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Accepted Version

Kumar, R., Oruna-Concha, M. J. ORCID: https://orcid.org/0000-0001-7916-1592, Methven, L. and Niranjan, K. ORCID: https://orcid.org/0000-0002-6525-1543 (2023) Modelling extraction kinetics of betalains from freeze dried beetroot powder into aqueous ethanol solutions. Journal of Food Engineering, 339. 111266. ISSN 0260-8774 doi: 10.1016/j.jfoodeng.2022.111266 Available at https://centaur.reading.ac.uk/107057/

To link to this article DOI: http://dx.doi.org/10.1016/j.jfoodeng.2022.111266

Publisher: Elsevier

Ms. Ref. No.: JFOODENG-D-22-01104 (R2: 30-08-2022)

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Abstract

The extraction kinetics of betalains (betacyanin and betaxanthin) from freeze dried beetroot powder into aqueous ethanol solutions is modelled by considering the concentration of a given betalain at any given time to result from a balance between the rate of its release from the solid phase and the rate of its chemical degradation in the extract phase. The mathematical model obtained shows that the concentration of the betalain peaks before progressively decreasing with time. The model was experimentally validated for various combinations of temperature (55-85 °C), ethanol concentration (10-30%) and particle size (120-300 µm). The ratio of betacyanin to betaxanthin in the liquid phase was approximately 1 over the duration of extraction at 55 and 65 °C. However, the ratio decreased at the higher temperatures of 75 and 85 °C. A maximum productivity rate of a given betalain was defined as its peak concentration divided by the time taken to reach the peak concentration, which was found to be relatively insensitive to the ethanol concentration below 75 °C.

Keywords: Beetroot; Betalain; Betaxanthin; Betacyanin; Extraction; Modelling

Nomenclature

A	Pre-exponential factor, Eqn 8, s^{-1},	L	Path length of cuvette, cm
A_{480}	Absorbance measured at 480 nm	MW	Molecular weight, g mol^{-1}
A_{538}	Absorbance measured at 538 nm	n	Number of observations for each experiment
A_{650}	Absorbance measured at 650 nm	p	Total number of predicted results from model
Adj-R^2	Adjusted coefficient of determination,	**P**	Betalain productivity rate, kg m^{-3} s^{-1}
BC	Betacyanin	R^2	Coefficient of determination
BX	Betaxanthin	**RMSE**	Root mean squared error
C_L	Concentration of betalain in the extract, kg m^{-3}	R	Universal gas constant, 8.314 $J\ mol^{-1}\ K^{-1}$
$(C_L)max$	The maximum or peak concentration of the extracted betalain, kg m^{-3}	RSS	Residual sum of square
C_s	Betalain concentration in the solid phase at any time, kg betalain (kg dry solid)$^{-1}$	S	Solid loading, kg dry powder m^{-3} extract
C_{si}	Initial concentration of betalain that is extractable, kg m^{-3}.	SSE	Sum of squared error
DF	Dilution Factor, Eqn 7	t	Time, s
T	Extraction Temperature, K	t^*	The time when C_L peaks, s
E_a	Activation Energy, Eqn 8, J mol^{-1}	TSS	Total sum of square
E	molar extinction co-efficient, Eqn 7, L mol^{-1} cm^{-1}	y_{exp}	Experimental results
k	First order rate constant for betalain degradation, s^{-1}	y_{model}	Predicted results from model
k_m	First order rate constant for exhaustion of the given betalain from the solid phase, s^{-1}		

1. Introduction

Beetroot (*Beta vulgaris L.*) is an herbaceous blooming biennial plant, native to Asia and Europe, that belongs to the Chenopodiaceae family and grown across seasons (Nirmal et al., 2021). It is widely consumed as a salad, as a juice, after pickling and as a cooked vegetable. It is known to contain high levels of nutritionally beneficial and bioactive compounds, such as nitrate, phenolics, and ascorbic acid, as well as vitamins, minerals, carbohydrates, fibre, protein, essential amino acids, fatty acids, phytosterols, alkaloids, steroids, carotenoids, and a significant level of pigments called betalains soluble in polar solvents (Fernandez et al., 2017a).

Industrial scale primary production, processing, packaging, retail market and household consumption of beetroot leads to a wastage of more than 30-50% across the world (Nirmal et al., 2021). One predominant approach to valorising beetroot waste is to extract the betalain pigments (Celli and Brooks, 2017). Betalains mainly consist of two nitrogenous components betacyanin (BC) and betaxanthins (BX). These two nitrogeneous compounds are of significant importance to food, pharmaceuticals, cosmetics and dye industries, where it is also known as “beetroot red” (Stintzing et al., 2003). Natural extracts from beetroot are also good for replacing synthetic colours in products such as confectionery and bakery, ice creams, yoghurts, and sweets (Azeredo, 2009a). However, the application of betalains, especially betaxanthin is limited due to limited production (Nestora et al., 2016). The stability of this colorant is pH and temperature dependent, and its application in high temperature processed products is limited. However, the ready availability of beetroot and low price seem to be driving forces for large scale applications of betalains in the food industry.

There have been many published reports on the extraction and analysis of betalains from beetroot. In addition, extraction, degradation, and stability of betalains from sources other than beetroot has been studied; (Merin et al., 1987) studied the stability of betacyanin as color extracted from prickly pear fruit and it was observed to be highly sensitive to temperature. (Wong and Siow, 2015) investigated the effect of heat, pH, antioxidant, agitation and light on betacyanin present in red-fleshed dragon fruit (*Hylocereus polyrhizus*) juice and concentrate. On the other hand, the stability of betalain pigments has been studied in a variety of food matrices such as milk, gummies, and beverages (Bassama et al., 2021). In general, these studies concluded that the stability of betalains during any processing such as extraction, storage, and thermal treatment was dependent on several factors including; genotype, part of the plant used, concentration of betalains, solvent employed and its pH, temperature, and water activity (Rodríguez-Sánchez et al., 2017). However, reports of the kinetics of extraction and degradation from beetroot are scarce. (Saguy, 1979) studied the thermostability of betanine (Betacyanin) and vulgaxanthin- I (betaxanthin) in blended beet juice at 61.5, 75.5 and 85.5 °C and pH range 4.8-6.2, and modelled the results using first order kinetics. The two pigments were found to exhibit maximum stability at pH of 5.8 , and betanine was found to be more stable than vulgaxanthin-I (Saguy, 1979). (Silva et al., 2020a) reported the extraction kinetics of the betalains from dried beetroot powder using Fick’s first law of diffusion, and noted that higher temperature and exposure time had a negative effect on the extraction rates. The temperature of extraction is critical in this process because of the location of the betalains within the beetroot tissue architecture. The betalains are mainly present inside vacuoles of the tissue, and the membrane protecting the vacuoles has to be broken to release the betalain (Nutter et al., 2021). A thermal shock is needed to rupture the membrane. Thus, a high temperature is needed in the process, however, the use of high temperature is also detrimental to the stability of the betalain released.

It is clear from a review of literature that there are only isolated studies providing insights into the extraction of betalains and their subsequent stability, but no systematic study which explores the kinetics of extraction in specified solvents and links it to the operational parameters such as the temperature. Since betalains are soluble in polar solvents, water and other aqueous solutions are effective media for extraction. Ethanolic solutions are usually preferred to water due to relatively lower extraction efficiency and stability of betalains (Roriz et al., 2017). The main objective of this paper is to develop a mechanistic model for the extraction of betalains from freeze dried beetroot into ethanolic solutions which takes into account: 1) the mechanisms operating during interfacial mass transfer of the betalains into a solvent phase and 2) the post-extraction chemical degradation in the solvent. The paper then reports extensive experimental data which validates the model, and discusses the effects of a range of operating parameters such as temperature, ethanol concentration and the particle size on extraction kinetics.

