

Modelling extraction kinetics of betalains from freeze dried beetroot powder into aqueous ethanol solutions

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5 Running Title: Modelling the extraction kinetics of betalains

6

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14 **Modelling extraction kinetics of betalains from freeze dried beetroot powder into**
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19 **Abstract**

20 The extraction kinetics of betalains (betacyanin and betaxanthin) from freeze dried beetroot
21 powder into aqueous ethanol solutions is modelled by considering the concentration of a given
22 betalain at any given time to result from a balance between the rate of its release from the solid
23 phase and the rate of its chemical degradation in the extract phase. The mathematical model
24 obtained shows that the concentration of the betalain peaks before progressively decreasing
25 with time. The model was experimentally validated for various combinations of temperature
26 (55-85 °C), ethanol concentration (10-30%) and particle size (120-300 µm). The ratio of
27 betacyanin to betaxanthin in the liquid phase was approximately 1 over the duration of
28 extraction at 55 and 65 °C. However, the ratio decreased at the higher temperatures of 75 and
29 85 °C. A maximum productivity rate of a given betalain was defined as its peak concentration
30 divided by the time taken to reach the peak concentration, which was found to be relatively
31 insensitive to the ethanol concentration below 75 °C.

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

33

Nomenclature

A	Pre-exponential factor, Eqn 8, s^{-1} ,	L	Path length of cuvette, cm
A₄₈₀	Absorbance measured at 480 nm	MW	Molecular weight, $g\ mol^{-1}$
A₅₃₈	Absorbance measured at 538 nm	n	Number of observations for each experiment
A₆₅₀	Absorbance measured at 650 nm	p	Total number of predicted results from model
Adj-R²	Adjusted coefficient of determination,	P	Betalain productivity rate, $kg\ m^{-3}\ s^{-1}$
BC	Betacyanin	R²	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}		

37 **1. Introduction**

38 Beetroot (*Beta vulgaris L.*) is an herbaceous blooming biennial plant, native to Asia and
39 Europe, that belongs to the Chenopodiaceae family and grown across seasons (Nirmal et al.,
40 2021). It is widely consumed as a salad, as a juice, after pickling and as a cooked vegetable. It
41 is known to contain high levels of nutritionally beneficial and bioactive compounds, such as
42 nitrate, phenolics, and ascorbic acid, as well as vitamins, minerals, carbohydrates, fibre,
43 protein, essential amino acids, fatty acids, phytosterols, alkaloids, steroids, carotenoids, and a
44 significant level of pigments called betalains soluble in polar solvents (Fernandez et al., 2017a).

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

58 There have been many published reports on the extraction and analysis of betalains from
59 beetroot. In addition, extraction, degradation, and stability of betalains from sources other
60 than beetroot has been studied; (Merin et al., 1987) studied the stability of betacyanin as color

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

83 It is clear from a review of literature that there are only isolated studies providing insights into
84 the extraction of betalains and their subsequent stability, but no systematic study which
85 explores the kinetics of extraction in specified solvents and links it to the operational

86 parameters such as the temperature. Since betalains are soluble in polar solvents, water and
87 other aqueous solutions are effective media for extraction. Ethanolic solutions are usually
88 preferred to water due to relatively lower extraction efficiency and stability of betalains (Roriz
89 et al., 2017). The main objective of this paper is to develop a mechanistic model for the
90 extraction of betalains from freeze dried beetroot into ethanolic solutions which takes into
91 account: 1) the mechanisms operating during interfacial mass transfer of the betalains into a
92 solvent phase and 2) the post-extraction chemical degradation in the solvent. The paper then
93 reports extensive experimental data which validates the model, and discusses the effects of a
94 range of operating parameters such as temperature, ethanol concentration and the particle size
95 on extraction kinetics.

96 **2. Modelling the transient concentration of betalains in the extract**

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

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

$$114 \quad C_S = C_{Si}e^{-k_m t} \quad (1)$$

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

$$118 \quad -S \frac{dC_S}{dt} = SC_{Si}k_m e^{-k_m t}. \quad (2)$$

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

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

$$131 \quad \frac{dC_L}{dt} = SC_{Si}k_m e^{-k_m t} - kC_L \quad (3)$$

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

133
$$C_L = \frac{k_m S C_{Si}}{(k - k_m)} [e^{-k_m t} - e^{-k t}] \quad (4)$$

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

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

140 whence:

141
$$t^* = \frac{1}{(k - k_m)} \ln \left(\frac{k}{k_m} \right) \quad (5)$$

142 Therefore, the maximum concentration of the extracted component is:

143
$$(C_L)_{max} = \frac{k_m S C_{Si}}{(k - k_m)} [e^{-k_m t^*} - e^{-k t^*}] \quad (6)$$

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

148 **3. Materials and Methods**

149 *3.1 Experimental design*

150 A random design was implemented for performing the extraction using different concentrations
151 of ethanol in water (10, 20, and 30%) as the solvent phase. For the purpose of comparison,
152 extraction was also undertaken using distilled water. The extraction temperatures investigated
153 were: 55, 65, 75, and 85 °C. The ethanol concentration range employed was consistent with

154 earlier studies (Celli and Brooks, 2017). The temperature range employed served to understand
155 the kinetics of betalain decomposition (Bengardino et al., 2019).

