

Elevated temperature extraction of β-Carotene from freeze dried carrot powder into sunflower oil: extraction kinetics and thermal stability

Article

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16 **Short version of title (running head)**: Extraction and stability of β -*Carotene*

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ABSTRACT: β -*Carotene*, a precursor of vitamin A, can alleviate the deficiency of this vitamin 19 prevalent worldwide. Earlier research studies have addressed the extraction of β -Carotene at 20 21 relatively low temperatures (up to 70 °C) due to its perceived instability at higher temperatures, as a result of which extraction rates recorded are relatively low. This study models the net rate of β -22 23 Carotene extraction by considering both extraction and degradation kinetics. The model developed, which accounts for degradation occurring in solid and extract phases, has been 24 25 experimentally validated for the extraction of β -Carotene from freeze dried carrot powder into sunflower oil over a range of temperatures 90-150 °C. This study also gives insights into the 26 application of sunflower oil as a carrier for β -Carotene during cooking and food processing, by 27 monitoring and modelling the thermal degradation and isomerisation of β -Carotene at 28 temperatures up to 220 °C. The modelling of extraction kinetics shows that it is possible to achieve 29 viable extraction rates by employing temperatures in the range (90-150 °C) for relatively short 30 times (< 5 mins). The degradation kinetics shows that almost 75% of the β -Carotene can survive 31 heating at 180 °C for 10 mins – indicating the possibility of using β -Carotene enriched edible oils 32 for frying. This study also reports on the formation of three isomers of β -Carotene identified using 33 HPLC: trans-, 9-cis and 13-cis. The reaction network model developed in this study was able to 34 account for the transient variation of the concentration of all three isomers. 35

36

37 *Keywords*: β -carotene; Extraction; Sunflower oil; Kinetics; Modelling.

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41 **Practical Application:**

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43 β -Carotene is a precursor of vitamin A and its consumption can potentially alleviate the 44 deficiency of this vitamin prevalent worldwide. This study validates a model for the extraction of 45 β -Carotene in sunflower oil which takes into account extraction as well as degradation occurring 46 during extraction, so that a rational method is available for the design of efficient extractors for 47 this purpose. This paper also establishes the thermal stability of β -Carotene under frying 48 conditions by quantifying its thermal degradation as well as isomerisation.

50 1. Introduction

51

 β -Carotene is a pigment found in fruits and vegetables that can be converted to vitamin A 52 in the body (Rodriguez-amaya, 1999; Marty and Berset, 1986). It has antioxidant properties that 53 can also protect against damage from harmful molecules (Elik et al., 2020). Consuming foods high 54 in β -Carotene is reported to have health benefits, such as reducing the risk of certain types of 55 cancer, improving immune function, and protecting against cardiovascular disease (Gul et al., 56 2015). β -Carotene is widely used as a colouring agent and a natural preservative in the food 57 industry (Yilmaz et al., 2017). It is also used as a natural colourant and skin conditioning agent in 58 the cosmetics industry (Strati & Oreopoulou, 2011). Vitamin A deficiency, which can be mitigated 59 60 by consuming β -Carotene, is a major public health concern worldwide, particularly in Asia and Africa. Worldwide, particularly in Asia and Africa, it is known to be one of the three most chronic 61 62 deficiencies, along with zinc and iron deficiencies (Harika et al., 2017). Globally, an estimated 250 million preschool children are vitamin A deficient (Chen et al., 2021; Tang et al., 2005). 63

64

Considering the health benefits, societal impact and industrial application, extraction of β -65 *Carotene* from natural plant sources has attracted considerable attention employing methods such 66 67 as microwave assisted extraction (Hiranvarachat & Devahastin, 2014), supercritical fluid extraction (M. Sun & Temelli, 2006a), ultrasound assisted extraction (Saini & Keum, 2018), 68 pulsed electric fields (Roohinejad et al., 2014), and others. The extraction of β -Carotene from 69 70 natural sources requires the use of nonpolar solvents (Hiranvarachat & Devahastin, 2014), some of which are not environmentally friendly and can also leave behind harmful residues in the extract 71 (Elik et al., 2020). Further, β -Carotene is also sensitive to light, heat, and oxygen, and can degrade 72

during extraction, resulting in a loss of its nutritional and functional properties (Gul *et al.*, 2015). Due to its significant bioactivity, there has been considerable interest in extracting β -*Carotene* into solvents that are efficient, safe, and environmentally friendly.

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Vegetable oils can be effective solvents due to their low cost and abundant availability all 77 78 over the world. Moreover, vegetable oils are biodegradable, non-toxic and do not leave any other harmful residues in the product. In addition, the absorption of β -Carotene in human body can be 79 enhanced between 4-12 fold by consuming it with edible oils and fats (Hornero-Méndez & 80 Mínguez-Mosquera, 2007). Earlier research has shown that vegetable oils can extract β -Carotene 81 from a range of sources, including fruits, vegetables, and microorganisms, while oils can provide 82 other health benefits such as unsaturated fatty acids and other nutrients (Chen and Meyers, 1982; 83 Sachindra and Mahendrakar, 2005; Sun and Temelli, 2006; Elik et al., 2020). The use of vegetable 84 oils for extracting β -Carotene also provides the opportunity to use the extract directly as a food 85 86 ingredient or for cooking processes such as frying.

87

A number of papers are available on the kinetics of β -Carotene extraction in various 88 89 organic solvents (Chumnanpaisont et al., 2014; Hiranvarachat & Devahastin, 2014; Humayoun Akhtar & Bryan, 2008; Purohit & Gogate, 2015a). These papers generally report on the use of 90 91 relatively low temperatures due to the nature of the solvent but more importantly due to the tendency of β -carotene to degrade during extraction (Gul et al., 2015). The common degradation 92 pathways include oxidation, thermal, and photochemical degradation (Achir et al., 2011; Gul et 93 al., 2015a). Photochemical degradation of β -Carotene can also lead to the formation of products, 94 such as apocarotenoids (Miękus et al., 2019). The excentric cleavage of β -Carotene produces 95

apocarotenoids and they cannot turned into vitamin A (Caris-Veyrat et al., 2001). Isomerization of 96 β-Carotene can form various geometric and structural isomers, such as 9-cis- and 13-cis (Achir et 97 al., 2011; Gul et al., 2015). They show potential bioactivity and colouring properties like β -98 *Carotene*. The use of low extraction temperatures to avoid such degradation reactions inevitably 99 results in low extraction rates being encountered and poor extraction efficiencies. Some 100 101 researchers have attempted to overcome this problem by superimposing ultrasound (Purohit & Gogate, 2015a), microwaves (Hiranvarachat & Devahastin, 2014), and pulsed electric field 102 (Roohinejad et al., 2014) which are capital intensive technologies and not easily scalable. 103 Moreover, all these technologies are claimed to be "green" in literature without any substantive 104 analysis of their environmental impacts. 105

106

In this paper, we hypothesize that the time-temperature conditions to be used for the 107 extraction of β -Carotene in any appropriate solvent can be rationally deduced by modelling the 108 kinetics of extraction. The net rate of extraction at any given temperature will be determined by a 109 balance between 1) the rate of transfer from the solid phase into the extraction medium and 2) the 110 111 rate of loss of β -Carotene due to degradation. The specific aims of this research are therefore 1) to develop for the first time a model which accounts for the transfer of β -Carotene from the solid 112 phase as well as its degradation in solid and extract phases during extraction in sunflower oil, 2) 113 to experimentally test the validity of the model over a range of temperatures, including high 114 temperatures not investigated in the literature so far, and 3) to investigate the thermal stability and 115 isomerization of β -Carotene in edible oil, particularly at high temperatures such as those 116 encountered during frying. The last aim of the research will inform on the possibility of using β -117

- *Carotene* enriched oil for cooking, which, if possible, will help considerably in alleviating vitamin
 A deficiency, especially in significant parts of Africa and Asia.
- 120