2. Modelling the transient concentration of betalains in the extract

If C_L (kg m^{-3}) is the concentration of a given betalain in the extract (betacyanin or betaxanthin) at any time, then the net rate of change of this concentration is given by the balance between: i) the rate at which the betalain is released from the solid phase and ii) the rate at which the betalain decomposes in the extract phase. The former rate depends on the intraparticle mass transfer characteristics, the liquid film mass transfer coefficient around the particles as well as the partition characteristics between the solid and extract phases. In addition, there could be other factors influencing the rate of release such as the location of betalain and the nature of its affinity within the particle phase cellular architecture. Thus, a detailed quantitative description of all such factors and how they influence the rate of release is expected to be complicated. However, as a simplified empirical description, it would be reasonable to assume that, at a given temperature, the rate of release of betalains is first order with respect to the concentration of betalain in the solid phase C_S (mg betalain per g dry matter). A key justification for this assumption is earlier well-documented experimental observations: e.g. in the case of sugars (Appiah-Nkansah et al., 2019), pectins (Leach et al, 1995) and total phenolic content

(Bengardino et al., 2019), where the solid-phase concentrations have been reported to vary in this manner. Thus, if C_{si} is the mean initial concentration of betalain that is extractable into a liquid medium, the mean extractable concentration at any time (C_s) will be given by:

$$C_S = C_{Si} e^{-k_m t} \tag{1}$$

where k_m (s^{-1}) is the first order rate constant for exhaustion of the given betalain from the solid phase. If S is the solid loading (g dry matter in the solid per m^3 of extraction medium), the rate at which the liquid phase gains betalain per unit volume is given by:

$$-S \frac{\mathrm{d}C_S}{\mathrm{d}t} = S C_{Si} k_m e^{-k_m t}. \tag{2}$$

It is necessary to note that C_{Si} is the initial mean concentration of betalain that is extractable into a given extraction medium, and not the initial solid phase concentration *per se*. Its value will depend on the nature of the solid phase, the chemical nature of the extraction medium as well as the temperature of extraction. C_{Si} is therefore a model parameter which must be experimentally determined. Likewise, the exhaustion rate constant k_m is not the mass transfer coefficient because it is not based on a driving force, but a mere rate constant. It may include within it, a measure of the solid phase resistance and the liquid film resistance to the transfer of the solutes.

At a given temperature, the rate at which the betalain degrades in the extraction medium can also be assumed to be a first order with respect to its concentration in the liquid phase i.e., kC_L where k is the rate constant for betalain degradation. Therefore, the net rate of change of a betalain concentration in the liquid phase is given by:

$$\frac{\mathrm{d}C_L}{\mathrm{d}t} = S C_{Si} k_m e^{-k_m t} - k C_L \tag{3}$$

which can be analytically solved with the initial condition: C_L=0 at t=0, to give:

$$C_L = \frac{k_m S C_{si}}{(k - k_m)} \left[e^{-k_m t} - e^{-kt} \right] \quad (4)$$

Experimentally determined C_L versus t data for a range of different conditions (described in the materials and methods section), will be fitted to Eqn (4) to validate the model, as well as determine the best-fitting values of the parameters k_m, k and C_{Si}.

Given the inflow of the betalain into the extract phase from the solid phase and its inherent decomposition in this phase, C_L goes through a maximum, and the time when the maximum value occurs, t^*, can be determined by differentiating Eqn (4) and setting $\frac{\mathrm{d}C_L}{\mathrm{d}t} = 0$, whence:

$$t^* = \frac{1}{(k - k_m)} \ln\left(\frac{k}{k_m}\right) \quad (5)$$

Therefore, the maximum concentration of the extracted component is:

$$(C_L)_{max} = \frac{k_m S C_{si}}{(k - k_m)} \left[e^{-k_m t^*} - e^{-kt^*} \right] \quad (6)$$

where t^* is given by Eqn (5). The maximum rate of productivity of betalain under any given set of solvent (i.e., liquid phase) and operating conditions can be approximated to $[(C_L)_{max}/t^*]$ and this value will be used to compare the productivity rate observed under different conditions of temperature and solvent composition.

3. Materials and Methods

3.1 Experimental design

A random design was implemented for performing the extraction using different concentrations of ethanol in water (10, 20, and 30%) as the solvent phase. For the purpose of comparison, extraction was also undertaken using distilled water. The extraction temperatures investigated were: 55, 65, 75, and 85 °C. The ethanol concentration range employed was consistent with earlier studies (Celli and Brooks, 2017). The temperature range employed served to understand the kinetics of betalain decomposition (Bengardino et al., 2019).

All extraction experiments were carried out in triplicate. Means and standard deviations of the data were calculated for each extraction condition. Data analysis was performed using XLSTAT version 2021.1 (AddinSoft, Paris, France). Fitting of the equations to the model and determination of the model constants were performed using MATLAB 2020b Academic version (Mathworks Inc., USA); further details are given below in section 3.7.

3.2 Preparation of beetroot powder

Fresh beetroot was purchased from a local supplier in Reading, United Kingdom. The beetroot was washed, cleaned, and chopped in a food processor (Kenwood Blend-X Fresh BLP41.A0GO). It was then transferred to an aluminium tray and subjected to blast freezing at –80 °C, for 24-36 hours. It was subsequently freeze dried (VirTis SP Scientific, UK) for 70-72 hours until the moisture content dropped below 3% (dry weight basis). After freeze drying samples were ground (Kenwood Prospero AT286 KW714229 Spice Mill) and sieved to obtain different particle sizes. Most of the experiments were performed using particles of average diameter 300 µm. To study the effect of particle size on extraction kinetics different sieved fractions were used, with average particle diameter of 300 ± 12.1, 230 ± 8.6, 180 ± 5.1 and 120 ± 3.3 µm.

It may be noted that freeze drying is an expensive process, and in practice, it would be better to use beetroot in its harvested form. However, for the purpose of this research work, a starting material was needed which had uniform and consistent betalain composition, so that the model developed could be validated over the range of operating conditions. Hence, it was decided to freeze dry the beetroot which avoided processing and storage losses of betalain and also yielded consistent initial concentration. The model developed above (section 2) can also be applied to extraction from beetroot in its harvested form. However, the model parameters may have to be experimentally determined.

3.3 Chemicals and reagents

Analytical grade ethanol, citric acid, sodium phosphate dibasic, and betanin standard were purchased from Merck Chemicals Limited (UK).

3.4 Extraction of betalains in aqueous ethanol solutions

All experiments were performed by contacting the solid and liquid phases in closed beakers and agitating these in a hot water shaking-bath operating at a frequency of 1.6 Hz. For each time point, a separate extraction was performed to determine the extract concentration. Arbitrarily 22 time points were selected so that sufficient concentration versus time data points could be obtained to fit the model. Each of these 22 extractions were performed in triplicate in order to determine the mean and standard deviation for each time point. Each extraction batch was prepared by adding 1 g of dehydrated beetroot powder to 100 ml of the solvent phase which was already pre-heated to the desired extraction temperature. After the desired extraction time, the extract was collected, immediately cooled to 4 ºC, and centrifuged at 14000 rpm for 40 mins at 5 ºC to obtain a clear supernatant. The extract was then stored at 4 °C for further analysis.

3.5 Spectrophotometric measurement of betalains

Betalains were determined using the methods described in literature (Wong and Siow, 2015). McIlvaine buffer was prepared by mixing 30 mL of 0.1 M citric acid with 70 mL of 0.2 M sodium phosphate dibasic. The clear extract from the centrifuge was diluted 10 times using McIlvaine buffer before the spectrophotometer measurement. The concentration of betalains was spectrophotometrically determined (Cecil CE1011 Spectrophotometer). Betaxanthin (BX) absorbance was measured at wavelength of 480 nm (A_{480}) and Betacyanin (BC) absorbance was measured at 538 nm (A_{538}). In addition, a measurement was also taken at 650 nm (A_{650}) to remove the effect of any impurities. The measurement of BX and BC at 480 nm and 538 nm, together account for more than 95% of the betalains present in beetroot (Janiszewska-Turak et al., 2021; Stintzing et al., 2002). The concentration of the betalain was determined as:

$$\text{Betalains (mg of BX or BC/litre of extract)} = \frac{A \times DF \times MW \times 1000}{E \times L} \tag{7}$$

where A=(A_{538}-A_{650}) for betacyanins (BC) or (A_{485}-A_{650}) for betaxanthins (BX); DF=dilution factor; MW (Molecular Weight) = 550 g/mol for betacyanin and 339 g/mol for betaxanthin; E=molar extinction co-efficient in L mol^{-1}cm^{-1}, and the values for betacyanins and betaxanthins are 60,000 and 48,000, respectively; L= path length of quartz cuvette in cm.