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

161 *3.2 Preparation of beetroot powder*

162 Fresh beetroot was purchased from a local supplier in Reading, United Kingdom. The beetroot
163 was washed, cleaned, and chopped in a food processor (Kenwood Blend-X Fresh
164 BLP41.A0GO). It was then transferred to an aluminium tray and subjected to blast freezing at
165 $-80\text{ }^{\circ}\text{C}$, for 24-36 hours. It was subsequently freeze dried (VirTis SP Scientific, UK) for 70-72
166 hours until the moisture content dropped below 3% (dry weight basis). After freeze drying
167 samples were ground (Kenwood Prospero AT286 KW714229 Spice Mill) and sieved to obtain
168 different particle sizes. Most of the experiments were performed using particles of average
169 diameter $300\text{ }\mu\text{m}$. To study the effect of particle size on extraction kinetics different sieved
170 fractions were used, with average particle diameter of 300 ± 12.1 , 230 ± 8.6 , 180 ± 5.1 and 120
171 $\pm 3.3\text{ }\mu\text{m}$.

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

178 extraction from beetroot in its harvested form. However, the model parameters may have to be
179 experimentally determined.

180 *3.3 Chemicals and reagents*

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

183 *3.4 Extraction of betalains in aqueous ethanol solutions*

184 All experiments were performed by contacting the solid and liquid phases in closed beakers
185 and agitating these in a hot water shaking-bath operating at a frequency of 1.6 Hz. For each
186 time point, a separate extraction was performed to determine the extract concentration.
187 Arbitrarily 22 time points were selected so that sufficient concentration versus time data points
188 could be obtained to fit the model. Each of these 22 extractions were performed in triplicate in
189 order to determine the mean and standard deviation for each time point. Each extraction batch
190 was prepared by adding 1 g of dehydrated beetroot powder to 100 ml of the solvent phase
191 which was already pre-heated to the desired extraction temperature. After the desired extraction
192 time, the extract was collected, immediately cooled to 4 °C, and centrifuged at 14000 rpm for
193 40 mins at 5 °C to obtain a clear supernatant. The extract was then stored at 4 °C for further
194 analysis.

195 *3.5 Spectrophotometric measurement of betalains*

196 Betalains were determined using the methods described in literature (Wong and Siow, 2015).
197 McIlvaine buffer was prepared by mixing 30 mL of 0.1 M citric acid with 70 mL of 0.2 M
198 sodium phosphate dibasic. The clear extract from the centrifuge was diluted 10 times using
199 McIlvaine buffer before the spectrophotometer measurement. The concentration of betalains
200 was spectrophotometrically determined (Cecil CE1011 Spectrophotometer). Betaxanthin (BX)
201 absorbance was measured at wavelength of 480 nm (A_{480}) and Betacyanin (BC) absorbance

202 was measured at 538 nm (A_{538}). In addition, a measurement was also taken at 650 nm (A_{650}) to
203 remove the effect of any impurities. The measurement of BX and BC at 480 nm and 538 nm,
204 together account for more than 95% of the betalains present in beetroot (Janiszewska-Turak et
205 al., 2021; Stintzing et al., 2002). The concentration of the betalain was determined as:

$$206 \text{ Betalains (mg of BX or BC/litre of extract)} = \frac{A \times DF \times MW \times 1000}{E \times L} \quad (7)$$

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

211 3.6 Activation energy (E_a) calculation for solid phase exhaustion and degradation of betalains

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

$$218 \ln(k) \text{ or } \ln(km) = \ln(A) - \frac{E_a}{RT} \quad (8)$$

219 3.7 Estimation of model parameters and goodness of fit

220 Experimentally determined C_L versus t data for a range of different conditions was fitted to
221 Eqn (4) to validate the model, as well as determine the best-fitting values of the parameters k_m ,
222 k and C_{Si} . A regular curve fitting tool from the toolbox of MATLAB 2020b was used
223 (Mathworks Inc., USA). The curve fitting tool works on the principle of reducing the sum

224 squared error (SSE) and minimizing the root of mean squared error (RMSE) and requires a
225 reasonable initial guess for k_m , k and C_{si} to obtain their best fit values.

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

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

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

242

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

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

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

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

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

250 **4. Results and Discussion**

251 *4.1 Validation of model*

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

266 It is clear from Fig 1 that the concentration of the extracted component rises sharply at the start
267 of the extraction, goes through a maximum value, and then gradually decreases with time. This
268 is consistent with the model, which hypothesises that the concentration of the betalain at any

269 time in the liquid phase is given by a balance between the rates of inflow from the solid phase
270 and the rate of chemical degradation of the component. It is also clear from the caption of Fig
271 1 that the first order rate constant for solid exhaustion of a given betalain, i.e., k_m , is orders of
272 magnitude greater than the rate constant for its chemical degradation. This indicates that the
273 components are readily released from the solid phase but its subsequent degradation in the
274 liquid phase is relatively slower. The fact that the betalain concentration peaks soon after
275 commencement of the extraction indicates that short-time extraction is preferable to longer
276 times. If this extraction is to be carried out continuously, then a reactor with tubular
277 configuration will be effective in controlling residence times at such low values. Sivakumar et
278 al. (2009) studied the extraction of beetroot coloring matter (combined betacyanin and
279 betaxanthin) from fresh beetroot into 50% ethanol solution at 45 °C, collecting extract samples
280 for analysis every 30 mins for 3 h. They found that the combined concentration of betalains
281 increased progressively before attaining a uniform value. Bengardino et al. (2019) studied the
282 extraction kinetics of betacyanin and betaxanthin separately from dehydrated beetroot leaves
283 which was cut into an average size of 1 mm² at 30 and 80 °C, over a period of 24 hours. The
284 betalain extraction profile was similar to the one reported in this study at 80 °C, except that the
285 maximum concentration was observed after around 10 minutes which is considerably longer
286 than the time observed in this study, which is due to the significantly larger particle size
287 employed. In another study extraction from dehydrated beet leaves a similar concentration
288 profile was observed for betaxanthin, but the extraction was assisted by ultrasound and the
289 temperature was not precisely controlled (Nutter et al., 2021).

