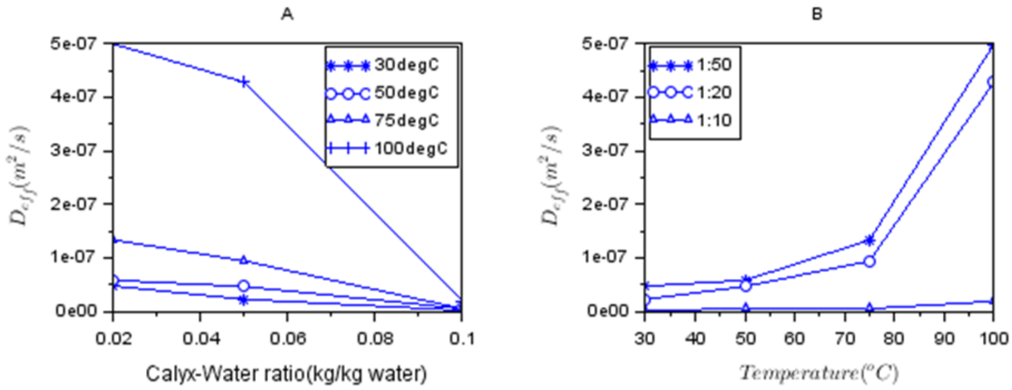

Figure 4. Diffusion coefficient versus calyx-water ratio (A), temperature (B).

leading to negligible external mass transfer resistance, and therefore, the internal transfer is rate limiting.

4. Conclusion

The kinetics of the extraction of vitamin C from roselle calyx under different conditions were determined. These conditions included time (5, 10, 15 mins) temperature (30°C, 50°C, 75°C and 100100°C) and calyx -water mass ratio (1:50, 1:20, 1:10). These factors were seen to have a significant ($p < 0.05$) linear, quadratic and interaction effect on the vitamin C content of the extract. Empirical equations were developed using the experimental data. The pseudo-first-order equation being the most suited was used to determine the equilibrium vitamin C content. Arrhenius model was used to estimate the activation energy of vitamin C during extraction and it shows that increasing the calyx-water ratio increased the activation energy of vitamin C from the calyces. The effective coefficient of diffusion, mass diffusivity and Biot number of vitamin C at different temperatures and calyx-water ratio was determined and shows that increased temperature increases the rate of diffusion of vitamin C into the extract.

Conflict of interests

The authors would like to declare no conflict of interest in the publication of this manuscript.

Acknowledgements

The authors would like to thank the Department of Agricultural and Bioresources Department, University of Nigeria, for their laboratory and technical support.

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