3.6 Activation energy (E_a) calculation for solid phase exhaustion and degradation of betalains

The rate constants k_m and k, obtained at any given temperature by fitting the experimentally obtained C_L *versus* t data to Eqn (2), are temperature dependent. An Arrhenius type equation was used to correlate the variation of the rate constants with temperature (Eqn 6 below), which involved plotting ln(k_m) and ln (k) separately against 1/T, where T is the extraction temperature in K, and determining the gradient and intercept of the best fitting line to yield activation energy E_a (J mol^{-1}) and pre-exponential factor, A (s^{-1}) (Zin and Bánvölgyi, 2021).

$$\ln(k) \ or \ \ln(km) = \ln(A) - \frac{E_a}{RT} \tag{8}$$

3.7 Estimation of model parameters and goodness of fit

Experimentally determined C_L versus t data for a range of different conditions was fitted to Eqn (4) to validate the model, as well as determine the best-fitting values of the parameters k_m, k and C_{Si}. A regular curve fitting tool from the toolbox of MATLAB 2020b was used (Mathworks Inc., USA). The curve fitting tool works on the principle of reducing the sum squared error (SSE) and minimizing the root of mean squared error (RMSE) and requires a reasonable initial guess for k_{m}, k and C_{si} to obtain their best fit values.

MATLAB 2020b uses Levenberg–Marquardt (LM) estimation algorithm with 95% confidence interval. This is the most significant method used in high accuracy software packages for model parameter optimization. The Levenberg-Marquardt algorithm is an iterative technique that locates the minima of error function and optimizes the model parameters. It is a standard technique for nonlinear least-squares problems and can be thought of as a combination of steepest descent and the Gauss-Newton methods.

The best-fit values of the three model parameters were based on 22×3 data points for each experimental condition. The 95% confidence interval for each model parameter was estimated to locate the parameter values precisely and obtain a unique set of values. The narrow range of joint confidence interval obtained (see Tables 1a and 1b) established the precision in estimating the parameters, and also reinforces the adequacy of the number of experimental data points used in the fitting exercise.

SSE and RMSE indicate model validity and goodness of fit between the experimental data and the proposed model. Further, the co-efficient of correlation, R^2 and adjusted R^2 were determined to indicate whether an adequate number of parameters have been used for fitting the model to the experimental data.

$$\text{Sum of squarred error (SSE)} = \sum (y_{exp} - y_{model})^2 \tag{9}$$

$$\text{Root mean squarred error (RMSE)} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_{exp} - y_{model})^2} \tag{10}$$

$$\text{Coefficient of determination } (R^2) = 1 - \frac{RSS}{TSS} \tag{11}$$

$$\text{Adjusted coefficient of determination } (Adj - R^2) = 1 - \frac{(1-R^2)(n-1)}{(n-p-1)} \qquad (12)$$

where, n=number of observations for each experiment; y_{exp} – Experimental results; Y_{model} – Predicted results from model; *RSS* – Residual sum of square; *TSS* – Total sum of square; *p*-total number of predicted results from model.

4. Results and Discussion

4.1 Validation of model

The transient variation in the extract or liquid phase concentration of betacyanin and betaxanthin is given by Eqn (4). Experimental data on the concentrations of betacyanin and betaxanthin, extracted from dehydrated beetroot powder at different temperatures (55, 65, 75 and 85 °C), into aqueous ethanol solutions (concentration: 10, 20, and 30%), were fitted to Eqn (4) to test the validity of the model. Figs 1 (a) and 1 (b) demonstrate the model fit with the experimental data at four specific temperatures; the best fitting values of the model parameters are also stated in the caption. Table 1 (a) and (b) confirms the validation of Eqn (4) for all the experimental conditions investigated in this study. The best-fit model parameters as well as statistical parameters indicating the goodness of fit are also given in Tables 1 (a) and (b) for each set of experimental conditions. Even though the high R^2 value illustrates a good fit between model and experimental data, other fitness parameters such as sum of squared error (SSE), root mean squared error (RMSE), and Adjusted R^2 were also calculated (Eqns. 7, 8 and 10). The distinctly evident lower values of error functions and higher values of determination coefficients (Table 1 (a) and (b)) enhances model validity.

It is clear from Fig 1 that the concentration of the extracted component rises sharply at the start of the extraction, goes through a maximum value, and then gradually decreases with time. This is consistent with the model, which hypothesises that the concentration of the betalain at any time in the liquid phase is given by a balance between the rates of inflow from the solid phase and the rate of chemical degradation of the component. It is also clear from the caption of Fig 1 that the first order rate constant for solid exhaustion of a given betalain, i.e., k_m, is orders of magnitude greater than the rate constant for its chemical degradation. This indicates that the components are readily released from the solid phase but its subsequent degradation in the liquid phase is relatively slower. The fact that the betalain concentration peaks soon after commencement of the extraction indicates that short-time extraction is preferable to longer times. If this extraction is to be carried out continuously, then a reactor with tubular configuration will be effective in controlling residence times at such low values. Sivakumar et al. (2009) studied the extraction of beetroot coloring matter (combined betacyanin and betaxanthin) from fresh beetroot into 50% ethanol solution at 45 °C, collecting extract samples for analysis every 30 mins for 3 h. They found that the combined concentration of betalains increased progressively before attaining a uniform value. Bengardino et al. (2019) studied the extraction kinetics of betacyanin and betaxanthin separately from dehydrated beetroot leaves which was cut into an average size of 1 mm^2 at 30 and 80 °C, over a period of 24 hours. The betalain extraction profile was similar to the one reported in this study at 80 °C, except that the maximum concentration was observed after around 10 minutes which is considerably longer than the time observed in this study, which is due to the significantly larger particle size employed. In another study extraction from dehydrated beet leaves a similar concentration profile was observed for betaxanthin, but the extraction was assisted by ultrasound and the temperature was not precisely controlled (Nutter et al., 2021).

Unlike earlier studies, the present study reports for the first time the transient concentration profiles of betaxanthin as well as betacyanin when extracted from beetroot powders into ethanolic solutions. Moreover, a model is also proposed and experimentally validated for the concentration profile observed.

4.2 Values of the model parameters k, k_m, and C_{si}

As mentioned in section 2, the value of k_m represents the rate constant for solid phase exhaustion of betalains and k represents the degradation rate constant. It is evident from Table 1 (a) and (b) that k_m is significantly greater than k for betacyanin as well as betaxanthin. A possible explanation for the rapid exhaustion of betalains from solid or particulate phase is the short diffusion path length resulting from the use of relatively small particle sizes (Alsaud and Farid, 2020). The betalains are mainly present in vacuoles of the beetroot cellular structure (Nutter et al., 2021). The protecting membranes of the vacuoles can be easily broken by heat in the presence of the solvent to release the betalains (Nutter et al., 2021). It is also possible that the freezing and freeze drying may have altered the cellular architecture to facilitate betalains release. The rapid release of betalains from particle have been reported in number of earlier studies (Silva et al., 2020a). The temperature dependence of k_m value for a given particulate phase and solvent can be expressed by Arrhenius model; the constants of the model for different particulate and solvent combinations is given in Table 2.

Betalains are thermolabile compound and their stability is known to decline considerably between 50-80 °C (Herbach et al., 2006). Betacyanin degrades by decarboxylation and dehydrogenation to produce stable yellow colorants known as neo-betacyanins (Herbach et al., 2006). Betaxanthin degrades by hydrolysis and isomerisation (Herbach et al., 2006). The k value for betacyanin and betaxanthin degradation are close to the rate constant values reported at 50 °C by (Rodríguez-Sánchez et al., 2017) . It is also evident from Table 1 that the k values for betacyanin increased more sharply with temperature than betaxanthin, irrespective of the ethanol concentration. The higher sensitivity to thermal degradation of betacyanin has also been reported by two previous studies (Herbach et al., 2006).

C_{si} as mentioned earlier in section 2, maybe considered to indicate the amount of betalain extractable under a given set of operating conditions. From Tables 1 (a) and (b) it is evident that concentration of extractable betalains (betacyanin and betaxanthin) is influenced by solvent and temperature. In a given solvent the value of C_{si} was greater at 65 °C than 75 °C but its value at 85 °C was lowest. One possible reason for this observation is the thermal degradation of betalain in particulate phase itself due to the higher temperature.