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

294 4.2 Values of the model parameters k , k_m , and C_{si}

295 As mentioned in section 2, the value of k_m represents the rate constant for solid phase
296 exhaustion of betalains and k represents the degradation rate constant. It is evident from Table
297 1 (a) and (b) that k_m is significantly greater than k for betacyanin as well as betaxanthin. A
298 possible explanation for the rapid exhaustion of betalains from solid or particulate phase is the
299 short diffusion path length resulting from the use of relatively small particle sizes (Alsaud and
300 Farid, 2020). The betalains are mainly present in vacuoles of the beetroot cellular structure
301 (Nutter et al., 2021). The protecting membranes of the vacuoles can be easily broken by heat
302 in the presence of the solvent to release the betalains (Nutter et al., 2021). It is also possible
303 that the freezing and freeze drying may have altered the cellular architecture to facilitate
304 betalains release. The rapid release of betalains from particle have been reported in number of
305 earlier studies (Silva et al., 2020a). The temperature dependence of k_m value for a given
306 particulate phase and solvent can be expressed by Arrhenius model; the constants of the model
307 for different particulate and solvent combinations is given in Table 2.

308 Betalains are thermolabile compound and their stability is known to decline considerably
309 between 50-80 °C (Herbach et al., 2006). Betacyanin degrades by decarboxylation and
310 dehydrogenation to produce stable yellow colorants known as neo-betacyanins (Herbach et al.,
311 2006). Betaxanthin degrades by hydrolysis and isomerisation (Herbach et al., 2006). The k
312 value for betacyanin and betaxanthin degradation are close to the rate constant values reported
313 at 50 °C by (Rodríguez-Sánchez et al., 2017) . It is also evident from Table 1 that the k values
314 for betacyanin increased more sharply with temperature than betaxanthin, irrespective of the
315 ethanol concentration. The higher sensitivity to thermal degradation of betacyanin has also
316 been reported by two previous studies (Herbach et al., 2006).

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

323 *4.3 Effect of Ethanol concentration on extraction kinetics and model parameters*

324 From Tables 1 (a) and (b), it can be observed that ethanol not only plays an important role in
325 the extraction of betalains but it also controls the maximum extractable betalains (C_{si}). It was
326 observed that for both betacyanin and betaxanthin C_{si} values were higher in the case of 20%
327 solution, than in 10 and 30%. The highest C_{si} value for the betacyanin and betaxanthin was
328 observed at 65 °C in 20% ethanol solution, and the values for betacyanin and betaxanthin were
329 0.0044 and 0.0049 kg/kg of dried beetroot powder, respectively. At the same temperature, C_{si}
330 value for extraction in pure water were determined as being 0.0037 kg/kg for betacyanin and
331 0.0035 kg/kg for betaxanthin; these values are significantly ($p < 0.05$) lower than the
332 corresponding values for ethanol solutions. In addition, the solid phase exhaustion rate constant
333 for pure water was also lower than for the ethanol solutions. The rate constants for degradation
334 of betacyanins in water are similar to the constants for ethanol solutions. Thus, we can reinforce
335 the conclusion that ethanol solutions act as better solvents than pure water for betalains (Roriz
336 et al., 2017). As mentioned earlier, $\ln(k)$ and $\ln(k_m)$, for each solvent, can be correlated with
337 temperature by employing Arrhenius type of equation. For the range of temperatures employed
338 in this study, the activation energy E_a varied with ethanol concentration, and the relevant values
339 are illustrated in Figure 2. The activation energy for k_m increased sharply with ethanol
340 percentage for both betacyanin and betaxanthin. On the other hand, the activation energy for k
341 was observed to be lower for 20% ethanol solution than for 10% and 30% solutions. It is

342 therefore clear that the use of a 20% ethanol solution as the extraction medium not only
343 facilitates mass transfer from the particulate phase, but also results in lesser post-extraction
344 degradation. Literature reports on the activation energy for betalain extraction are scarce, but
345 the values are extensively reported for other solutes such as polyphenols. Balyan and Sarkar
346 (2017) reported an activation energy for polyphenols from extraction jamun seeds in the range
347 of 5.45-12.1 kJ/mol for the temperature range of 34.8-85.2 °C. Hobbi et al. (2021) reported a
348 value of 12.4 kJ/mol for the extraction of polyphenols from apple pomace in the temperature
349 range 40-85°C. The values of activation energy for betaxanthin and betacyanin shown in Fig.
350 2 are consistent with the values reported in literature. For example, Güneşer (2016) reported
351 E_a value of 42.449 kJ/mol for betalain degradation from beetroot extracted into milk.
352 Rodríguez-Sánchez et al. (2017) reported E_a values of 66.25 kJ/mol for the degradation of
353 betaxanthin extracted from *S. pruinosus*. Kayın et al. (2019) reported E_a values of 66.13 and
354 92.04 kJ/mol for the degradation of betacyanin and betaxanthin, respectively, in red beet juice,
355 which are consistent with the values given in Fig. 2(b).