121 **2. Modelling the extraction kinetics of** β *-Carotene* in sunflower oil

122

If C_s ((kg (kg dry matter)⁻¹) is the average concentration of β-*Carotene* in the solid phase at any time *t*, the instantaneous rate at which this changes is a balance between the rate at which β-*Carotene* degrades in the solid phase and the rate at which the solute is transferred to the liquid phase. If M_s represents the instantaneous rate of transfer of β-*Carotene* to the liquid phase (kg s⁻¹), and the rate of degradation in the solid phase is assumed to be first order (Achir et al., 2010; Mba et al., 2017), i.e. proportional to the concentration of β-*Carotene*, we have:

129

130
$$-\frac{dC_s}{dt}X_{dm} = M_s + k_1C_sX_{dm}$$
 (1)

131

where X_{dm} is the dry matter content of the carrot powder and k_1 is the rate constant for β -*Carotene* degradation in the solid phase (s⁻¹). It is reasonable to hypothesize that C_s is an exponential function of time. This assumption is supported by previous experimental observations that have been welldocumented. For instance, in the case of sugars (Appiah-Nkansah et al., 2016), pectins (Leach et al., 1994), and total phenolic content (Bengardino et al., 2019), solid-phase concentrations have been reported to exhibit this type of release kinetics. Therefore:

138

$$139 C_s = C_{si} e^{-k_M t} (2)$$

where $C_{\rm si}$ is the initial average concentration of β -Carotene in the solid phase and $k_{\rm M}$ (s⁻¹) is a rate 141 constant for solid phase exhaustion of β -Carotene. It is arguable whether C_{si} represents the initial 142 concentration of β -Carotene per se in the solid phase. Experiments were conducted to determine 143 the total mass of β -Carotene that could be extracted from freeze dried carrot powder into different 144 solvents such as tetrahydrofuran, hexane and coconut oil. These experiments involved extraction 145 146 over very long periods of time (4 h) at 25 °C and repeated extractions using fresh solvents until no more β -Carotene extraction was possible. The amount extracted into each solvent was different. 147 For example, the maximum amount of β -Carotene extracted into tetrahydrofuran – in which β -148 Carotene is known to be most soluble (Purohit & Gogate, 2015b) – was 865.68 μ g g⁻¹ powder. 149 Likewise, the maximum amount extracted into hexane was $752.54 \ \mu g \ g^{-1}$; and that extracted into 150 coconut oil was 722.36 µg g⁻¹ This suggests that C_{si} represents the concentration of β -Carotene 151 that is extractable into a given solvent under a given set of operating conditions. It is therefore 152 reasonable to hypothesize that C_{si} is a model parameter which can potentially be estimated from 153 the experimental data. By differentiating eqn (2) and substituting the values of the derivative and 154 $C_{\rm s}$ into eqn (1), we get: 155

156

157
$$M_s = X_{dm} C_{si} e^{k_m t} (k_m - k_1)$$
(3)

158

159 which is the net rate of transfer of β -Carotene to the liquid or extract phase.

A mass balance equation for β -Carotene in the liquid phase can also be developed by assuming that the rate of change of β -Carotene concentration in the liquid phase C_L (kg m⁻³) is the difference between the rates of transfer from the solid phase (i.e. M_s) and the rate at which β -Carotene degrades in the liquid phase. The latter can also be assumed to follow first order kinetics with a rate constant given by, say, k_2 (s⁻¹). Thus, we have:

165

$$166 \qquad V\frac{dC_L}{dt} = M_s - k_2 C_L V \tag{4}$$

167

where *V* is the volume of the extraction medium, in this case, the volume of sunflower oil taken (m³). By substituting for M_s from eqn (4), a first order ordinary differential equation is obtained which can be solved using the initial condition $C_L = 0$ at t = 0, to give:

171

172
$$C_L = \frac{SC_{si}(k_M - k_1)}{(k_2 - k_M)} [e^{-k_M t} - e^{-k_2 t}]$$
(5)

where, $\frac{X_{dm}}{V} = S$ represents the solid loading in the extractor (kg of carrot powder per m³ of sunflower oil).

175

For the developed model the experimental conditions can be variable given the degradation kinetics of β -Carotene in solid and liquid phases during extraction. Hence, there could be two special cases for the model apart from the basic assumption of degradation of β -Carotene differently in different phases.

180

It is interesting to note that the rate constant for β -Carotene degradation in the solid and extract phases, i.e. k_1 and k_2 , have been assumed to take different values in the model. It is known that β -Carotene degradation may be attributable to temperature (Achir et al., 2011) and oxidation (Burton et al., 2014). If β -Carotene degradation is induced by both these factors, i.e. temperature and oxidation, the values of k_1 and k_2 will be different because the oxidative environments in the solid and oil phases are different. If, on the other hand, temperature induced degradation dominates, then one expects k_1 and k_2 values to be the same since the temperatures in the solid and extract phases are not different. Thus k_1 and k_2 can be set to equal to k in eqn (5), to yield:

189

190
$$C_L = SC_{si}[e^{-kt} - e^{-k_M t}]$$
 (6)

191

In this study, k_1 and k_2 will initially be assumed to take different values; the outcome of the analysis of experimental data will inform whether k_1 and k_2 are the same or different. Regardless, it is interesting to note that the model (i.e. eqns 5 and 6) predict that the plot of C_L versus *t* goes through a turning point, which is a maxima, when $dC_L/dt = 0$. The time *t** at which this maximum value occurs is given by:

197

198
$$t^* = \frac{1}{(k_M - k_2)} \ln\left(\frac{k_M}{k_2}\right)$$
 (7)

199

If the experimental conditions are such that there is no significant degradation of β -Carotene either in the solid or liquid phases, e.g. extraction at relatively low temperatures, then k_1 and k_2 can both be set equal to zero in eqn 5, to yield:

203

204
$$C_L = SC_{si}(1 - e^{-k_M t})$$
 (8)

205

Thus, the plot of $C_{\rm L}$ versus *t* will increase monotonically before asymptotically converging to a value of $C_{\rm L}=SC_{\rm si}$. Experimentally determined C_L versus *t* data for a range of different conditions (described in materials and method section), will be fitted to eqn (5) or eqn (6) or eqn (8) to deduce the bestfitting values of parameters C_{si} , k_M , k_1 , and k_2 . Under experimental conditions resulting in β -Carotene degradation, the values of k_2 can also be directly determined at different temperatures by dissolving a known quantity in oil and monitoring its transient concentration. Thus, the experimentally determined value of k_2 can also be compared with the values indirectly deduced from the model.

216

217 **3. Materials and Methods**

218 **3.1 Design of experiments**

219

Extraction of β -Carotene was performed by implementing a random design using sunflower 220 oil as solvent phase. The extraction temperatures employed were: 90, 115, 135 and 150 °C. The 221 use of higher extraction temperatures than those employed by earlier researchers, aimed to 222 accelerate the extraction process and investigate the extent to which β -Carotene degradation 223 occurred under such conditions. All extraction experiments were carried out in triplicate to 224 estimate means and standard deviations. Data analysis was performed using XLSTAT version 225 2021.1 (AddinSoft, Paris, France). Fitting of the experimental data to the model (eqns. (5) and (8)) 226 and the determination of the model constants were undertaken using MATLAB 2022a Academic 227 version (Mathworks Inc., USA); further details are given below in section 3.6. 228

229

230 3.2 Preparation of freeze-dried carrot powder and purchase of sunflower oil

Fresh carrots (Daucus carota L.), purchased from a local supplier in Reading (United 232 Kingdom) were washed, cleaned, and chopped in a food processor (Kenwood Blend-X Fresh 233 BLP41.A0GO) and subjected to blast freezing at -80 °C, for 24-36 h. The frozen material was 234 subsequently freeze dried at pressure 0.420 mbar and temperature -35 °C (VirTis SP Scientific, 235 UK, Pressure range: 0.001-6.11 mbar; Temperature range: 0.01 to -76 °C) for 70-72 h until the 236 moisture content dropped below 3% (dry weight basis). The freeze dried material was ground 237 using a spice mill (Kenwood Prospero AT286 KW714229) and sieved to obtain three cuts with 238 mean particle size of 0. 35, 0.75 and 1.40 mm. Sunflower oil, Flora (100% Natural, Suitable for 239 All Cooking, Made with pure sunflower oil) was purchased from a local supermarket in Reading 240 (United Kingdom). 241