4.3 Effect of Ethanol concentration on extraction kinetics and model parameters

From Tables 1 (a) and (b), it can be observed that ethanol not only plays an important role in the extraction of betalains but it also controls the maximum extractable betalains (C_{si}). It was observed that for both betacyanin and betaxanthin C_{si} values were higher in the case of 20% solution, than in 10 and 30%. The highest C_{si} value for the betacyanin and betaxanthin was observed at 65 °C in 20% ethanol solution, and the values for betacyanin and betaxanthin were 0.0044 and 0.0049 kg/kg of dried beetroot powder, respectively. At the same temperature, C_{si} value for extraction in pure water were determined as being 0.0037 kg/kg for betacyanin and 0.0035 kg/kg for betaxanthin; these values are significantly ($p<0.05$) lower than the corresponding values for ethanol solutions. In addition, the solid phase exhaustion rate constant for pure water was also lower than for the ethanol solutions. The rate constants for degradation of betacyanins in water are similar to the constants for ethanol solutions. Thus, we can reinforce the conclusion that ethanol solutions act as better solvents than pure water for betalains (Roriz et al., 2017). As mentioned earlier, $\ln(k)$ and $\ln(k_m)$, for each solvent, can be correlated with temperature by employing Arrhenius type of equation. For the range of temperatures employed in this study, the activation energy E_a varied with ethanol concentration, and the relevant values are illustrated in Figure 2. The activation energy for k_m increased sharply with ethanol percentage for both betacyanin and betaxanthin. On the other hand, the activation energy for k was observed to be lower for 20% ethanol solution than for 10% and 30% solutions. It is therefore clear that the use of a 20% ethanol solution as the extraction medium not only facilitates mass transfer from the particulate phase, but also results in lesser post-extraction degradation. Literature reports on the activation energy for betalain extraction are scarce, but the values are extensively reported for other solutes such as polyphenols. Balyan and Sarkar (2017) reported an activation energy for polyphenols from extraction jamun seeds in the range of 5.45-12.1 kJ/mol for the temperature range of 34.8-85.2 °C. Hobbi et al. (2021) reported a value of 12.4 kJ/mol for the extraction of polyphenols from apple pomace in the temperature range 40-85°C. The values of activation energy for betaxanthin and betacyanin shown in Fig. 2 are consistent with the values reported in literature. For example, Güneşer (2016) reported E_a value of 42.449 kJ/mol for betalain degradation from beetroot extracted into milk. Rodríguez-Sánchez et al. (2017) reported E_a values of 66.25 kJ/mol for the degradation of betaxanthin extracted from *S. pruinosus*. Kayın et al. (2019) reported E_a values of 66.13 and 92.04 kJ/mol for the degradation of betacyanin and betaxanthin, respectively, in red beet juice, which are consistent with the values given in Fig. 2(b).

4.4 Variation of the ratio of betalains (betacyanin and betaxanthin) with time in the extract phase

It is interesting to note from Figs 1 (a) and (b) that the extraction profile for betaxanthin mirrors that for betacyanin, with the solid exhaustion rate constant values (i.e., k_m) being similar. However, the rate constant for betaxanthin degradation is somewhat lower than that for betacyanin. In other words, the betacyanin released by the solid is expected to suffer greater levels of degradation over time. Fig 3 shows the variation of the ratio of the concentrations of betacyanin to betaxanthin with time at four different temperatures. At 55 and 65°C, the ratio does not vary significantly with time ($p<0.05$). However, at the higher temperatures of 75 and 85°C, a linear decreasing trend is observed that is consistent with the fact that betacyanin degrades much faster, especially at higher temperatures. The ratio of the concentrations of betacyanin to betaxanthin is important from the point of view of extract composition. Even though the concentration of betacyanin is greater than betaxanthin in beetroot powder (Fernandez et al., 2017), this ratio is maintained at the lower temperatures of 55 and 65°C, and for shorter extraction times at the higher temperatures of 75 and 85°C (Fig 3).

4.5 Effect of particle size on the extraction kinetics and model parameters

Effect of particle size was investigated by separating four sieve fractions in the range of 120-300 µm to give average particle sizes of 300 ± 12.1, 230 ± 8.6, 180 ± 5.1, and 120 ± 3.3 µm. The model parameters as a function of mean particle size are given in Table 3. The values of C_{si} and the rate constant for betalain degradation k are not expected to vary significantly with particle size. Table 3 confirms this fact for betacyanin and betaxanthin, except that the value of k for 300 µm particle size is somewhat higher. Although a specific reason for this observation has not been identified, a similar observation has been made by (Alsaud and Farid, 2020) who noted that the bioactive degradation rate is higher in the case of very fine particles (<200 µm).

The critical parameter influenced by the particle size is the rate constant for solid exhaustion (k_m). As mentioned in section 4.1, the value of this parameter is strongly influenced by the solute diffusion path length, which drops sharply with particle size. Thus, k_m is expected to increase with a decrease in particle size, which is observed in Table 3. However, the data for the smallest mean particle size, i.e., 120 µm, shows an anomaly. The value of K_m for this particle size is lower than the value for the next higher mean particle size, i.e., 180 µm. In other words, the solute transfer rate from 180 µm particles is faster than the transfer from 120 µm. This observation is not uncommon when very fine particle sizes are employed. Such fine particles tend to increase the effective suspension viscosity of the liquid phase and slow down mass transfer. Similar observations have been reported earlier (Asai et al., 1988). To conclude, it is worth noting that smaller particle sizes lower diffusion path length and increase rates of mass transfer from the particulate phase; however, when the particle size falls below a critical value, the particle hold-up for a given solid loading becomes very high and tends to increase the suspension viscosity which adversely affects mass transfer rates.

4.6 Maximum productivity rate of betalains extraction into the liquid phase

As mentioned in section 2, the concentration of a given betalain peaks at a given time (t*) and a betalain productivity rate, P, can be evaluated as the maximum concentration of the betalain divided by the time taken to reach this maximum value. Fig 4 plots the productivity rate of betacyanin and betaxanthin against temperature for different ethanol concentrations. It is interesting to note that similar productivity values can be achieved in all ethanol solutions at 55, 65 and 75°C. However, at 85°C, the betalain productivity rate in 20 and 30 % ethanol solutions are significantly higher than the values at other temperatures, with the value in 20% ethanol solution being higher than in 30% solution. This graph shows that similar betalain productivity values can be achieved at different temperatures and ethanol concentrations. In fact, a very high productivity can also be achieved at a temperature as high as 85°C, provided the residence time can be accurately controlled. In practice, achieving precise control of residence times is challenging and deviations from t* will result in betalain degradation due to the high temperature.

5. Conclusions

Based on the results obtained in this study and discussed above, the following conclusions can be derived.

1. A three-parameter model representing the balance between; 1) the rate of betalain inflow into a solvent phase and 2) the rate of betalain degradation in the solvent by a first order reaction, has been developed. The three model parameters are solid phase

exhaustion rate constant (k_m), the first order betalain degradation constant (k) and the concentration of extractable betalain in the solid phase (C_{si})

2. The model has been experimentally validated for the extraction of betacyanin and betaxanthin into 10, 20 and 30% ethanol solutions at 55, 65, 75 and 85°C.
3. The rate constants for solid phase exhaustion (k_m) and liquid phase degradation of betaxanthin and betacyanin (k) were correlated with temperature by an Arrhenius type equation.
4. The ratio of betacyanin to betaxanthin in the extract phase did not vary with extraction time significantly at 55 and 65°C, but it decreased with time at higher temperatures due to the more thermolabile nature of betacyanin.
5. The rate constant for solid phase exhaustion (k_m) increased with decrease in particle size for a given solid loading, except for the smallest particle size i.e., 120 µm, where k_m was lower, probably due to increase in suspension viscosity.
6. The betalain productivity rate at t* - the time when the concentration peaks - did not vary significantly at temperatures of 55, 65 and 75 °C in all ethanol solutions studied. However, at 85°C, the productivity value in 20 and 30% ethanol solutions was significantly higher. Therefore, similar betalain productivity values can be achieved at different temperatures and ethanol concentrations provided the residence time for extraction can be precisely controlled.
7. It is clear that Betalains are thermolabile; and high temperature processes, no doubt, pose a risk. But these processes are not beyond the realm of possibility. For example, UHT processes work very satisfactorily even at significantly higher temperatures and at shorter residence times than the values reported in this study. Thus, Food Engineering Operations are well-tuned to deal with such time-temperature combinations.

Acknowledgments

Author Rahul KUMAR is grateful for the financial support provided by ***Felix Trust*** through Felix Scholarship program. He would also like to thank the Society of Chemical Industry (SCI) for support towards training through Sydney Andrew Scholarship.