356 *4.4 Variation of the ratio of betalains (betacyanin and betaxanthin) with time in the extract* 357 *phase*

358 It is interesting to note from Figs 1 (a) and (b) that the extraction profile for betaxanthin mirrors
359 that for betacyanin, with the solid exhaustion rate constant values (i.e., k_m) being similar.
360 However, the rate constant for betaxanthin degradation is somewhat lower than that for
361 betacyanin. In other words, the betacyanin released by the solid is expected to suffer greater
362 levels of degradation over time. Fig 3 shows the variation of the ratio of the concentrations of
363 betacyanin to betaxanthin with time at four different temperatures. At 55 and 65°C, the ratio
364 does not vary significantly with time ($p < 0.05$). However, at the higher temperatures of 75 and
365 85°C, a linear decreasing trend is observed that is consistent with the fact that betacyanin
366 degrades much faster, especially at higher temperatures. The ratio of the concentrations of

367 betacyanin to betaxanthin is important from the point of view of extract composition. Even
368 though the concentration of betacyanin is greater than betaxanthin in beetroot powder
369 (Fernandez et al., 2017), this ratio is maintained at the lower temperatures of 55 and 65°C, and
370 for shorter extraction times at the higher temperatures of 75 and 85°C (Fig 3).

371 *4.5 Effect of particle size on the extraction kinetics and model parameters*

372 Effect of particle size was investigated by separating four sieve fractions in the range of 120-
373 300 µm to give average particle sizes of 300 ± 12.1 , 230 ± 8.6 , 180 ± 5.1 , and 120 ± 3.3 µm.
374 The model parameters as a function of mean particle size are given in Table 3. The values of
375 C_{si} and the rate constant for betalain degradation k are not expected to vary significantly with
376 particle size. Table 3 confirms this fact for betacyanin and betaxanthin, except that the value
377 of k for 300 µm particle size is somewhat higher. Although a specific reason for this observation
378 has not been identified, a similar observation has been made by (Alsaud and Farid, 2020) who
379 noted that the bioactive degradation rate is higher in the case of very fine particles (<200 µm).
380 The critical parameter influenced by the particle size is the rate constant for solid exhaustion
381 (k_m). As mentioned in section 4.1, the value of this parameter is strongly influenced by the
382 solute diffusion path length, which drops sharply with particle size. Thus, k_m is expected to
383 increase with a decrease in particle size, which is observed in Table 3. However, the data for
384 the smallest mean particle size, i.e., 120 µm, shows an anomaly. The value of K_m for this
385 particle size is lower than the value for the next higher mean particle size, i.e., 180 µm. In other
386 words, the solute transfer rate from 180 µm particles is faster than the transfer from 120 µm.
387 This observation is not uncommon when very fine particle sizes are employed. Such fine
388 particles tend to increase the effective suspension viscosity of the liquid phase and slow down
389 mass transfer. Similar observations have been reported earlier (Asai et al., 1988). To conclude,
390 it is worth noting that smaller particle sizes lower diffusion path length and increase rates of

391 mass transfer from the particulate phase; however, when the particle size falls below a critical
392 value, the particle hold-up for a given solid loading becomes very high and tends to increase
393 the suspension viscosity which adversely affects mass transfer rates.

394 *4.6 Maximum productivity rate of betalains extraction into the liquid phase*

395 As mentioned in section 2, the concentration of a given betalain peaks at a given time (t^*) and
396 a betalain productivity rate, P , can be evaluated as the maximum concentration of the betalain
397 divided by the time taken to reach this maximum value. Fig 4 plots the productivity rate of
398 betacyanin and betaxanthin against temperature for different ethanol concentrations. It is
399 interesting to note that similar productivity values can be achieved in all ethanol solutions at
400 55, 65 and 75°C. However, at 85°C, the betalain productivity rate in 20 and 30 % ethanol
401 solutions are significantly higher than the values at other temperatures, with the value in 20%
402 ethanol solution being higher than in 30% solution. This graph shows that similar betalain
403 productivity values can be achieved at different temperatures and ethanol concentrations. In
404 fact, a very high productivity can also be achieved at a temperature as high as 85°C, provided
405 the residence time can be accurately controlled. In practice, achieving precise control of
406 residence times is challenging and deviations from t^* will result in betalain degradation due to
407 the high temperature.

408 **5. Conclusions**

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

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

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

416 2. The model has been experimentally validated for the extraction of betacyanin and
417 betaxanthin into 10, 20 and 30% ethanol solutions at 55, 65, 75 and 85°C.

418 3. The rate constants for solid phase exhaustion (k_m) and liquid phase degradation of
419 betaxanthin and betacyanin (k) were correlated with temperature by an Arrhenius type
420 equation.

421 4. The ratio of betacyanin to betaxanthin in the extract phase did not vary with extraction
422 time significantly at 55 and 65°C, but it decreased with time at higher temperatures due
423 to the more thermolabile nature of betacyanin.

424 5. The rate constant for solid phase exhaustion (k_m) increased with decrease in particle
425 size for a given solid loading, except for the smallest particle size i.e., 120 μm , where
426 k_m was lower, probably due to increase in suspension viscosity.

427 6. The betalain productivity rate at t^* - the time when the concentration peaks - did not
428 vary significantly at temperatures of 55, 65 and 75 °C in all ethanol solutions studied.
429 However, at 85°C, the productivity value in 20 and 30% ethanol solutions was
430 significantly higher. Therefore, similar betalain productivity values can be achieved at
431 different temperatures and ethanol concentrations provided the residence time for
432 extraction can be precisely controlled.

433 7. It is clear that Betalains are thermolabile; and high temperature processes, no doubt,
434 pose a risk. But these processes are not beyond the realm of possibility. For example,
435 UHT processes work very satisfactorily even at significantly higher temperatures and
436 at shorter residence times than the values reported in this study. Thus, Food Engineering
437 Operations are well-tuned to deal with such time-temperature combinations.