242

243 **3.3 Determination of extraction kinetics**

244

Extraction kinetics was determined by measuring the concentration of β -*Carotene* dissolved in the oil phase at different time points. A separate extraction was performed for each time point. The time points were arbitrarily selected so that sufficient concentration versus time data points could be obtained to fit the model. Each of these 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 2 g of dehydrated carrot powder sample to 100 ml of the solvent phase (sunflower oil) which was already pre-heated to the desired extraction temperature using magnetic heating and stirring plate. The beaker was then placed on a hot plate to control the temperature and constantly agitated using a magnetic stirrer operating at 300 rpm. After the desired extraction time, the beaker and its content were immediately cooled to 4 °C in an ice-bath. The cooled mixture was then centrifuged (Eppendorf MiniSpin Plus Centrifuge, fisher scientific, UK) at 14000 rpm for 40 mins, whilst maintaining its temperature at 4 °C, to obtain a clear supernatant which was then stored at 4 °C until further analysis.

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3.4 Measurement and characterization of β-Carotene in sunflower oil

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The concentration of β -*Carotene* in the extract phase was determined by taking 0.25 ml of the stored oil extract, mixing it with 3.75 ml of hexane and measuring the absorbance of the mixture against a blank solution of hexane and plain sunflower oil at 450 nm using a spectrophotometer (Cecil CE1011 Spectrophotometer) (Li et al., 2013a). A standard calibration curve ($R^2 = 0.99$) was prepared by dissolving pure β -Carotene (Tokyo Chemical Industry UK Ltd, 98 %) at various concentrations (0.5 µg/ml to 12 µg/ml) in a mixture of 8:1 (v/v) hexane and plain sunflower oil and measuring the absorbance at 450 nm.

268

In general, the β -*Carotene* extract can consist of *cis* and, *trans* isomers due to the high temperature applied during extraction (B. H. Chen & Liu, 1998). The extract solutions were therefore characterized by using a HPLC based method described by Achir *et al.*, (2010) and Syamila *et al.*, (2019). This procedure involved crystallizing out the sunflower oil triglycerides by mixing 0.5 ml of stored extract with 4.5 mL acetone, vortexing the mixture for 10 s and leaving it overnight at -20 °C. The triacylglycerols were separated by rapid sampling and filtration through

a 0.2 µm PES filter (Fisher Scientific, China). The triacylglycerol-free mixture was then directly 275 injected into the HPLC column - a polymeric YMC-30 (4.6 mm id ×250 mm, 5 mm particle size) 276 277 (YMC, Wilmington, NC, USA). Elution was performed with a quaternary pump. The mobile phase consisted of methanol, tert-butyl-methyl-ether (TBME), and milli-Q water (50:45:5, v/v/v at a 278 flow rate of 1 mL/min under isocratic conditions. A UV- visible photodiode array detector (Dionex 279 UVD 340U) was used to analyze the chromatograms at a detection wavelength of 450 nm. Analysis 280 were done in triplicate. The quantification was done against a standard calibration curve (R^2 = 281 0.99) in a concentration range between 0.5 μ g/ml to 12 μ g/ml in a mixture of 8:1 (v/v) hexane and 282 plain sunflower oil. 283

284

285 **3.5 Degradation kinetics of** β *-Carotene* in sunflower oil under frying conditions

286

As mentioned earlier, a key purpose of this research is to explore the possibility of using β -287 Carotene enriched oil in cooking and food processing. It was therefore thought desirable to 288 investigate the degradation kinetics of β -Carotene at different temperatures which included 289 common frying temperatures (135, 150, 160, 180, 200 and 220 °C), by measuring the concentration 290 of β -Carotene remaining in the sunflower oil after exposure to the temperature for a stipulated 291 time. At each temperature, the concentration of β -Carotene was measured after 5, 10, 15, 20, 25, 292 and 30 mins, in addition to the initial concentration. A separate batch of β -Carotene in oil, 293 contained in a heat stable test tube (Pyrex, UK), was used for each time point. 9 ml of commercially 294 available sunflower oil (Flora, United Kingdom) was first heated to the desired temperature and 1 295 ml of β -Carotene enriched sunflower oil was added to it, so as to result in an initial β -Carotene 296 concentration of 200 mg kg⁻¹. This procedure ensured that the β -Carotene attained the pre-297

determined temperature in the shortest possible time, which was less than 10 s. The test tube was then maintained at this temperature for the desired time. It was then rapidly cooled to in an ice bath, and stored at 4 °C temperature in an amber vial which protected it from light degradation until further analysis. The transient concentrations of β -Carotene, determined at each temperature, were fitted to the first order equation to deduce the rate constant:

303

$$304 \quad \ln\left(\frac{c_0}{c_t}\right) = kt \tag{9}$$

305

where, c_t and c_0 are the concentrations of β -*Carotene* at any time t and initially, respectively, and k is the first order isothermal rate constant, assumed to vary with temperature (*T*) according to the well-known Arrhenius equation: $k = A \exp(\frac{-E_a}{RT})$ where, A is the pre-exponential factor (s⁻¹); E_a is the activation energy (J mol⁻¹); and *R* is the universal gas constant (8.314 J mol⁻¹ K⁻¹).

310

311 3.6 Statistical analysis

312

The validity of the model was tested by fitting eqns. (5) and (8) to the experimentally 313 determined c_L versus t data using MATLAB 2020b's curve fitting tool for 95% confidence interval. 314 The tool works by minimizing the sum squared error and root mean squared error, and requires an 315 initial guess for the model parameters. The Levenberg-Marquardt algorithm is used to optimize 316 the model parameters, and the best-fit values were based on 15×3 data points (in triplicates) for 317 each experimental condition. This article also explains that the SSE and RMSE values indicate 318 model validity and goodness of fit, and the co-efficient of correlation and adjusted R^2 are 319 determined to ensure an adequate number of parameters have been used. The narrow range of joint 320

confidence intervals obtained reinforces the precision in estimating the parameters and the
 adequacy of the number of experimental data points used in the fitting exercise.

323

324 Sum of squarred error (SSE) =
$$\sum (y_{exp} - y_{model})^2$$
 (10)

325 Root mean squarred error (RMSE) =
$$\sqrt{\frac{1}{n} \sum_{i=1}^{n} (y_{exp} - y_{model})^2}$$
 (11)

326 Coefficient of determination
$$(R^2) = 1 - \frac{RSS}{TSS}$$
 (12)

327 Adjusted coefficient of determination
$$(Adj - R^2) = 1 - \frac{(1-R^2)(n-1)}{(n-p-1)}$$
 (13)

328

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.

332

4. Results and Discussion

4.1 Validation of the model

335

Experimentally determined C_L versus *t* data were fitted to eqn (5). At temperatures of 90 and 115 °C, the C_L values increased with time before reaching asymptotic values (p<0.05) – as shown in Fig 1. This trend suggests that the degradation of β -*Carotene* during extraction is negligible at these temperatures. In other words, k_1 and k_2 can be considered to be negligible in eqn (5), and eqn (8) represents the variation of concentration with time. Fig 1 also shows the fit between the experimental data at these temperatures with eqn (6) and (8), and the model constants are reported in the caption of Fig 1.