References:

Alsaud, N., Farid, M., 2020. Insight into the influence of grinding on the extraction efficiency of selected bioactive compounds from various plant leaves. Applied Sciences (Switzerland) 10. https://doi.org/10.3390/APP10186362

Appiah-Nkansah, N. B., Li, J., Zhang, K., Zhang, M., & Wang, D. (2019). Study on mass transfer kinetics of sugar extraction from Sweet Sorghum biomass via diffusion process and ethanol yield using SSF. Processes, *7*(3). https://doi.org/10.3390/pr7030137

Asai, S., Konishi, Y., Sasaki, Y., 1988. Mass transfer between fine particles and liquids in agitated vessels. Journal of Chemical Engineering of Japan 21, 107–112. https://doi.org/10.1252/jcej.21.107

Azeredo, H.M.C., 2009a. Betalains: Properties, sources, applications, and stability - A review. International Journal of Food Science and Technology 44, 2365–2376. https://doi.org/10.1111/j.1365-2621.2007.01668.x

Bassama, J., Tamba, A., Ndong, M., Sarr, K.D.D., Cissé, M., 2021. Degradation kinetics of betacyanins during the pasteurization and storage of cactus pear (Opuntia dillenii haw.) juice using the arrhenius, eyring, and ball models. Beverages 7, 1–12. https://doi.org/10.3390/beverages7010002

Balyan, U., & Sarkar, B. (2017). Aqueous extraction kinetics of phenolic compounds from jamun (Syzygium cumini L.) seeds. *International Journal of Food Properties, 20*(2), 372–389. https://doi.org/10.1080/10942912.2016.1163266

Bengardino, M.B., Fernandez, M. V., Nutter, J., Jagus, R.J., Agüero, M. V., 2019. Recovery of bioactive compounds from beet leaves through simultaneous extraction: Modelling and process optimization. Food and Bioproducts Processing 118, 227–236. https://doi.org/10.1016/j.fbp.2019.09.013

Celli, G.B., Brooks, M.S.L., 2017. Impact of extraction and processing conditions on betalains and comparison of properties with anthocyanins — A current review. Food Research International 100, 501–509. https://doi.org/10.1016/j.foodres.2016.08.034

Fernandez, M. V, Jagus, R.J., Agüero, M. V, 2017. Evaluation and Characterization of Nutritional , Microbiological and Sensory Properties of Beet Greens. Acta Scientific Nutritional Health 1, 37–45.

Güneşer, O., 2016. Pigment and color stability of beetroot betalains in cow milk during thermal treatment. Food Chemistry 196, 220–227. https://doi.org/10.1016/j.foodchem.2015.09.033

Herbach, K.M., Stintzing, F.C., Carle, R., 2006. Betalain stability and degradation - Structural and chromatic aspects. Journal of Food Science 71, 41–50. https://doi.org/10.1111/j.1750-3841.2006.00022.x

Hobbi, P., Okoro, O. V., Delporte, C., Alimoradi, H., Podstawczyk, D., Nie, L., Bernaerts, K. v., & Shavandi, A. (2021). Kinetic modelling of the solid–liquid extraction process of polyphenolic compounds from apple pomace: influence of solvent composition and temperature. In *Bioresources and Bioprocessing* (Vol. 8, Issue 1). https://doi.org/10.1186/s40643-021-00465-4

Leach G., Pyle D.L., Niranjan K. (1995). Effective diffusivity of total solids and pectic substances from apple tissue. International Journal of Food Science & technology, 29(6), 687-697.

Merin, U., Gagel, S., Popel, G., Bernstein, S., Rosenthal, I., 1987. Thermal Degradation Kinetics of Prickly-Pear-Fruit Red Pigment. Journal of Food Science 52, 485–486. https://doi.org/10.1111/j.1365-2621.1987.tb06646.x

Nestora, S., Merlier, F., Prost, E., Haupt, K., Rossi, C., Tse Sum Bui, B., 2016. Solid-phase extraction of betanin and isobetanin from beetroot extracts using a dipicolinic acid molecularly imprinted polymer. Journal of Chromatography A 1465, 47–54. https://doi.org/10.1016/j.chroma.2016.08.069

Nirmal, N.P., Mereddy, R., Maqsood, S., 2021. Recent developments in emerging technologies for beetroot pigment extraction and its food applications. Food Chemistry 356, 129611. https://doi.org/10.1016/j.foodchem.2021.129611

Nutter, J., Fernandez, M. V., Jagus, R.J., Agüero, M. V., 2021. Development of an aqueous ultrasound-assisted extraction process of bioactive compounds from beet leaves: a proposal for reducing losses and increasing biomass utilization. Journal of the Science of Food and Agriculture 101, 1989–1997. https://doi.org/10.1002/jsfa.10815

Rodríguez-Sánchez, J.A., Cruz y Victoria, M.T., Barragán-Huerta, B.E., 2017b. Betaxanthins and antioxidant capacity in Stenocereus pruinosus: Stability and use in food. Food Research International 91, 63–71. https://doi.org/10.1016/j.foodres.2016.11.023

Roriz, C.L., Barros, L., Prieto, M.A., Barreiro, M.F., Morales, P., Ferreira, I.C.F.R., 2017. Modern extraction techniques optimized to extract betacyanins from Gomphrena globosa L. Industrial Crops and Products 105, 29–40. https://doi.org/10.1016/j.indcrop.2017.05.008

Saguy, I., 1979a. Thermostability of Red Beet Pigments (Betanine and Vulgaxanthin–I): Influence of pH and Temperature. Journal of Food Science 44, 1554–1555. https://doi.org/10.1111/j.1365-2621.1979.tb06488.x

Silva, J.P.P., Bolanho, B.C., Stevanato, N., Massa, T.B., da Silva, C., 2020a. Ultrasound-assisted extraction of red beet pigments (Beta vulgaris L.): Influence of operational parameters and kinetic modeling. Journal of Food Processing and Preservation 1–10. https://doi.org/10.1111/jfpp.14762

Sivakumar, V., Anna, J. L., Vijayeeswarri, J., & Swaminathan, G. (2009). Ultrasound assisted enhancement in natural dye extraction from beetroot for industrial applications and natural dyeing of leather. *Ultrasonics Sonochemistry*, *16*(6), 782–789. https://doi.org/10.1016/j.ultsonch.2009.03.009

Stintzing, F.C., Schieber, A., Carle, R., 2003. Evaluation of colour properties and chemical quality parameters of cactus juices. European Food Research and Technology 216, 303–311. https://doi.org/10.1007/s00217-002-0657-0

Wong, Y.M., Siow, L.F., 2015. Effects of heat, pH, antioxidant, agitation and light on betacyanin stability using red-fleshed dragon fruit (Hylocereus polyrhizus) juice and concentrate as models. Journal of Food Science and Technology 52, 3086–3092. https://doi.org/10.1007/s13197-014-1362-2

Zin, M.M., Bánvölgyi, S., 2021. Emerging technology approach for extractability and stability of betalains from the peel of beetroot (Beta vulgaris L.). Biomass Conversion and Biorefinery. https://doi.org/10.1007/s13399-021-01975-z

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Figure 1: Extraction of Betalains from beet root powder at 65 and 85 °C into 10% ethanol water solution (a) Betacyanin concentration, (b) Betaxanthin concentration. Solid loading =10 kg/m^3, Particle size – 300±12.1 µm. Values of model parameters for betaxanthin at (i) 65 °C: $k = 1.411 \times 10^{-4}$ s^{-1}, K_m= 0.275 s^{-1}, C_{si} = 0.0042 kg/kg, R^2 = 0.996, and at (ii) 85 °C: $k = 4.29 \times 10^{-4}$ s^{-1}, K_m= 0.290 s^{-1}, C_{si} = 0.0041 kg/kg, R^2 = 0.954; Values of model parameters for betacyanin (i) 65 °C: $k = 1.052 \times 10^{-4}$ s^{-1}, K_m= 0.271 s^{-1}, C_{si} = 0.0038 kg/kg, R^2 = 0.997, and at (ii) 85 °C: $k = 6.64 \times 10^{-4}$ s^{-1}, K_m= 0.297 s^{-1}, C_{si} = 0.0040 kg/kg, R^2 = 0.988. The points indicate experimental values of the concentration and the solid line represents the model, i.e., concentration given by Eqn 4. Values of the model parameters for the other temperature range and ethanol-water solution are shown in Table 1 (a) and (b).

Figure 2: Effect of ethanol concentration on (a) Activation energy for k_m; (b) Activation energy for k. The experiments were performed with particle size of 300 ± 12.1 µm and solid loading of 10 kg m^{-3}.

Figure 3: Variation of betalains ratio (i.e., concentration of betacyanin/concentration of betaxanthin) with time at 55, 65, 75, 85 °C into 10% ethanol in water. The experiments were performed with particle size of 300 ± 12.1 µm and solid loading of 10 kg m^{-3}.

Figure 4: Variation of betalain productivity rate with temperature for different ethanol concentrations (a) betaxanthin and (b) betacyanin. The experiments were performed with particle size of 300 ± 12.1 µm and solid loading of 10 kg m^{-3}.