438

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536

537 **List of figure captions**

538 **Figure 1:** Extraction of Betalains from beet root powder at 65 and 85 °C into 10% ethanol
539 water solution (a) Betacyanin concentration, (b) Betaxanthin concentration. Solid loading =10
540 kg/m³, Particle size – 300±12.1 µm. Values of model parameters for betaxanthin at (i) 65 °C:
541 $k = 1.411 \times 10^{-4} \text{ s}^{-1}$, $K_m = 0.275 \text{ s}^{-1}$, $C_{si} = 0.0042 \text{ kg/kg}$, $R^2 = 0.996$, and at (ii) 85 °C: $k = 4.29$
542 $\times 10^{-4} \text{ s}^{-1}$, $K_m = 0.290 \text{ s}^{-1}$, $C_{si} = 0.0041 \text{ kg/kg}$, $R^2 = 0.954$; Values of model parameters for
543 betacyanin (i) 65 °C: $k = 1.052 \times 10^{-4} \text{ s}^{-1}$, $K_m = 0.271 \text{ s}^{-1}$, $C_{si} = 0.0038 \text{ kg/kg}$, $R^2 = 0.997$, and
544 at (ii) 85 °C: $k = 6.64 \times 10^{-4} \text{ s}^{-1}$, $K_m = 0.297 \text{ s}^{-1}$, $C_{si} = 0.0040 \text{ kg/kg}$, $R^2 = 0.988$. The points
545 indicate experimental values of the concentration and the solid line represents the model, i.e.,
546 concentration given by Eqn 4. Values of the model parameters for the other temperature range
547 and ethanol-water solution are shown in Table 1 (a) and (b).

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

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

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

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558 **List of table captions**

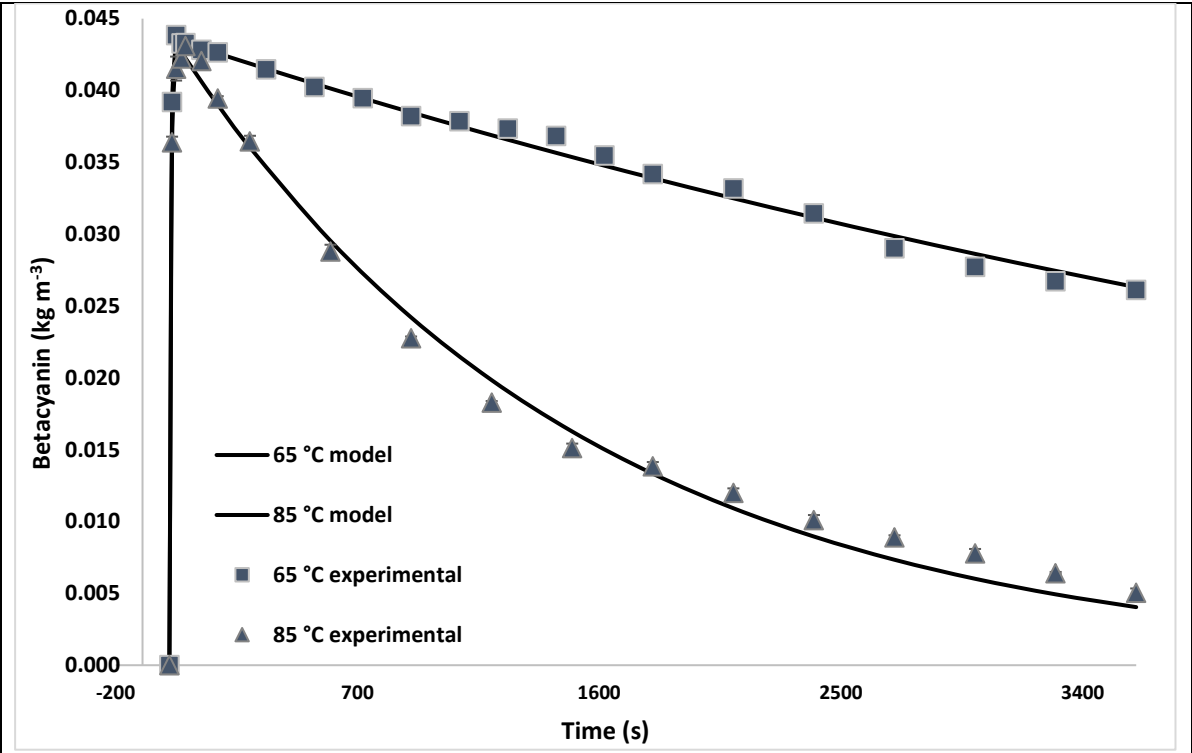
559 **Table 1 (a):** Values of model parameters fitting Eqn (4) for Betacyanin. Experiments
560 performed with solid to liquid ratio 10 kg m^{-3} and particle size $300 \pm 12.1 \text{ }\mu\text{m}$.

561 **Table 1 (b):** Values of model parameters fitting Eqn (4) for Betaxanthin. Experiments
562 performed with solid to liquid ratio 10 kg m^{-3} and particle size $300 \pm 12.1 \text{ }\mu\text{m}$.