At the higher temperatures of 135 and 150 °C, the concentration goes through a maximum 344 value which is consistent with eqn (5) and also confirms the occurrence of β -Carotene degradation 345 during extraction. The acceptable fit between the eqn (5) and the experimental data at these 346 temperatures is shown in Table 1S (supplementary data), along with the corresponding best-fit 347 values of the model constants as well as goodness of fit. Even though the high R^2 value illustrates 348 a good fit between model and experimental data, other fitness parameters such as sum of squared 349 error (SSE), root mean squared error (RMSE), and adjusted R² were also estimated (Eqns. 9, 10 350 and 12). The distinctly lower values of the statistical error and higher values of determination 351 coefficients (Table 1S) enhance the model validity. 352

353

The values of the constant k_2 – which represents the first order rate constant for the 354 degradation of β -Carotene in the oil phase – were estimated by fitting the C_L versus t data as 355 356 mentioned above, as well as by undertaking separate β -Carotene degradation experiments, already stated earlier under materials and methods (section 3.6). ANOVA (pair comparison test) was run 357 to check the null hypothesis of a significant difference existing between k_2 values given by the 358 model and the experimentally determined values of k_2 ; the p value obtained was greater than 0.05 359 which negates the null hypothesis. Thus, k_2 values deduced from eqn (5) and experimental values 360 are statistically the same – which further reinforces the model hypothesis that degradation kinetics 361 of β -Carotene in oil follows first order between 135-220 °C. 362

363

364 It is evident from Table 1S that the values of k_1 and $(k_2)_{exp}$ are very close. An ANOVA was 365 therefore run to check whether k_1 and $(k_2)_{exp}$ were significantly different or not, which resulted in

a p value for the null hypothesis greater than 0.05 suggesting the rejection of the hypothesis. Thus, 366 k_1 and $(k_2)_{exp}$ can be assumed to be equal in eqn (5), which indicates that the general variation of 367 368 β -Carotene concentration in oil is given by eqn (6) where k_1 and k_2 are considered to be equal and both replaced by k. The insignificant difference between k_1 and k_2 values also suggests that the 369 degradation is predominantly thermal by nature, and any differences in the structural environments 370 371 of the two phases do not play a significant role in the degradation process. Thus, the experimentally determined $C_{\rm L}$ versus t data were fitted to eqn (6) to generate Table 1 which shows the best fit 372 model parameters as well as the goodness of fit. Fig 1 illustrates the fit of the experimental data 373 against equations 6 and 8, for all the temperatures and particle sizes investigated in this work. 374

375

As mentioned in section 2, the value of k_m represents the rate constant for solid phase exhaustion of β -*Carotene* and k represents its degradation rate constant. It is evident from Table 1 that k_m is significantly greater than k which suggests that chemical degradation of β -*Carotene* in sunflower oil is relatively slow in comparison with its transfer from the solid phase, even at temperatures as high as 135 or 150 °C, which enables rapid and efficient extraction to be carried out at such high temperatures. If this extraction is to be carried out continuously, then a reactor with tubular configuration will be effective to control residence times.

383

Earlier work on β -*Carotene* extraction has largely been undertaken using organic solvents such as hexane (Y. Sun et al., 2010), Tetrahydrofuran (Y. Sun et al., 2010), ethyl acetate (Y. Sun et al., 2010), dichloromethane (Y. Sun et al., 2010) and ethanol (Purohit & Gogate, 2015a) where rapid degradation of β -*Carotene* has been noted, prompting the use of relatively low temperatures (30-60 °C) and, in some cases, the use of devices such as microwaves or ultrasound (Chutia &

Mahanta, 2020; Demiray & Tulek, 2017; Stupar et al., 2021). The rates of extraction observed in 389 the present study are significantly greater than those observed in some earlier studies. For example, 390 Purohit and Gogate (2015) have reported an extraction time of around 50 mins to attain a yield of 391 70% (based on the total extractable β -Carotene) in ultrasound assisted ethanol solutions at 392 temperature of 30°C, when using carrot particles of sizes comparable with the sizes used in the 393 394 present study. By employing higher temperatures such as those used in this work, similar yields can be obtained in a matter of 5-6 mins. Chumnanpaisont et al (2014) have also reported extraction 395 times of 2-5 mins for the extraction of β -Carotene from carrots using microwave power, operating 396 either continuously or intermittently. Table (2) shows a comparison between the net rate of 397 extraction determined using various extraction methods and the values observed in this work 398 399 employing solely thermal heating. It is clear that the extraction rates at 135 and 150 °C are higher or comparable with the values obtained employing energy intensive extraction methods such as 400 microwave, pulsed electric field and electrohydrodynamic combined with ultra sound. 401

402

403 **4.2 Composition of the sunflower oil extract**

404

405 β -Carotene can exist in three isomeric forms in oil: *trans*, 9-*cis and* 13-*cis* (Achir et al., 406 2011). HPLC analysis was performed for each extract and the concentrations of the three isomers 407 in the extract are shown as a function of time in Fig. 2 (a)-(d). The concentration of 9-*cis* in the 408 extract was below the detection limit, therefore the concentrations of only the other two isomers 409 are shown. A similar result was reported earlier by Achier et. al., (2011). It is also interesting to 400 note that the sum of the concentrations of the two isomers is the total β -Carotene concentration 411 determined spectrophotometrically; this is also shown in Fig. 2 (a)-(d). At higher extraction

temperatures the concentration of 13-cis increases initially, but decreases to virtually zero soon 412 after the peak concentration is reached. Therefore, longer extraction durations only result in trans 413 414 isomers. In general, the extract is dominated by the *trans*-isomer with its percentage varying between 70-87% of the β -Carotene in the extract. This implies that the percentage of cis isomers 415 ranged between 13-30%, which is somewhat lower than the value of 40% reported for copra fat 416 417 and palm olein by Achir et al (2011). could the higher value for these materials may be attributed to the higher concentration of β -Carotene used and the application of more severe treatment. It 418 may be noted that these observations are valid for all the particle sizes employed in this study (data 419 not shown). 420

421

The three isomers have been reported to possess similar vitamin A forming potentials and colouring attributes (Rodriguez-amaya, 1999). Therefore, the relative concentrations of the isomers may not be critical from applications point of view. However, Figs. 2 (a)-(d) provide insights into the distribution of the isomers in the extract phase under different operating conditions.

427

428 **4.3 Effect of temperature and particle size on the extraction kinetics**

429

430 β -*Carotene* is mainly present in chromo- and chloroplast, and protected by the cellulose 431 and pectin layers of the cellular structure (Thürmann et al., 2002). Smaller particle sizes imply 432 shorter diffusion path length and greater accessibility of the β -Carotene. Therefore, k_m increases 433 with decrease in particle size, which is confirmed in Table 1. Higher temperatures, on the other 434 hand, improve accessibility by rupturing the protecting membranes (Nutter et al., 2021). Therefore $k_{\rm m}$ also increases with temperature, but, as Table 1 shows, the increase is not as marked as the effect of particle size.

437

438 4.4 Degradation kinetics of β-Carotene in sunflower oil, especially at normal frying 439 temperatures

440

 β -Carotene degradation experiments were performed by dissolving commercially available 441 trans- β -Carotene in sunflower oil and allowing the β -Carotene to degrade at the desired 442 443 temperatures (section 3.6). For all heating treatments, the concentration of trans- β -Carotene decreased as a function of the heating time. This disappearance was visible macroscopically by a 444 445 loss of color, and it was more rapid as the temperature increased around 200 °C. Fig 3 shows a semi-log plot of normalized β -Carotene concentration against time over a range of temperatures 446 between 135 and 220 °C. The linear nature of the plots confirm that the degradation follows first 447 order isothermal kinetics; the rate constants values are given in Table 3, which also reports the 448 Arrhenius constants: activation energy and pre-exponential factor. Table 3 shows that the 449 activation energy value over the temperatures 135-220 °C is 56.65 kJ/mol (R²=0.91), which is 450 consistent with the reported values of 48 kJ/mol for β -Carotene degradation in palm olein (Achir 451 452 et al., 2010).