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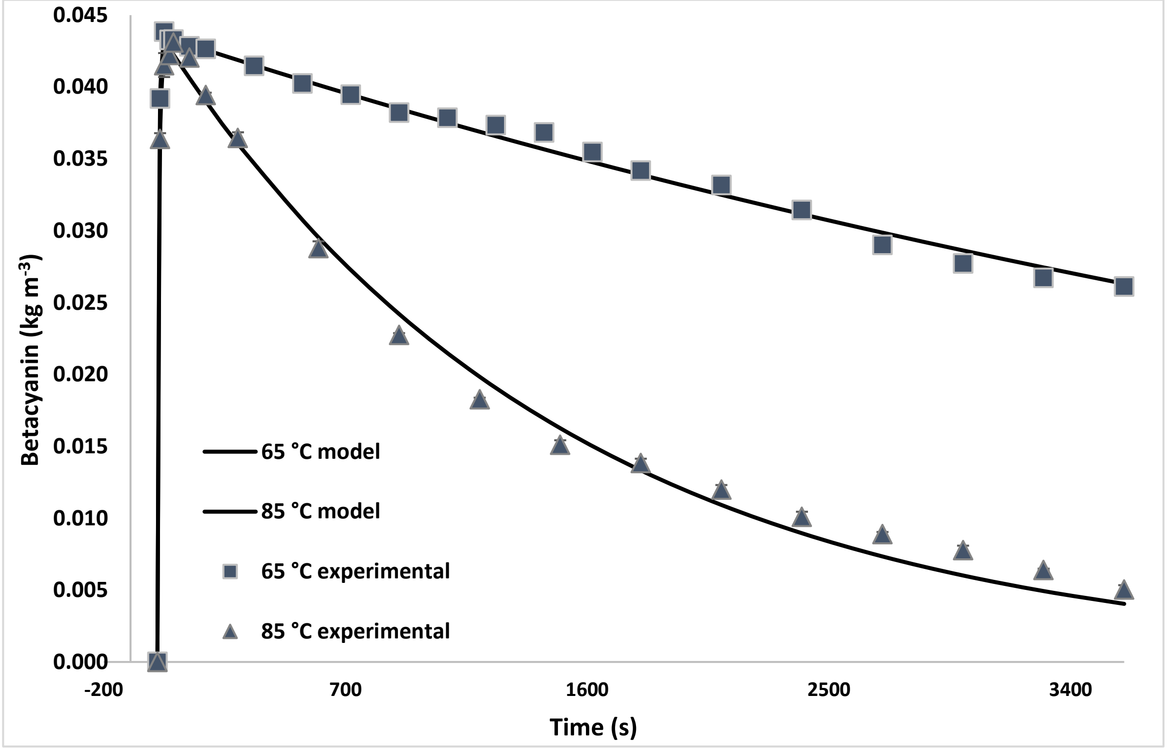
Table 1 (b): Values of model parameters fitting Eqn (4) for Betaxanthin. Experiments performed with solid to liquid ratio 10 kg m^{-3} and particle size 300±12.1 µm.

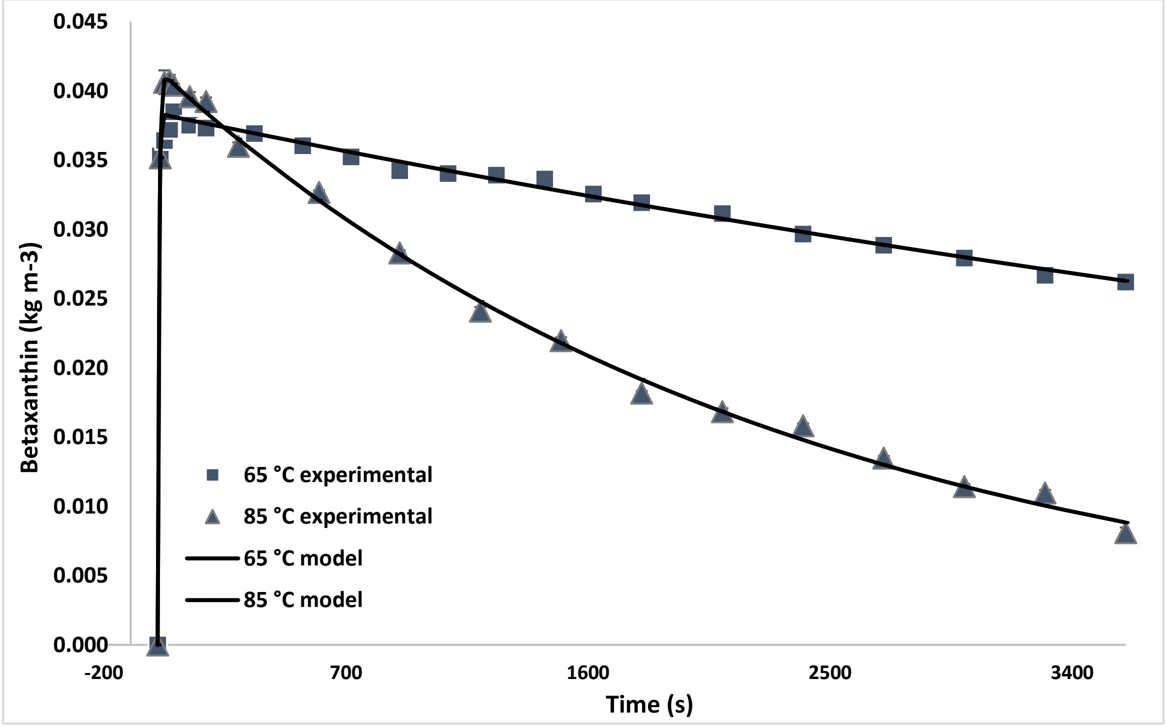
Table 2: Variation of Arrhenius parameters (Eqn 8) with ethanol concentration for betacyanin and betaxanthin. Experiments performed with solid loading of 10 kg m^{-3} and mean particle size 300 ± 12.1 µm.

Table 3 (a): Effect of particle size on the values of model parameters of Eqn (4) at 65 °C and ethanol Concentration of 20% in water for betacyanin.

Table 3 (b): Effect of particle size on the values of model parameters of Eqn (4) at 65 °C and ethanol Concentration of 20% in water for betaxanthin.



(a)



(b)

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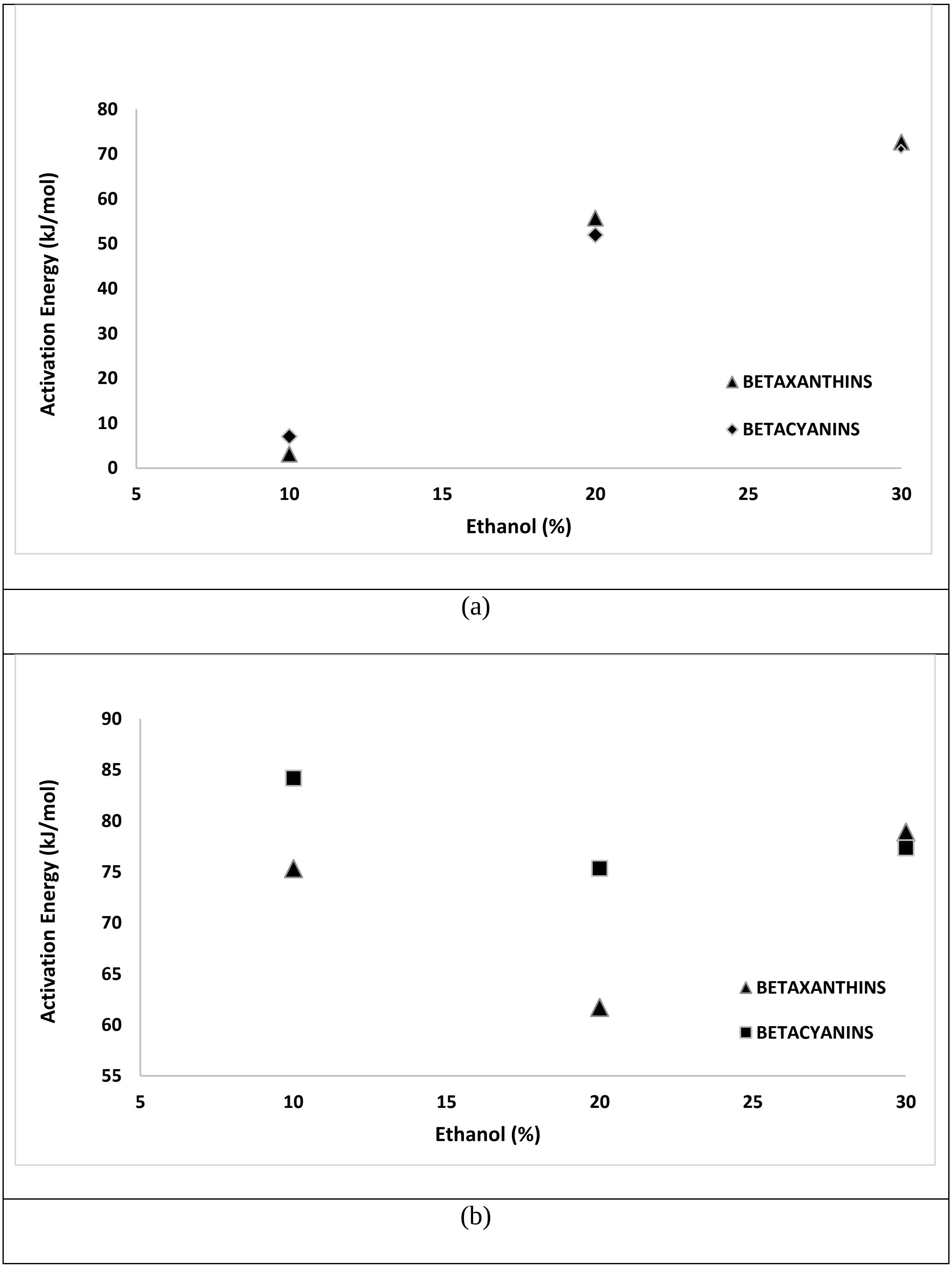