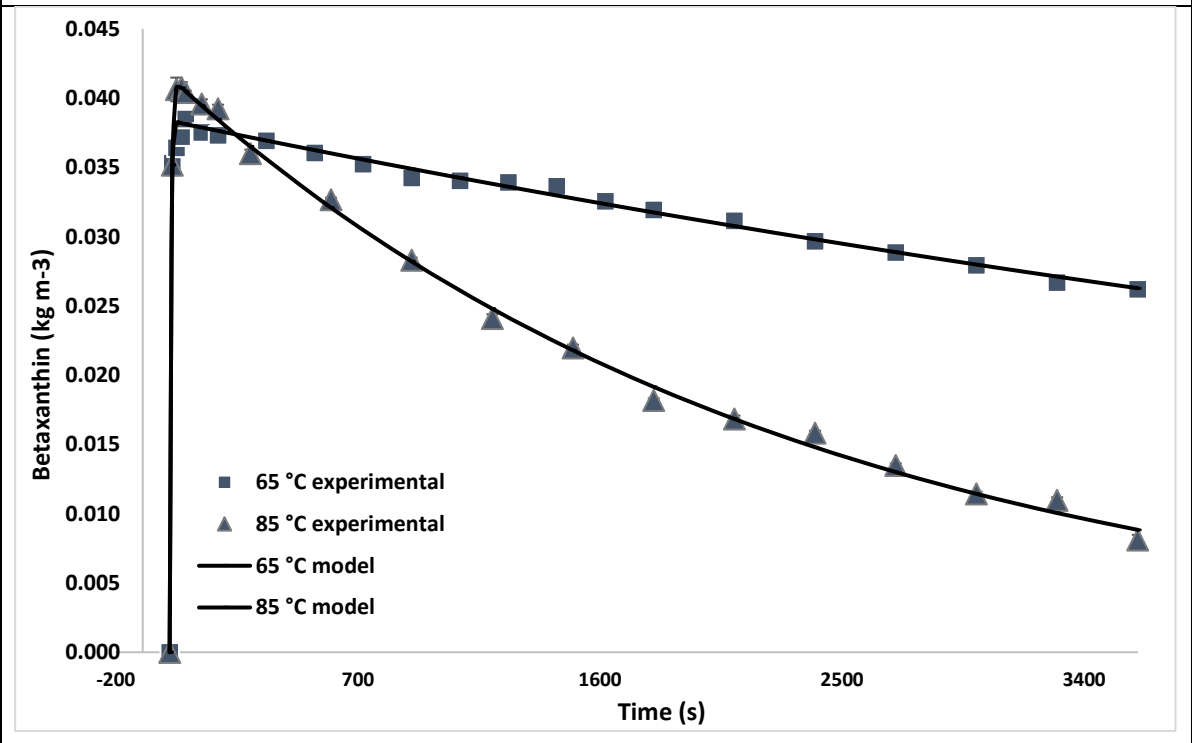
563 **Table 2:** Variation of Arrhenius parameters (Eqn 8) with ethanol concentration for betacyanin
564 and betaxanthin. Experiments performed with solid loading of 10 kg m^{-3} and mean particle size
565 $300 \pm 12.1 \text{ }\mu\text{m}$.

566 **Table 3 (a):** Effect of particle size on the values of model parameters of Eqn (4) at $65 \text{ }^\circ\text{C}$ and
567 ethanol Concentration of 20% in water for betacyanin.

568 **Table 3 (b):** Effect of particle size on the values of model parameters of Eqn (4) at $65 \text{ }^\circ\text{C}$ and
569 ethanol Concentration of 20% in water for betaxanthin.



(a)



(b)

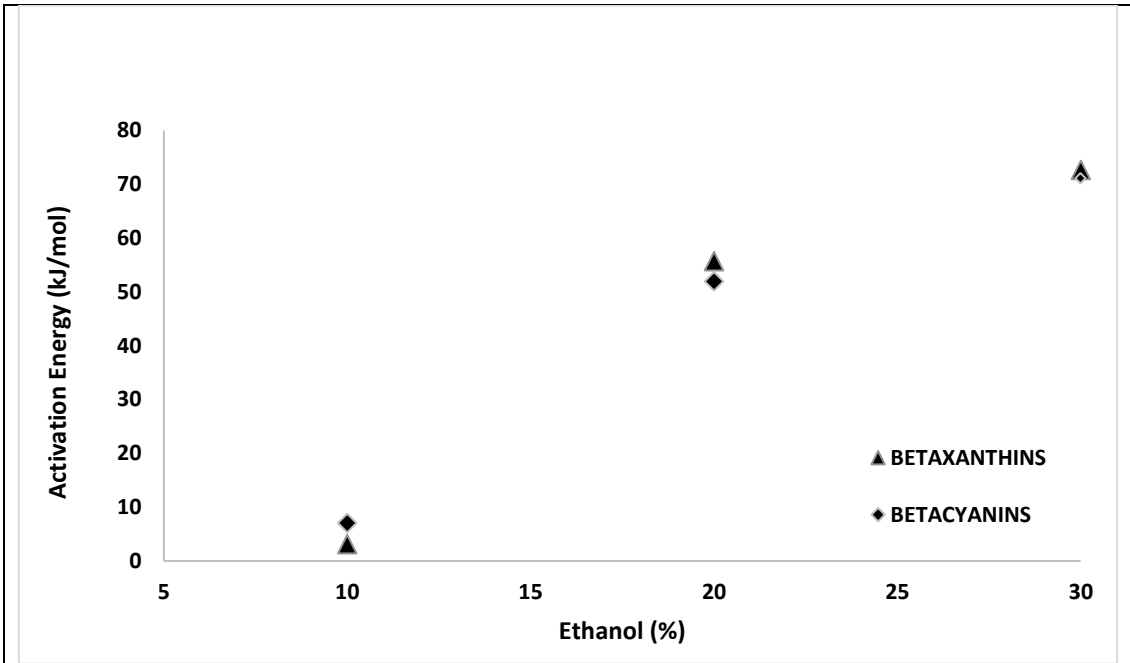
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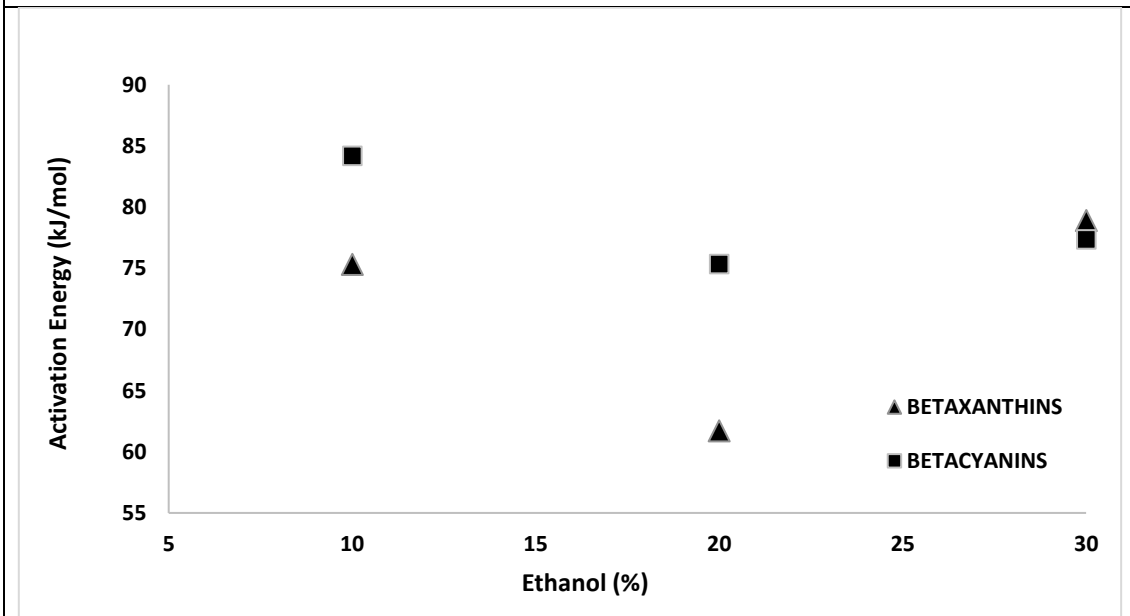
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(a)