453

The choice of temperature and time employed in this study were intended to cover values encountered during the use of sunflower oil for deep fat frying (Totani et al., 2013). The values of degradation rate constant given in Table 3 are in close agreement with the values previously reported by (Achir et al. 2011). But the values of the rate constants observed in this study are

significantly lower than the values reported by Sun et al., (2010) for trans β -Carotene degradation 458 in dichloromethane under the influence of ultrasound at temperatures in the range -5-25 °C. It is 459 unclear whether the chemical nature of the solvent medium plays a role in influencing kinetics, 460 but these studies suggest that there is a role played by the solvent. Further experiments are needed 461 to confirm solvent effects. Regardless, it is clear that in sunflower oil, β -Carotene undergoes 462 degradation at frying temperatures, the extent depending on the time-temperature combination 463 employed. If we assume a typical frying temperature of 180 °C for 10 mins (e.g. for frying French 464 fries), the percentage of β -Carotene remaining in the oil, based on the rate constant values reported 465 in this work is 75%, which suggests that β -Carotene fortified vegetable oils can be used, in 466 practice, for frying and other food processing applications. At such high temperatures, the heating 467 time needs to be over 30 minutes for 90% of β -Carotene to be destroyed (Achir et al., 2010). 468

469

470 During heating, β -Carotene degradation is reported to be accompanied by concomitant isomerization, as well as oxidation to produce epoxy- and hydroxy- β -Carotene, and cleavage 471 products such as apocarotenals and apocarotenones (Mordi, 1993); Caris-Veyrat et al., 2001). In 472 this study, the development of trans-, 9-cis and 13-cis isomer concentrations were monitored with 473 time, at various temperatures, using HPLC-DAD (section 3.4). Achir et al (2011) have proposed 474 plausible reaction networks leading to the formation of the isomers and thermal degradation 475 products. A simplified network model scheme is presented in Fig 4, which assumes that, at any 476 given temperature, the trans isomer can reversibly change either to 9-cis or13-cis isomer, each of 477 which can also undergo subsequent thermal degradation. Each reaction in the network shown in 478 Fig. 4 is also assumed to be first order with corresponding rate constants. It is also reasonable to 479 assume that the thermal degradation rate constants for all three isomers are the same at a given 480

temperature, as suggested by Achir (2011). Based on these assumptions, an instantaneous mass
balance can be written for each of the isomers as follows:

484
$$\frac{dC_{trans}}{dt} = -(k_1 + k_3 + k_5) \times C_{trans} + k_2 \times C_{9-cis} + k_4 \times C_{13-cis}$$
(14)

485
$$\frac{dC_{9-cis}}{dt} = k_1 \times C_{trans} - (k_2 + k_5) \times C_{9-cis}$$
(15)

486
$$\frac{dC_{13-cis}}{dt} = k_3 \times C_{trans} - (k_2 + k_5) \times C_{13-cis}$$
(16)

487

The above set of differential equations was used to model the concentration of the three 488 isomers with respect to reaction (processing/cooking) time and temperature, the initial conditions 489 being $C_{\text{trans}} = C_{\beta\text{-Carotene}}$ and $C_{9\text{-cis}} = C_{13\text{-cis}} = 0$. Multiresponse modelling to obtain the best estimates 490 of the rate constants from k_1 through to k_5 and their corresponding activation energies was 491 performed by by non-linear regression using the Bayesian approach and the determinant criterion 492 (van Boekel 2008), included in the modelling software Athena Visual Studio software package 493 (Athena Visual Software Inc., Naperville, IL). The minimisation of the determinant criterion 494 (Stewart, Caracotsios, & Sørensen, 1992) is ideal for multiresponse studies since it removes the 495 need for the statistical compliance that is required for the typical minimization of the sum of 496 squares (van Bokel, 2008). Each of the rate constants from k_1 through to k_5 was assumed to follow 497 Arrhenius behaviour with respect to temperature and was reparametrized as follows: 498

499

500
$$k = k_{\text{ref}} \exp(\frac{-E_a}{R}(\frac{1}{T} - \frac{1}{T_{\text{ref}}}))$$

501

502 where:

503 *k*: rate constant at any temperature T ($^{\circ}$ K)

504 k_{ref} : rate constant at reference temperature T_{ref} (set at 473 °K, i.e. 200 °C)

505 *Ea*: activation energy (Joules/mole)

- 506 R: Universal gas constant (8.314 J mol⁻¹ K⁻¹)
- 507

Three models were compared, which differ on the number of parameters employed: The 508 first model had explicit activation energies attributed to each rate constant, in the second model k_1 509 and k_2 shared the same activation energy, and the same applied to k_3 and k_4 , while in the third 510 candidate model all rate constants shared the same activation energy. The sum of squares of the 511 residuals (RSS) is a measure of the discrepancy between the experimental and model data, with a 512 lower value indicating a better fit between the two. In addition, the Akaike information criterion 513 (AIC) was also employed to discriminate between the different candidate models. Out of the three 514 models, the first one was the best since it had the lowest RSS as well as the lowest AIC value. The 515 516 best estimates of the parameters of the first model - i.e. the rate constants at 200 °C and their activation energies - along with their 95% confidence intervals are presented in Table 4. 517

518

A graphical comparison between the experimentally determined concentrations of different β -Carotene isomers and the values predicted by the model is shown in Figs. 5 (a)-(d). The rate constants for back isomerization of the trans isomer from 9-*cis* and 13-*cis* are greater than the corresponding values for the forward reaction, which accounts for the significantly higher concentrations of the *trans* isomer in the mixture at all the temperatures, except 220 °C where the concentrations become comparable. This observation of generating higher concentrations of trans isomers at higher temperatures is consistent with Achir et al (2011), who reported lower rate

526	constants for the back isomerization of cis . The rate constant k_5 representing irreversible therma
527	degradation of all three isomers can be compared with the rate constant values obtained by
528	measuring absorbance values as a function of time (section 3.4) and a parity plot of values obtained
529	at different temperatures is shown in Fig 6.
530	
531	The significance of isomer formation during degradation to potential bioactivity is no
532	conclusive. According to Rodriguez-Amaya (1999), all three isomers are capable of synthesizing
533	vitamin A, and can impart coloration to food materials. However, Castenmiller & West (1998) has
534	stated that the <i>trans</i> -isomer is more active at synthesizing vitamin A than the <i>cis</i> -isomers. It is
535	therefore evident that further research is needed to conclusively establish the role played by each
536	isomer in this regard.
537	
538	4. Conclusion
539	
540	1. Elevated temperatures (upto 150 °C) can be used viably to extract β -Carotene in edible oil
541	Despite thermal degradation of β -Carotene at high temperatures, the net rates of extraction
542	observed in this study were found to be significantly higher or comparable with the rates
543	observed in earlier studies using energy intensive technologies such as pulsed electric field
544	microwave and electrohydrodynamic in combination with ultrasound.
545	2. A model developed to determine the transient concentration of β -Carotene in sunflowe
546	oil, which accounts for thermal degradation occurring in the solid and extract phases, gave
547	a good fit with the experimental data. This kinetic model can potentially be used to design
548	and size extractors.

- 549 3. β-Carotene enriched sunflower can be used as a frying medium to enrich the nutritional
 550 value of fried products.
- 4. A reaction network model was developed to explain kinetics of formation and degradation
- for each β -*Carotene* isomer during thermal degradation.