(a)

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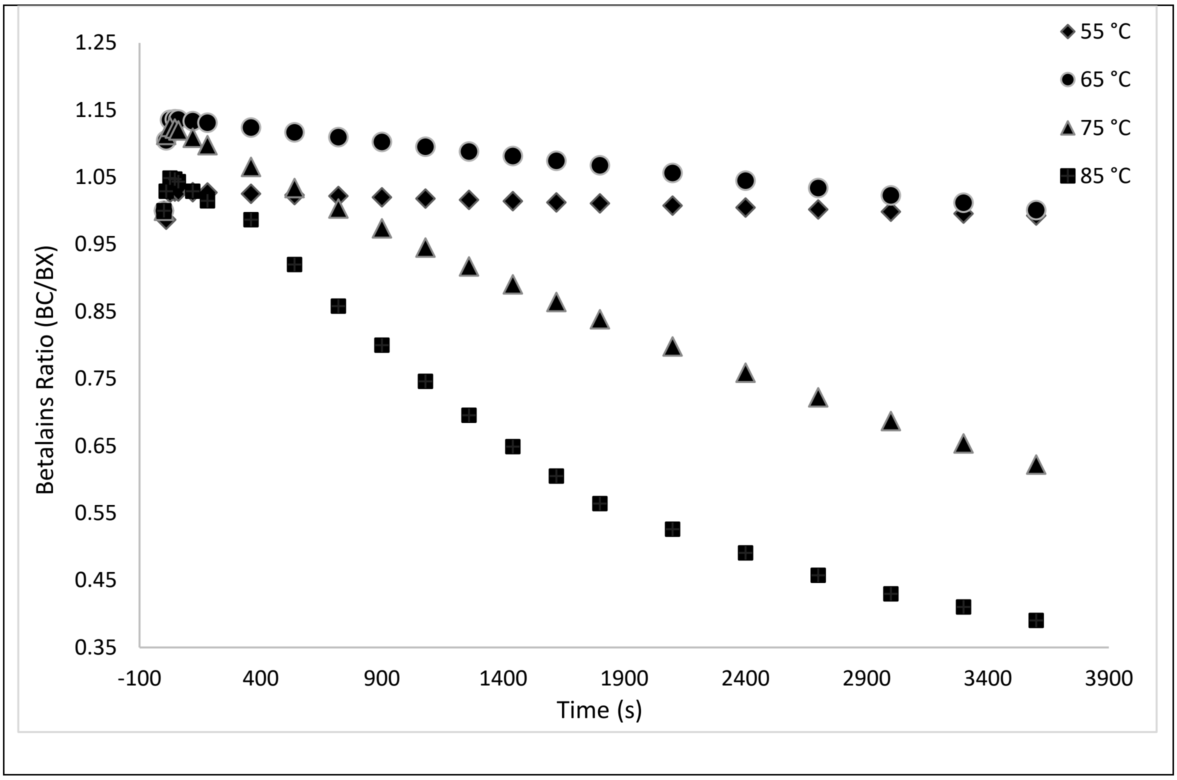

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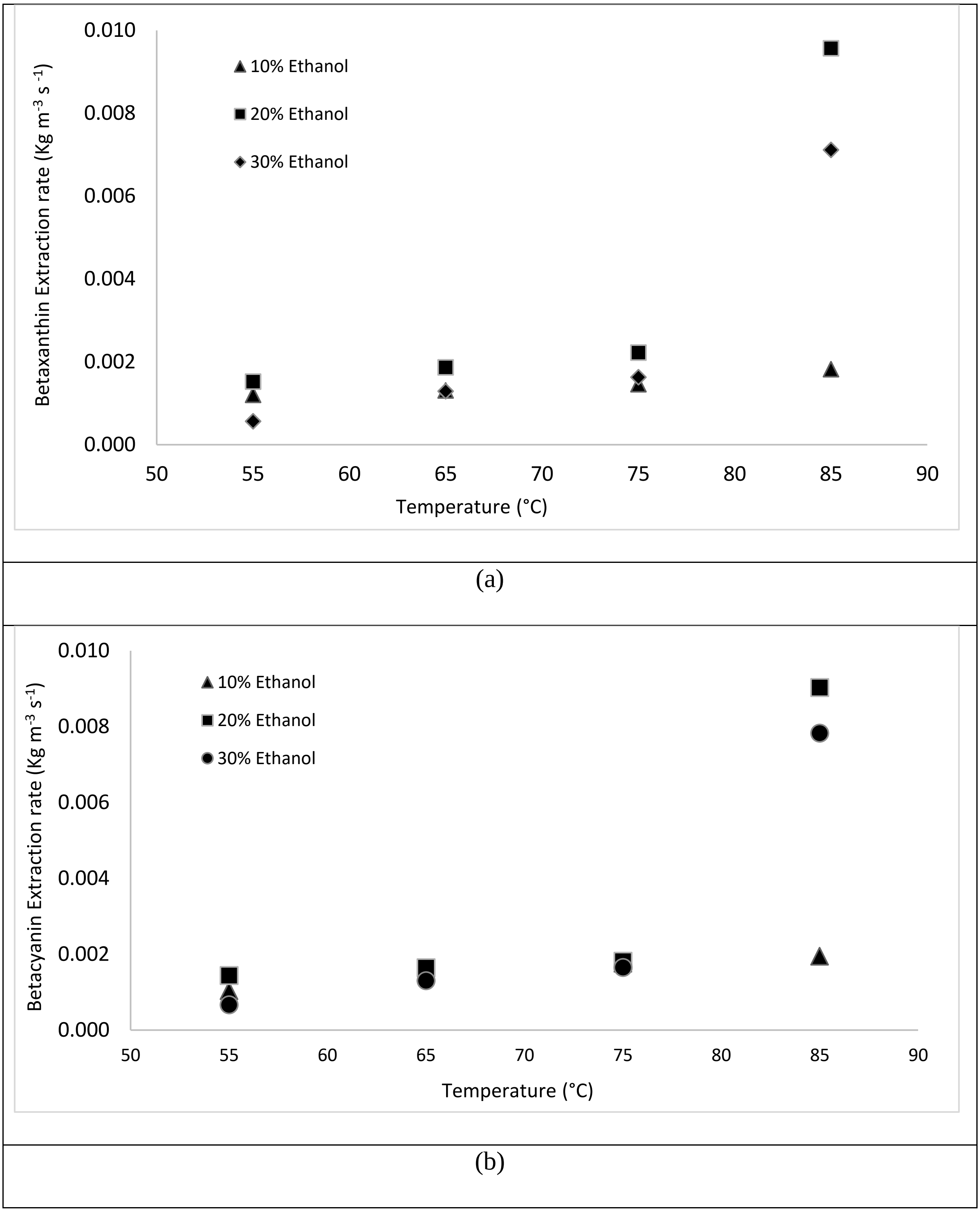

(a)

(b)

Figure 4: Variation of betalain productivity rate with temperature for different ethanol concentrations (a) betaxanthin and (b) betacyanin. The experiments were performed with particle size of 300 ± 12.1 µm and solid loading of 10 kg m^{-3}.