(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 $300 \pm 12.1 \mu\text{m}$ and solid loading of 10 kg m^{-3} .

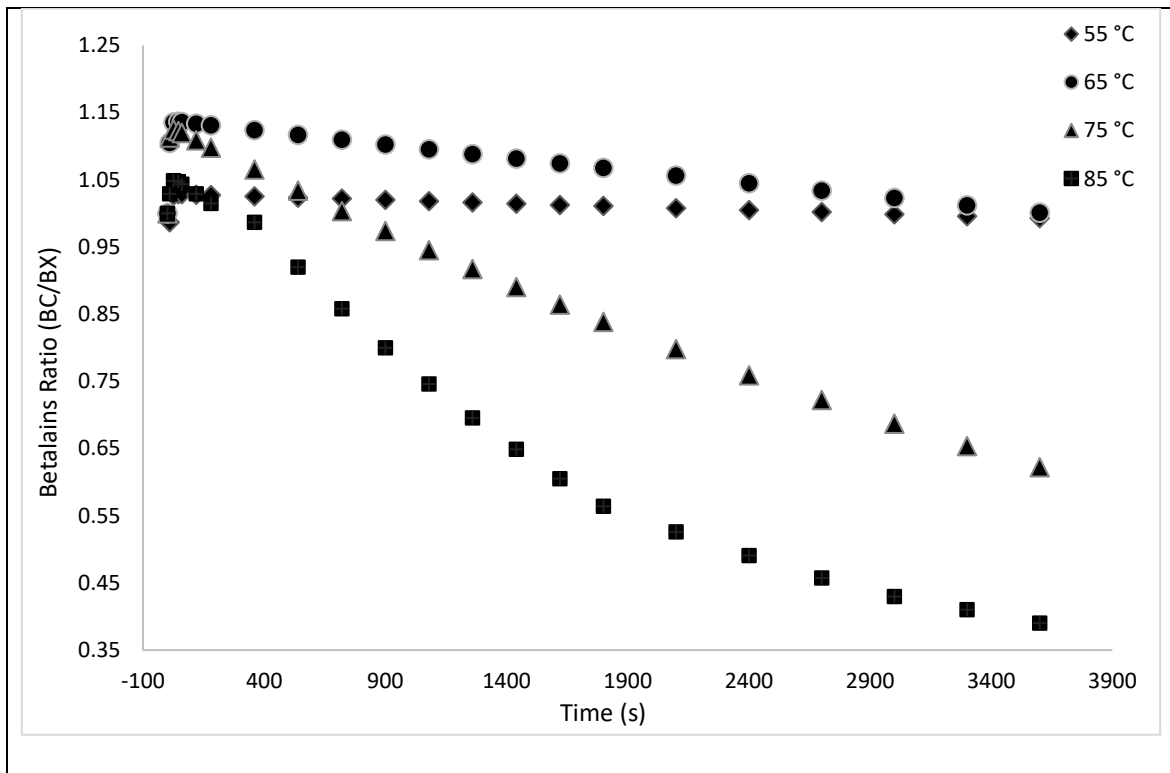
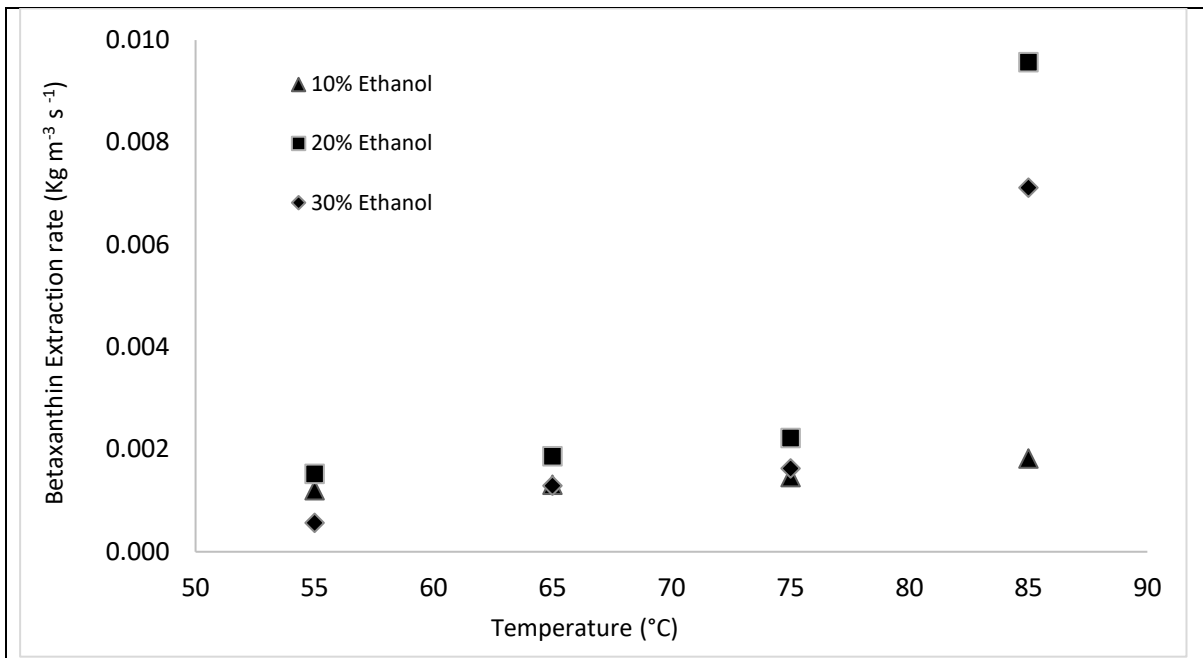
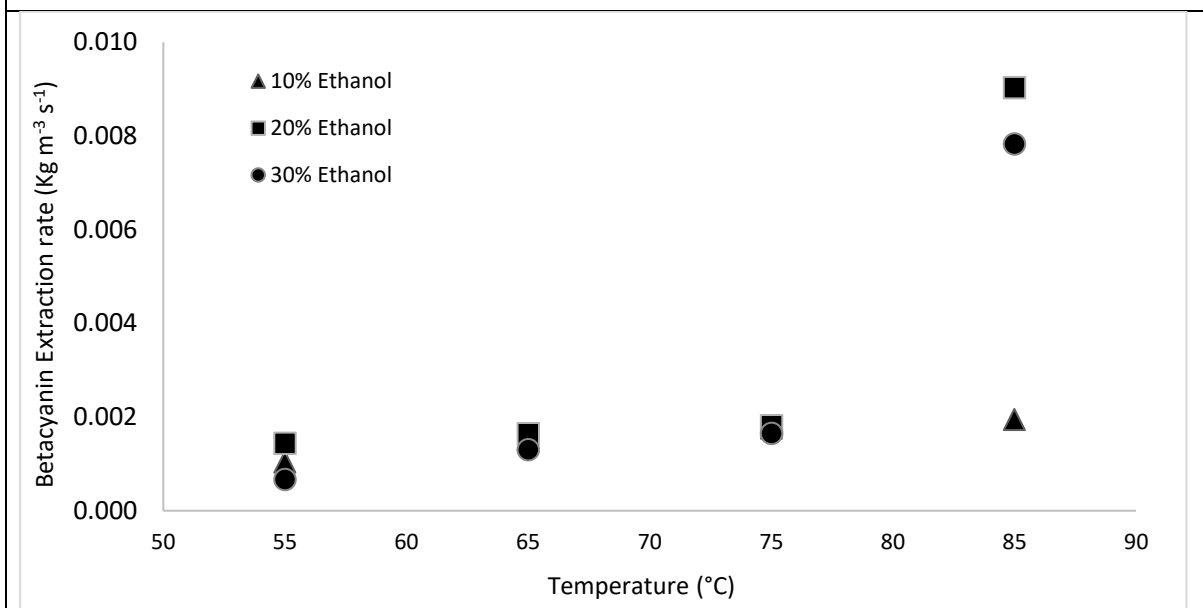


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(a)



(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 $300 \pm 12.1 \mu\text{m}$ 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 $\times 10^{-5}$ (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

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

579 k – Degradation rate constant (s⁻¹)

580 k_m – Solid exhaustion rate constant (s⁻¹)

581 SSE – Sum of squared errors

582 R² – Co-efficient of determination

583 Adj. R² – Adjusted Co-efficient of determination

584 RMSE – Root mean squared error

585 CI – Confidence Interval

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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.

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 $\times 10^{-5}$ (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 $300 \pm 12.1 \text{ }\mu\text{m}$.

Betaxanthin (BX)							Betacyanin (BC)					
k_m				k			k_m			k		
Ethanol (%)	$A \text{ (s}^{-1}\text{)}$	$E_a \text{ (kJ mol}^{-1}\text{)}$	R^2	$A \text{ (s}^{-1}\text{)}$	$E_a \text{ (kJ mol}^{-1}\text{)}$	R^2	$A \text{ (s}^{-1}\text{)}$	$E_a \text{ (kJ mol}^{-1}\text{)}$	R^2	$A \text{ (s}^{-1}\text{)}$	$E_a \text{ (kJ mol}^{-1}\text{)}$	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

589 A – Pre-exponential factor (s^{-1})

590 E_a – Activation energy (kJ mol^{-1})

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

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

595 SSE – Sum of squared errors

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

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

598 R^2 – Co-efficient of determination

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

600 RMSE – Root mean squared error

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