554 Nomenclature

	Dra averagential factor al		Total number of predicted results from		
A	Pre-exponential factor, s ,	р	model		
AIC	Akaike information criterion	R^2	Coefficient of determination		
Adj-R ²	Adjusted coefficient of determination,	rpm	Revolution per minute, min ⁻¹		
C ₀	Concentrations of β -Carotene initially, µg/ml	RMSE	Root mean squared error		
C	Concentration of <i>b</i> -Carotene in the	D	Universal gas constant, 8.314 J mol ⁻¹		
C_L	extract, kg m ⁻³	ĸ	<i>K</i> ⁻¹		
	β -Carotene concentration in the solid				
Cs	phase at any time, kg betalain (kg dry	RSS	Residual sum of square		
	solid) ⁻¹				
C	Initial concentration of <i>b</i> -Carotene that is		Sum of squared error		
C _{si}	extractable, kg m ⁻³ .	55 E	Sum of squared error		
0	Concentrations of β - <i>Carotene</i> at any time	THE	Tetrahydrofuran		
Ct	t, µg/ml	1111			
E_a	Activation Energy, Eqn 6, J mol ⁻¹	t	Time, s		
1	First order rate constant for b-Carotene	* *	The time when C neeks		
<i>k</i> 1	degradation in the solid phase, s ⁻¹	l	The time when C_L peaks, s		
V-	First order rate constant for <i>b</i> -Carotene	TCC	Total sum of square		
N2	degradation in the extract phase, s^{-1}		rotal sum of square		

k _m	First order rate constant for exhaustion of the given β -Carotene from the solid phase, s ⁻¹	Т	Extraction and degradation temperature, °C				
k	First order isothermal degradation rate constant for <i>b</i> -Carotene, s^{-1}	V	Volume of the solvent, m ³				
(k2)exp	Experimentally determined first order rate constant for β - <i>Carotene</i> degradation in the extract phase, s ⁻¹	X _{dm}	Dry matter content of the carrot powder, kg				
k _{ref}	Rate constant at reference temperature (s ⁻	Yexp	Experimental results				
Ms	instantaneous rate of transfer of b -Carotene to the liquid phase, kg s ⁻¹	Ymodel	Predicted results from model				
n	Number of observations for each experiment						
555							

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561

562 **Conflicts of Interest**

563 There are none to declare

565 **References**

- Achir, N., Pénicaud, C., Avallone, S., & Bohuon, P. (2011). Insight into β-carotene thermal
 degradation in oils with multiresponse modeling. *JAOCS, Journal of the American Oil Chemists' Society*, 88(12), 2035–2045. https://doi.org/10.1007/s11746-011-1864-2
- 569 Achir, N., Randrianatoandro, V. A., Bohuon, P., Laffargue, A., & Avallone, S. (2010). Kinetic
- study of β-carotene and lutein degradation in oils during heat treatment. *European Journal of Lipid Science and Technology*, *112*(3), 349–361. https://doi.org/10.1002/ejlt.200900165
- Appiah-Nkansah, N. B., Zhang, K., Rooney, W., & Wang, D. (2016). Model study on extraction
 of fermentable sugars and nonstructural carbohydrate from sweet sorghum using diffusion
 process. *Industrial Crops and Products*, 83, 654–662.
 https://doi.org/10.1016/j.indcrop.2015.12.056
- 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
- Burton, G. W., Daroszewski, J., Nickerson, J. G., Johnston, J. B., Mogg, T. J., & Nikiforov, G. B.
 (2014). β-Carotene autoxidation: Oxygen copolymerization, non-vitamin A products, and
 immunological activity. *Canadian Journal of Chemistry*, 92(4), 305–316.
 https://doi.org/10.1139/cjc-2013-0494
- Caris-Veyrat, C., Amiot, M. J., Ramasseul, R., & Marchon, J. C. (2001). Mild oxidative cleavage
 of β,β-carotene by dioxygen induced by a ruthenium porphyrin catalyst: Characterization of
 products and of some possible intermediates. *New Journal of Chemistry*, 25(2), 203–206.
 https://doi.org/10.1039/b006975m
- Chen, B. H., & Liu, M. H. (1998). Relationship between chlorophyll a and β-carotene in a lipidcontaining model system during illumination. *Food Chemistry*, 63(2), 207–213.
 https://doi.org/10.1016/S0308-8146(98)00006-5
- Chen, H. -M, & Meyers, S. P. (1982). Extraction of Astaxanthin Plgment from Crawfish Waste
 Using a Soy Oil Process. *Journal of Food Science*, 47(3), 892–896.
 https://doi.org/10.1111/j.1365-2621.1982.tb12739.x

- Chen, Q., Liu, Y., Chen, L., Chen, J., Yang, T., Cheng, Q., & Li, T. (2021). Vitamin A Levels
 Among Pre-School Children of Central and Western China. *Frontiers in Public Health*,
 9(September), 1–9. https://doi.org/10.3389/fpubh.2021.694106
- 597 Chumnanpaisont, N., Niamnuy, C., & Devahastin, S. (2014). Mathematical model for continuous
- and intermittent microwave-assisted extraction of bioactive compound from plant material:
- 599 Extraction of β -carotene from carrot peels. *Chemical Engineering Science*, 116, 442–451.
- 600 https://doi.org/10.1016/j.ces.2014.05.010
- Chutia, H., & Mahanta, C. L. (2020). Green ultrasound and microwave extraction of carotenoids
 from passion fruit peel using vegetable oils as a solvent: Optimization, comparison, kinetics,
 and thermodynamic studies. *Innovative Food Science and Emerging Technologies*, *xxxx*,
 102547. https://doi.org/10.1016/j.ifset.2020.102547
- Demiray, E., & Tulek, Y. (2017). Degradation kinetics of β-carotene in carrot slices during
 convective drying. *International Journal of Food Properties*, 20(1), 151–156.
 https://doi.org/10.1080/10942912.2016.1147460
- Elik, A., Yanık, D. K., & Göğüş, F. (2020). Microwave-assisted extraction of carotenoids from
 carrot juice processing waste using flaxseed oil as a solvent. *Lwt*, *123*(December 2019).
 https://doi.org/10.1016/j.lwt.2020.109100
- Gul, K., Tak, A., Singh, A. K., Singh, P., Yousuf, B., & Wani, A. A. (2015). Chemistry,
 encapsulation, and health benefits of β-carotene A review. *Cogent Food and Agriculture*, *1*(1). https://doi.org/10.1080/23311932.2015.1018696
- Harika, R., Faber, M., Samuel, F., Kimiywe, J., Mulugeta, A., & Eilander, A. (2017). 614 Micronutrient status and dietary intake of iron, Vitamin A, iodine, folate and zinc in women 615 of reproductive age and pregnant women in Ethiopia, Kenya, Nigeria and South Africa: A 616 systematic review of data from 2005 2015. 9(10). 617 to Nutrients. https://doi.org/10.3390/nu9101096 618
- Hiranvarachat, B., & Devahastin, S. (2014). Enhancement of microwave-assisted extraction via
 intermittent radiation: Extraction of carotenoids from carrot peels. *Journal of Food Engineering*, *126*, 17–26. https://doi.org/10.1016/j.jfoodeng.2013.10.024
- Hornero-Méndez, D., & Mínguez-Mosquera, M. I. (2007). Bioaccessibility of carotenes from

- carrots: Effect of cooking and addition of oil. *Innovative Food Science and Emerging Technologies*, 8(3), 407–412. https://doi.org/10.1016/j.ifset.2007.03.014
- Humayoun Akhtar, M., & Bryan, M. (2008). Extraction and quantification of major carotenoids in
 processed foods and supplements by liquid chromatography. *Food Chemistry*, *111*(1), 255–
 261. https://doi.org/10.1016/j.foodchem.2008.03.071
- Leach, G., Pyle, L., & Niranjan, K. (1994). Effective diffusivity of total solids and pectic
 substances from apple tissue. *International Journal of Food Science & Technology*, 29(6),
 687–697. https://doi.org/10.1111/j.1365-2621.1994.tb02110.x
- Li, Y., Fabiano-Tixier, A. S., Tomao, V., Cravotto, G., & Chemat, F. (2013). Green ultrasoundassisted extraction of carotenoids based on the bio-refinery concept using sunflower oil as an
 alternative solvent. *Ultrasonics Sonochemistry*, 20(1), 12–18.
 https://doi.org/10.1016/j.ultsonch.2012.07.005
- Mba, O. I., Dumont, M. J., & Ngadi, M. (2017). Thermostability and degradation kinetics of
 tocochromanols and carotenoids in palm oil, canola oil and their blends during deep-fat
 frying. LWT Food Science and Technology, 82, 131–138.
 https://doi.org/10.1016/j.lwt.2017.04.027
- Miękus, N., Iqbal, A., Marszałek, K., Puchalski, C., & Świergiel, A. (2019). Green chemistry 639 extractions of carotenoids from daucus carota L.-Supercritical carbon dioxide and enzyme-640 23). **MDPI** assisted methods. In Molecules (Vol. 24, Issue AG. 641 https://doi.org/10.3390/molecules24234339 642
- Purohit, A. J., & Gogate, P. R. (2015). Ultrasound-Assisted Extraction of β-Carotene from Waste
 Carrot Residue: Effect of Operating Parameters and Type of Ultrasonic Irradiation. *Separation Science and Technology (Philadelphia)*, 50(10), 1507–1517.
 https://doi.org/10.1080/01496395.2014.978472
- 647 Rodriguez-amaya, D. B. (n.d.). a Guide To Analysis in. In Life Sciences.
- Roohinejad, S., Oey, I., Everett, D. W., & Niven, B. E. (2014). Evaluating the Effectiveness of βCarotene Extraction from Pulsed Electric Field-Treated Carrot Pomace Using Oil-in-Water
 Microemulsion. *Food and Bioprocess Technology*, 7(11), 3336–3348.
 https://doi.org/10.1007/s11947-014-1334-6