Table 1 (a): Values of model parameters fitting Eqn (4) for Betacyanin. Experiments performed with solid to liquid ratio 10 kg m^{-3} and particle size 300±12.1 μm.

Sl. No.	Temperature (°C)	Ethanol (%)	$k \times 10^{-4}$ (s^{-1})	$k \times 10^{-4}$ (s^{-1}) with 95% CI	k_m (s^{-1})	k_m (s^{-1}) with 95% CI	C_{si} (kg/kg)	C_{si} (kg/kg) with 95% CI	SSE × 10^{-5} (Eqn 9)	R^2 (Eqn 11)	Adj. R^2 (Eqn 12)	RMSE (Eqn 10)
1.	55	10	0.541	0.440-0.642	0.234	0.216-0.252	0.0037	0.0035-0.0039	1.022	0.985	0.983	0.00073
		20	0.823	0.792-0.854	0.278	0.227-0.329	0.0042	0.0041-0.0043	1.071	0.993	0.992	0.00081
		30	0.692	0.618-0.766	0.146	0.122-0.170	0.0035	0.0033-0.0037	0.166	0.875	0.859	0.00322
2.	65	10	1.411	1.011-1.811	0.275	0.245-0.305	0.0042	0.0039-0.0045	6.787	0.996	0.995	0.00065
		20	1.576	1.470-1.670	0.281	0.269-0.293	0.0044	0.0041-0.0047	5.373	0.997	0.996	0.00057
		30	1.934	1.791-2.077	0.206	0.152-0.261	0.0043	0.0042-0.0044	2.049	0.991	0.990	0.00113
3.	75	10	4.311	3.669-4.953	0.277	0.265-0.289	0.0041	0.00405-0.00415	7.842	0.997	0.996	0.00070
		20	3.913	2.927-4.899	0.285	0.278-0.292	0.0042	0.0040-0.0044	1.375	0.994	0.993	0.00092
		30	4.595	4.152-5.038	0.279	0.217-0.341	0.0039	0.0035-0.0043	1.187	0.996	0.995	0.00086
4.	85	10	6.647	4.892-8.402	0.297	0.199-0.395	0.0040	0.0038-0.0042	4.793	0.988	0.987	0.00173
		20	7.934	6.534-9.334	1.692	1.452-1.932	0.0041	0.0037-0.0045	0.244	0.958	0.953	0.00390
		30	7.238	6.167-8.309	1.544	1.348-1.741	0.0039	0.0038-0.0040	0.187	0.961	0.956	0.00342

C_{si} – Maximum extractable betalains (kg of dried betalains/kg of dried beetroot powder).

k – Degradation rate constant (s^{-1})

k_m – Solid exhaustion rate constant (s^{-1})

SSE – Sum of squared errors

R^2 – Co-efficient of determination

Adj. R^2 – Adjusted Co-efficient of determination

RMSE – Root mean squared error

CI – Confidence Interval

Table 1 (b): Values of model parameters fitting Eqn (4) for Betaxanthin. Experiments performed with solid to liquid ratio 10 kg m^{-3} and particle size 300±12.1 µm.

Sl. No.	Temperature (°C)	Ethanol (%)	$k \times 10^{-4}$ (s^{-1})	$k \times 10^{-4}$ (s^{-1}) with 95% CI	k_m (s^{-1})	k_m (s^{-1}) with 95% CI	C_{si} (kg/kg)	C_{si} (kg/kg) with 95% CI	SSE × 10^{-5} (Eqn 9)	R^2 (Eqn 11)	Adj. R^2 (Eqn 12)	RMSE (Eqn 10)
1.	55	10	0.544	0.511-0.577	0.261	0.252-0.271	0.0041	0.0039-0.0045	0.725	0.995	0.994	0.00061
		20	0.823	0.684-0.962	0.297	0.273-0.321	0.0042	0.0040-0.0044	0.417	0.997	0.997	0.00051
		30	0.275	0.245-0.305	0.133	0.121-0.145	0.0037	0.0036-0.0038	0.165	0.899	0.887	0.00321
2.	65	10	1.052	0.917-1.187	0.271	0.255-0.287	0.0038	0.0035-0.0041	0.411	0.997	0.996	0.00050
		20	0.956	0.776-1.136	0.307	0.291-0.323	0.0049	0.0046-0.0052	0.469	0.997	0.996	0.00054
		30	1.038	0.901-1.175	0.214	0.156-0.272	0.0047	0.0045-0.0049	0.902	0.995	0.995	0.00075
3.	75	10	2.659	2.132-3.186	0.274	0.249-0.299	0.0037	0.0035-0.0039	1.545	0.991	0.989	0.00098
		20	2.314	1.965-2.663	0.405	0.312-0.498	0.0042	0.0039-0.0045	2.045	0.989	0.987	0.00113
		30	2.187	1.857-2.517	0.286	0.266-0.306	0.0041	0.0040-0.0042	1.943	0.991	0.989	0.00110
4.	85	10	4.291	3.845-4.737	0.290	0.265-0.315	0.0041	0.0039-0.0043	0.138	0.954	0.948	0.00294
		20	5.094	4.458-5.730	1.87	1.473-2.267	0.0043	0.0040-0.0046	0.228	0.952	0.946	0.00378
		30	0.356	0.312-0.400	1.484	1.221-1.747	0.0040	0.0036-0.0044	0.203	0.947	0.941	0.00356

Table 2: Variation of Arrhenius parameters (Eqn 8) with ethanol concentration for betacyanin and betaxanthin. Experiments performed with solid loading of 10 kg m^{-3} and mean particle size 300 ± 12.1 µm.

	Betaxanthin (BX)						Betacyanin (BC)					
	k_m			k			k_m			k		
Ethanol (%)	A (s^{-1})	E_a (kJ mol^{-1})	R^2	A (s^{-1})	E_a (kJ mol^{-1})	R^2	A (s^{-1})	E_a (kJ mol^{-1})	R^2	A (s^{-1})	E_a (kJ mol^{-1})	R^2
10	1.66	3.14	0.93	2.87	75.32	0.98	0.15	7.06	0.87	3.05	84.20	0.98
20	2.93	55.76	0.87	2.57	61.73	0.92	2.85	52.00	0.89	2.91	75.35	0.99
30	3.19	72.72	0.94	2.99	78.90	0.96	3.17	71.14	0.91	2.93	77.37	0.98

A – Pre-exponential factor (s^{-1})

E_a – Activation energy (kJ mol^{-1})

R^2 – Coefficient of Determination

Table 3 (a): Effect of particle size on the values of model parameters of Eqn (4) at 65 °C and ethanol Concentration of 20% in water for betacyanin.

Average Particle size (μm)	k_m (s^{-1})	k x 10^{-3} (s^{-1})	C_{si} (kg/kg)	SSE x 10^{-5}	R^2	Adj. R^2	RMSE
300±12.1	0.28	0.161	0.0044	0.531	0.997	0.996	0.0006
230±8.6	0.627	0.255	0.0041	3.728	0.984	0.982	0.0015
180±5.1	1.758	0.265	0.0039	5.362	0.975	0.972	0.0018
120±3.3	0.522	0.256	0.0038	2.762	0.986	0.984	0.0013

C_{si} – Maximum extractable betalains (kg of dried betalains/kg of dried beetroot powder).

SSE – Sum of squared errors

k – Degradation rate constant (s^{-1})

k_m – Solid exhaustion rate constant (s^{-1})

R^2 – Co-efficient of determination

Adj. R^2 – Adjusted Co-efficient of determination

RMSE – Root mean squared error

Table 3 (b): Effect of particle size on the values of model parameters of Eqn (4) at 65 °C and ethanol concentration of 20% in water for betaxanthin.

Average Particle size (μm)	k_m (s^{-1})	k x 10^{-3} (s^{-1})	C_{si} (kg/kg)	SSE x 10^{-5}	R^2	Adj. R^2	RMSE
300±12.1	0.307	0.095	0.0049	0.469	0.997	0.996	0.000541
230±8.6	0.474	0.146	0.0037	2.551	0.982	0.980	0.00126
180±5.1	1.411	0.145	0.0034	2.594	0.978	0.975	0.00127
120±3.3	0.412	0.156	0.0033	1.413	0.988	0.986	0.00093