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Elevated Temperature Extraction of *β-Carotene* from Freeze Dried Carrot Powder into Sunflower Oil: Extraction Kinetics and Thermal Stability

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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 prevalent worldwide. Earlier research studies have addressed the extraction of *β-Carotene* at 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-Carotene* extraction by considering both extraction and degradation kinetics. The model developed, which accounts for degradation occurring in solid and extract phases, has been 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 application of sunflower oil as a carrier for *β-Carotene* during cooking and food processing, by monitoring and modelling the thermal degradation and isomerisation of *β-Carotene* at temperatures up to 220 °C. The modelling of extraction kinetics shows that it is possible to achieve viable extraction rates by employing temperatures in the range (90-150 °C) for relatively short times (< 5 mins). The degradation kinetics shows that almost 75% of the *β-Carotene* can survive heating at 180 °C for 10 mins – indicating the possibility of using *β-Carotene* enriched edible oils for frying. This study also reports on the formation of three isomers of *β-Carotene* identified using HPLC: *trans*-, 9-*cis* and 13-*cis*. The reaction network model developed in this study was able to account for the transient variation of the concentration of all three isomers.

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

Practical Application:

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

1. Introduction

β-Carotene is a pigment found in fruits and vegetables that can be converted to vitamin A in the body (Rodriguez-amaya, 1999; Marty and Berset, 1986). It has antioxidant properties that can also protect against damage from harmful molecules (Elik et al., 2020). Consuming foods high in *β-Carotene* is reported to have health benefits, such as reducing the risk of certain types of cancer, improving immune function, and protecting against cardiovascular disease (Gul *et al.*, 2015). *β-Carotene* is widely used as a colouring agent and a natural preservative in the food industry (Yilmaz *et al.*, 2017). It is also used as a natural colourant and skin conditioning agent in the cosmetics industry (Strati & Oreopoulou, 2011). Vitamin A deficiency, which can be mitigated 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 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).

Considering the health benefits, societal impact and industrial application, extraction of *β-Carotene* from natural plant sources has attracted considerable attention employing methods such as microwave assisted extraction (Hiranvarachat & Devahastin, 2014), supercritical fluid extraction (M. Sun & Temelli, 2006a), ultrasound assisted extraction (Saini & Keum, 2018), pulsed electric fields (Roohinejad et al., 2014), and others. The extraction of *β-Carotene* from 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 (Elik et al., 2020). Further, *β-Carotene* is also sensitive to light, heat, and oxygen, and can degrade

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.

Vegetable oils can be effective solvents due to their low cost and abundant availability all 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 enhanced between 4-12 fold by consuming it with edible oils and fats (Hornero-Méndez & Mínguez-Mosquera, 2007). Earlier research has shown that vegetable oils can extract *β-Carotene* from a range of sources, including fruits, vegetables, and microorganisms, while oils can provide other health benefits such as unsaturated fatty acids and other nutrients (Chen and Meyers, 1982; Sachindra and Mahendrakar, 2005; Sun and Temelli, 2006; Elik *et al.*, 2020). The use of vegetable oils for extracting *β-Carotene* also provides the opportunity to use the extract directly as a food ingredient or for cooking processes such as frying.

A number of papers are available on the kinetics of *β-Carotene* extraction in various organic solvents (Chumnanpaisont et al., 2014; Hiranvarachat & Devahastin, 2014; Humayoun Akhtar & Bryan, 2008; Purohit & Gogate, 2015a). These papers generally report on the use of 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 pathways include oxidation, thermal, and photochemical degradation (Achir et al., 2011; Gul et al., 2015a). Photochemical degradation of *β-Carotene* can also lead to the formation of products, such as apocarotenoids (Mięku s et al., 2019). The excentric cleavage of *β-Carotene* produces

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

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

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

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

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:

$$-\frac{dC_s}{dt}X_{dm} = M_s + k_1 C_s X_{dm} \quad (1)$$

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 well-documented. 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:

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

where C_{si} is the initial average concentration of β-Carotene in the solid phase and k_M (s^{-1}) is a rate constant for solid phase exhaustion of β-Carotene. It is arguable whether C_{si} represents the initial concentration of β-Carotene *per se* in the solid phase. Experiments were conducted to determine the total mass of β-Carotene that could be extracted from freeze dried carrot powder into different solvents such as tetrahydrofuran, hexane and coconut oil. These experiments involved extraction 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. For example, the maximum amount of β-Carotene extracted into tetrahydrofuran – in which β-Carotene is known to be most soluble (Purohit & Gogate, 2015b) – was 865.68 µg g^{-1} powder. Likewise, the maximum amount extracted into hexane was 752.54 µg g^{-1} ; and that extracted into coconut oil was 722.36 µg g^{-1} This suggests that C_{si} represents the concentration of β-Carotene that is extractable into a given solvent under a given set of operating conditions. It is therefore reasonable to hypothesize that C_{si} is a model parameter which can potentially be estimated from the experimental data. By differentiating eqn (2) and substituting the values of the derivative and C_s into eqn (1), we get:

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

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:

$$V\frac{dC_L}{dt} = M_s - k_2 C_L V \quad (4)$$

where V is the volume of the extraction medium, in this case, the volume of sunflower oil taken (m^3). 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:

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

where, $\frac{X_{dm}}{V} = S$ represents the solid loading in the extractor (kg of carrot powder per m^3 of sunflower oil).

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.

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:

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

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:

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

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:

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

Thus, the plot of C_L versus t will increase monotonically before asymptotically converging to a value of $C_L = SC_{si}$.

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 best-fitting 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.

3. Materials and Methods

3.1 Design of experiments

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

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

3.3 Determination of extraction kinetics

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 ^{o}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 ^{o}C, to obtain a clear supernatant which was then stored at 4 °C until further analysis.

3.4 Measurement and characterization of β-Carotene in sunflower oil

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.

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 injected into the HPLC column - a polymeric YMC-30 (4.6 mm id ×250 mm, 5 mm particle size) (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 flow rate of 1 mL/min under isocratic conditions. A UV- visible photodiode array detector (Dionex UVD 340U) was used to analyze the chromatograms at a detection wavelength of 450 nm. Analysis were done in triplicate. The quantification was done against a standard calibration curve (R^2 = 0.99) in a concentration range between 0.5 µg/ml to 12 µg/ml in a mixture of 8:1 (v/v) hexane and plain sunflower oil.

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

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

298 determined temperature in the shortest possible time, which was less than 10 s. The test tube was
299 then maintained at this temperature for the desired time. It was then rapidly cooled to in an ice
300 bath, and stored at 4 ℃ temperature in an amber vial which protected it from light degradation
301 until further analysis . The transient concentrations of β-Carotene, determined at each temperature,
302 were fitted to the first order equation to deduce the rate constant:
303
304 $$\ln\left(\frac{C_0}{C_t}\right) = kt \quad (9)$$
305
306 where, c_t and c_0 are the concentrations of *β-Carotene* at any time t and initially, respectively, and
307 k is the first order isothermal rate constant, assumed to vary with temperature (T) according to the
308 well-known Arrhenius equation: $k = A\ exp(\frac{-E_a}{RT})$ where, A is the pre-exponential factor (s^{-1}); E_a is
309 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
313 The validity of the model was tested by fitting eqns. (5) and (8) to the experimentally
314 determined c_L versus t data using MATLAB 2020b's curve fitting tool for 95% confidence interval.
315 The tool works by minimizing the sum squared error and root mean squared error, and requires an
316 initial guess for the model parameters. The Levenberg-Marquardt algorithm is used to optimize
317 the model parameters, and the best-fit values were based on 15×3 data points (in triplicates) for
318 each experimental condition. This article also explains that the SSE and RMSE values indicate
319 model validity and goodness of fit, and the co-efficient of correlation and adjusted R^2 are
320 determined to ensure an adequate number of parameters have been used. The narrow range of joint

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.

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

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

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

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

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

4. Results and Discussion

4.1 Validation of the model

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

The values of the constant k_2 – which represents the first order rate constant for the degradation of *β-Carotene* in the oil phase – were estimated by fitting the C_L versus t data as 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 to check the null hypothesis of a significant difference existing between k_2 values given by the model and the experimentally determined values of k_2; the p value obtained was greater than 0.05 which negates the null hypothesis. Thus, k_2 values deduced from eqn (5) and experimental values are statistically the same – which further reinforces the model hypothesis that degradation kinetics of *β-Carotene* in oil follows first order between 135-220 ℃.