- Sachindra, N. M., & Mahendrakar, N. S. (2005). Process optimization for extraction of carotenoids
 from shrimp waste with vegetable oils. *Bioresource Technology*, *96*(10), 1195–1200.
 https://doi.org/10.1016/j.biortech.2004.09.018
- Saini, R. K., & Keum, Y. S. (2018). Carotenoid extraction methods: A review of recent
 developments. *Food Chemistry*, 240(April 2017), 90–103.
 https://doi.org/10.1016/j.foodchem.2017.07.099
- Salehi, L., & Taghian Dinani, S. (2020). Application of electrohydrodynamic-ultrasonic procedure
 for extraction of β-carotene from carrot pomace. *Journal of Food Measurement and Characterization*, 14(6), 3031–3039. https://doi.org/10.1007/s11694-020-00542-w
- Stewart, W. E., Caracotsios, M., & Sørensen, J. P. (1992). Parameter estimation from
 multiresponse data. AIChe Journal, 38, 641–650.
- Strati, I. F., & Oreopoulou, V. (2011). Process optimisation for recovery of carotenoids from
 tomato waste. *Food Chemistry*, *129*(3), 747–752.
 https://doi.org/10.1016/j.foodchem.2011.05.015
- Stupar, A., Šeregelj, V., Ribeiro, B. D., Pezo, L., Cvetanović, A., Mišan, A., & Marrucho, I. (2021).
 Recovery of β-carotene from pumpkin using switchable natural deep eutectic solvents. In
 Ultrasonics Sonochemistry (Vol. 76). https://doi.org/10.1016/j.ultsonch.2021.105638
- Sun, M., & Temelli, F. (2006). Supercritical carbon dioxide extraction of carotenoids from carrot
 using canola oil as a continuous co-solvent. *Journal of Supercritical Fluids*, *37*(3), 397–408.
 https://doi.org/10.1016/j.supflu.2006.01.008
- Sun, Y., Ma, G., Ye, X., Kakuda, Y., & Meng, R. (2010). Stability of all-trans-β-carotene under
 ultrasound treatment in a model system: Effects of different factors, kinetics and newly
 formed compounds. *Ultrasonics Sonochemistry*, 17(4), 654–661.
 https://doi.org/10.1016/j.ultsonch.2009.12.005
- Syamila, M., Gedi, M. A., Briars, R., Ayed, C., & Gray, D. A. (2019). Effect of temperature,
 oxygen and light on the degradation of β-carotene, lutein and α-tocopherol in spray-dried
 spinach juice powder during storage. *Food Chemistry*, 284(September 2018), 188–197.
 https://doi.org/10.1016/j.foodchem.2019.01.055
- Tang, G., Qin, J., Dolnikowski, G. G., Russell, R. M., & Grusak, M. A. (2005). Spinach or carrots

- can supply significant amounts of vitamin A as assessed by feeding with intrinsically
 deuterated vegetables. *American Journal of Clinical Nutrition*, 82(4), 821–828.
 https://doi.org/10.1093/ajcn/82.4.821
- Thürmann, P. A., Steffen, J., Zwernemann, C., Aebischer, C. P., Cohn, W., Wendt, G., & Schalch,
- 685 W. (2002). Plasma concentration response to drinks containing β -carotene as carrot juice or
- formulated as a water dispersible powder. *European Journal of Nutrition*, 41(5), 228–235.
- 687 https://doi.org/10.1007/s00394-002-0381-3
- Totani, N., Yawata, M., Mori, T., & Hammond, E. G. (2013). Oxygen content and oxidation in
 frying oil. *Journal of Oleo Science*, 62(12), 989–995. https://doi.org/10.5650/jos.62.989
- Van Boekel, M.A.J.S. (2008), Kinetic Modeling of Food Quality: A Critical Review.
 Comprehensive Reviews in Food Science and Food Safety, 7: 144158. https://doi.org/10.1111/j.1541-4337.2007.00036.x
- Yilmaz, T., Kumcuoglu, S., & Tavman, S. (2020). Ultrasound-assisted extraction of lycopene and
 β-carotene from tomato-processing wastes. In *Ital. J. Food Sci* (Vol. 29).

Tables

Table 1: Values of model parameters fitting to eqns. (6) and (8). Experiments performed with solid to liquid ratio 20 kg m⁻³ and for different particle sizes at different temperatures.

Sl. No.	Particle size (mm)	Temperature (°C)	C _{si} × 10 ^{−4} (kg of BC/kg dry matter)	$k \times 10^{-4}$ (Degradation rate constant in the solid phase, s ⁻¹)	$k_m \times 10^{-3}$ (Exhaustion rate constant of BC in the liquid phase, s ⁻¹)	SSE × 10 ⁻⁶ (Eqn. 10)	R ² (Eqn. 12)	Adjusted-R ² (Eqn. 13)	RMSE × 10 ⁻⁴ (Eqn. 11)	
		90	7.52±0.16		8.0±0.71	17.60	0.94	0.94	10.83	
1	0.350	115	7.61±0.19		8.0±0.77	14.07	0.95	0.95	9.68	
		135 8	8.03±0.15	5.70±0.11	18.0±1.22	20.17	0.89	0.87	36.67	
			150	6.67±0.13	4.64±0.07	22.0±1.31	62.18	0.92	0.90	20.36
		90	4.98±0.09		10.0±0.83	5.92	0.96	0.95	6.28	
	0.750	115	6.27±0.11		16.0±1.09	90.82	0.99	0.99	2.46	
2		135	5.77±0.12	0.51±0.03	17.0±1.04	3.73	0.97	0.97	5.16	
		150	6.82±0.17	0.62 ± 0.07	20.0±1.53	35.95	0.99	0.99	16.03	
	1 400	90	4.68±0.02		6.0±0.21	0.99	0.99	0.99	2.57	
3	1.400	1.400	115	5.05±0.06		9.0±0.55	5.88	0.96	0.96	6.26

135	4.51±0.08	1.35±0.09	13.0±0.33	86.01	0.98	0.98	2.47
150	4.68±0.02	4.08±0.11	15.0±1.19	14.74	0.92	0.90	10.26

- Note: the model parameters were predicted by using average value of triplicate experimental dataset (n=3).
- 701 C_{si} Maximum extractable betalains (kg of dried β -*Carotene*/kg of dried carrot powder).
- 702 k Degradation rate constant (s⁻¹)
- 703 k_m Solid exhaustion rate constant (s⁻¹)
- 704SSE Sum of squared errors
- 705 R^2 Co-efficient of determination
- 706 Adj. R^2 Adjusted Co-efficient of determination
- 707 RMSE Root mean squared error

Authors	Solvent Used	Extraction method	Operating parameters	Maximum extraction rate (kg β -Carotene (kg of dry matter) ⁻¹ s ⁻¹	Comment
(Li et al. 2013)	Sunflower oil	Ultrasound Extraction	Solid/liquid = 1/20, Time = 30 min	2.0×10 ⁻⁶	Particle size was not mentioned
(Roohinejad et al., 2014)	Glycerol monocaprylocaprat e+Posphate Buffer + Tween 20	Pulsed Electric Field Treatment as pre-treatment	Solid/liquid = 1/30, Time = 60 min	2.0×10 ⁻⁶	Particle size was not mentioned
(Hiranvarach at & Devahastin, 2014)	Hexane (50%), acetone (25%), Ethanol (25%)	Microwave Extraction	180 W/75 ml, Time = 4 min	4.6×10 ⁻⁶	Particle size was not mentioned

Table 2: Comparison of maximum extraction rates reported in literature with values observed in this research.