It is evident from Table 1S that the values of k_1 and $(k_2)_{exp}$ are very close. An ANOVA was 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, k_1 and $(k_2)_{exp}$ can be assumed to be equal in eqn (5), which indicates that the general variation of *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 degradation is predominantly thermal by nature, and any differences in the structural environments of the two phases do not play a significant role in the degradation process. Thus, the experimentally determined C_L versus t data were fitted to eqn (6) to generate Table 1 which shows the best fit model parameters as well as the goodness of fit. Fig 1 illustrates the fit of the experimental data against equations 6 and 8, for all the temperatures and particle sizes investigated in this work.

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 ^oC, 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.

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 ^oC) 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 the present study are significantly greater than those observed in some earlier studies. For example, Purohit and Gogate (2015) have reported an extraction time of around 50 mins to attain a yield of 70% (based on the total extractable *β*-Carotene) in ultrasound assisted ethanol solutions at temperature of 30^{o}C, when using carrot particles of sizes comparable with the sizes used in the 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 times of 2 -5 mins for the extraction of *β-Carotene* from carrots using microwave power, operating either continuously or intermittently. Table (2) shows a comparison between the net rate of extraction determined using various extraction methods and the values observed in this work 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 microwave, pulsed electric field and electrohydrodynamic combined with ultra sound.

4.2 Composition of the sunflower oil extract

β-Carotene can exist in three isomeric forms in oil: *trans,* 9-*cis and* 13-*cis* (Achir et al., 2011). HPLC analysis was performed for each extract and the concentrations of the three isomers in the extract are shown as a function of time in Fig. 2 (a)-(d). The concentration of 9-*cis* in the extract was below the detection limit, therefore the concentrations of only the other two isomers are shown. A similar result was reported earlier by Achier et. al., (2011). It is also interesting to note that the sum of the concentrations of the two isomers is the total *β-Carotene* concentration 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 after the peak concentration is reached. Therefore, longer extraction durations only result in *trans* 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 ranged between 13-30%, which is somewhat lower than the value of 40% reported for copra fat 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 may be noted that these observations are valid for all the particle sizes employed in this study (data not shown).

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.

4.3 Effect of temperature and particle size on the extraction kinetics

β-Carotene is mainly present in chromo- and chloroplast, and protected by the cellulose and pectin layers of the cellular structure (Thürmann et al., 2002). Smaller particle sizes imply shorter diffusion path length and greater accessibility of the *β*-Carotene. Therefore, k_m increases with decrease in particle size, which is confirmed in Table 1. Higher temperatures, on the other hand, improve accessibility by rupturing the protecting membranes (Nutter et al., 2021). Therefore

435 k_m also increases with temperature, but, as Table 1 shows, the increase is not as marked as the
436 effect of particle size.

437

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

440

441 *β-Carotene* degradation experiments were performed by dissolving commercially available
442 *trans-β-Carotene* in sunflower oil and allowing the *β-Carotene* to degrade at the desired
443 temperatures (section 3.6). For all heating treatments, the concentration of *trans-β-Carotene*
444 decreased as a function of the heating time. This disappearance was visible macroscopically by a
445 loss of color, and it was more rapid as the temperature increased around 200 °C. Fig 3 shows a
446 semi-log plot of normalized *β-Carotene* concentration against time over a range of temperatures
447 between 135 and 220 °C. The linear nature of the plots confirm that the degradation follows first
448 order isothermal kinetics; the rate constants values are given in Table 3, which also reports the
449 Arrhenius constants: activation energy and pre-exponential factor. Table 3 shows that the
450 activation energy value over the temperatures 135-220 °C is 56.65 kJ/mol (R^2=0.91), which is
451 consistent with the reported values of 48 kJ/mol for *β-Carotene* degradation in palm olein (Achir
452 et al., 2010).