(Salehi & Taghian Dinani, 2020)	Ethanol	Ultrasound- electrohydrodyna mic	Solid/liquid = 1/10, Time = 60 min	3.5 ×10 ⁻⁶	Particle size was not mentioned
This Study	Sunflower oil	Hot plate Stirring	(a) 90-150 °C, 4 min		Particle size
This Study	Sumower on	Extraction	extraction time	3.1×10 ⁻⁶	= 350 µm.

Temperature (°C)	Degradation rate constants, k (s ⁻¹)	Half-life, $(t_{1/2})$ (min)	R ²	Activation Energy, E_a (kJ/mol ⁻¹)	Pre-exponential Factor, A (s ⁻¹)
135	0.0001	115.5	0.87		
150	0.0002	57.75	0.99		
160	0.0004	28.87	0.96		7.6
180	0.0006	19.25	0.96	36.63	/.0
200	0.0009	12.83	0.98		
220	0.0022	5.25	0.97		

Table 3: Effect of temperature on the rate constants for thermal degradation of β -*Carotene* in sunflower oil, and Arrhenius constants.

712	Note: the model parameters were deduced by plotting average value of triplicate
713	experimental dataset (n=3).
714	
715	k – First order isothermal degradation rate constant of β -Carotene (s ⁻¹)
716	$t_{1/2}$ - Half life time for degradation of β -Carotene (min)
717	R ² - Co-efficient of determination
718	$E_{\rm a}$ – Activation energy of degradation for β -Carotene (kJ/mole ⁻¹)
719	A - Pre-exponential Factor, (s ⁻¹)

Reaction		Activation	Goodness of fit							
rate constants (min ⁻¹)	nts 160 180 200 220		Energy (kJ mol ⁻¹)	RSS	n					
k_1	0.079±0.001	0.188±0.002	0.415±0.002	0.860±0.006	70.51					
k_2	0.148 ± 0.001	0.330±0.002	0.689 ± 0.005	1.356±0.010	65.52					
<i>k</i> ₃	0.166±0.001	0.232±0.001	0.316±0.001	0.418±0.003	27.24	0.005	288			
k_4	0.461 ± 0.002	0.569±0.003	0.689±0.001	0.821±0.005	17.04					
k_5	0.011±0.001	0.027 ± 0.001	0.060 ± 0.004	0.125±0.002	70.16					
Note	Note: the model parameters were predicted by using average value of triplicate experimental dataset (n=3).									
RSS	RSS – Sum of square of the residuals (RSS = $\sum_{i=1}^{n} ([X_{optipred}] - [X_{exp}]))$, where n is the									
num	number of data points, $[X_{exp}]$ the experimental result, and $[X_{optipred}]$ the optimized simulated									
resu	lt.									
n-1	n – no. of datapoints model was evaluated.									

Table 4: Rate constant values and Arrhenius parameters for the reaction network described in Fig 4.

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Figure 1: Extraction of β -*Carotene* from carrot powders at 90, 115, 135 and 150 °C into sunflower oil, Solid loading =20 kg/m³, a) Particle size – 0.35 mm, b) Particle size – 0.75 mm, (c) Particle size – 1.40 mm. The points indicate experimental values of the concentration and the solid line represents the model, i.e., concentration given by Eqns. (6) and (8). Values of the model parameters for the other particle sizes with temperature range in sunflower oil are shown in Table 1. Standard deviation was included for triplicates (n=3).

Figure 2: Composition of β -Carotene isomers, trans and 13-cis, in sunflower oil extract at different extraction temperatures (a) 90 °C, (b) 115 °C, (c) 135 and (d) 150 °C. It may be noted that 13-cis isomer was only observed in the initially stages of extraction at higher temperatures of 135, and 150 °C (Figs c and d). 9-cis isomer was not detected in the extracts. Standard deviation was included for triplicates (n=3).

Figure 3: Degradation kinetics of β -*Carotene* in sunflower oil at different temperatures. Temperature range was selected to reflect normal frying and cooking conditions. Solid lines indicate first-order kinetic fit. The rate constant at different temperatures are reported in Table (3). Note: the model parameters were deduced by plotting average value of triplicate experimental dataset (n=3).

Figure 4: Schematic representation of the reaction network which includes degradation and

isomerization of β -*Carotene* during heating in sunflower oil.

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Figure 5: Concentration of β -*Carotene* isomers during thermal degradation of trans- β -*Carotene* in sunflower oil at different temperatures (a) 160 °C, (b) 180 °C, (c) 200 °C, and (d) 220 °C. Solid lines passing through the experimental points are deduced from indicate Athena Visual Software applied to reaction network shown in Fig 4. All experimental datasets were used for predictive modelling. Hence, no standard deviations applied.

Figure 6: Parity plot showing consistency between the rate constant values obtained by absorbance measurement during β -*Carotene* degradation, and the value of k_5 estimated by Athena Visual Software.

744 supplemental Information

Table 1S: Values of degradation rate constants in extract and solid phases for β -*Carotene* after fitting to eqn. (5) for Betacarotene. Experiments performed with solid to liquid ratio 20 kg m⁻³ and for different particle sizes at different temperatures.

Sl. No.	Particle size (mm)	Temperature (°C)	$k_1 \times 10^{-4}$ (Degradation rate constant in the solid phase, s ⁻¹)	$k_2 \times 10^{-4}$ (Degradation rate constant in the liquid phase, s ⁻¹)	$(k_2)exp \times 10^{-4}$ (Degradation rate constant in the liquid phase experimental, s ⁻¹)	SSE × 10 ⁻⁶ (Eqn. 10)	R ² (Eqn. 12)	Adjusted- R ² (Eqn. 13)	RMSE × 10 ⁻⁴ (Eqn. 11)
1	0.35	90				17.60	0.94	0.94	10.83
		115				14.07	0.95	0.95	9.68
		135	1.41±0.01	5.84±0.39	1.00	20.17	0.89	0.87	36.67
		150	1.33±0.02	4.68±0.21	2.00	62.18	0.92	0.90	20.36
2	0.75	90				5.92	0.96	0.95	6.28
		115				90.82	0.99	0.99	2.46
		135	1.22±0.01	0.51±0.02	1.00	3.73	0.97	0.97	5.16
		150	1.01±0.01	0.62±0.02	2.00	35.95	0.99	0.99	16.03
3	1.40	90				0.99	0.99	0.99	2.57
		115				5.88	0.96	0.96	6.26
		135	1.42±0.06	1.34±0.05	1.00	86.01	0.98	0.98	2.47

	150	1.45 ± 0.07	4.08±0.10	2.00	14.74	0.92	0.90	10.26		
745										
746	Note: the model parameters were predicted by using average value of triplicate experimental dataset (n=3).									
747	k_I – Degradation rate constant in liquid phase (s ⁻¹)									
748	k_2 - Degradation rate constant in solid phase (s ⁻¹)									
749	$(k_2)_{exp}$ -Degradation rate constant in liquid phase experimental (s ⁻¹)									
750	SSE – Sum of squared errors									
751	R^2 – Co-efficient of determination									
752	Adj. R ² – Adjusted Co-efficient of determination									
753	RMSE – Root mean	squared error								