453

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

458 significantly lower than the values reported by Sun et al., (2010) for trans *β-Carotene* degradation
459 in dichloromethane under the influence of ultrasound at temperatures in the range -5-25 ^{o}C. It is
460 unclear whether the chemical nature of the solvent medium plays a role in influencing kinetics,
461 but these studies suggest that there is a role played by the solvent. Further experiments are needed
462 to confirm solvent effects. Regardless, it is clear that in sunflower oil, *β-Carotene* undergoes
463 degradation at frying temperatures, the extent depending on the time-temperature combination
464 employed. If we assume a typical frying temperature of 180 °C for 10 mins (e.g. for frying French
465 fries), the percentage of *β-Carotene* remaining in the oil, based on the rate constant values reported
466 in this work is 75%, which suggests that *β-Carotene* fortified vegetable oils can be used, in
467 practice, for frying and other food processing applications. At such high temperatures, the heating
468 time needs to be over 30 minutes for 90% of *β-Carotene* to be destroyed (Achir et al., 2010).
469
470 During heating, *β-Carotene* degradation is reported to be accompanied by concomitant
471 isomerization, as well as oxidation to produce epoxy- and hydroxy-*β*-Carotene, and cleavage
472 products such as apocarotenals and apocarotenones (Mordi, 1993); Caris-Veyrat *et al.*, 2001). In
473 this study, the development of *trans*-, 9-*cis* and 13-*cis* isomer concentrations were monitored with
474 time, at various temperatures, using HPLC-DAD (section 3.4). Achir et al (2011) have proposed
475 plausible reaction networks leading to the formation of the isomers and thermal degradation
476 products. A simplified network model scheme is presented in Fig 4, which assumes that, at any
477 given temperature, the trans isomer can reversibly change either to 9-*cis* or13-*cis* isomer, each of
478 which can also undergo subsequent thermal degradation. Each reaction in the network shown in
479 Fig. 4 is also assumed to be first order with corresponding rate constants. It is also reasonable to
480 assume that the thermal degradation rate constants for all three isomers are the same at a given

481 temperature, as suggested by Achir (2011). Based on these assumptions, an instantaneous mass
482 balance can be written for each of the isomers as follows:
483
484 $$\frac{dC_{trans}}{dt} = -(k_1 + k_3 + k_5) \times C_{trans} + k_2 \times C_{9-cis} + k_4 \times C_{13-cis} \quad (14)$$
485 $$\frac{dC_{9-cis}}{dt} = k_1 \times C_{trans} - (k_2 + k_5) \times C_{9-cis} \quad (15)$$
486 $$\frac{dC_{13-cis}}{dt} = k_3 \times C_{trans} - (k_2 + k_5) \times C_{13-cis} \quad (16)$$
487
488 The above set of differential equations was used to model the concentration of the three
489 isomers with respect to reaction (processing/cooking) time and temperature, the initial conditions
490 being $C_{trans} = C_{\beta\text{-Carotene}}$ and $C_{9\text{-cis}} = C_{13\text{-cis}} = 0$. Multiresponse modelling to obtain the best estimates
491 of the rate constants from k_1 through to k_5 and their corresponding activation energies was
492 performed by by non-linear regression using the Bayesian approach and the determinant criterion
493 (van Boekel 2008), included in the modelling software Athena Visual Studio software package
494 (Athena Visual Software Inc., Naperville, IL). The minimisation of the determinant criterion
495 (Stewart, Caracotsios, & Sørensen, 1992) is ideal for multiresponse studies since it removes the
496 need for the statistical compliance that is required for the typical minimization of the sum of
497 squares (van Bokel, 2008). Each of the rate constants from k_1 through to k_5 was assumed to follow
498 Arrhenius behaviour with respect to temperature and was reparametrized as follows:
499
500 $$k = k_{\text{ref}} \exp\left(\frac{-E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right)$$
501
502 where:

503 k: rate constant at any temperature T (^{o}K)

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

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

506 R: Universal gas constant (8.314 J mol^{-1} K^{-1})

507

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

518

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

constants for the back isomerization of *cis*. The rate constant k_5 representing irreversible thermal degradation of all three isomers can be compared with the rate constant values obtained by measuring absorbance values as a function of time (section 3.4) and a parity plot of values obtained at different temperatures is shown in Fig 6.

The significance of isomer formation during degradation to potential bioactivity is not conclusive. According to Rodriguez-Amaya (1999), all three isomers are capable of synthesizing vitamin A, and can impart coloration to food materials. However, Castenmiller & West (1998) has stated that the *trans*-isomer is more active at synthesizing vitamin A than the *cis*-isomers. It is therefore evident that further research is needed to conclusively establish the role played by each isomer in this regard.

4. Conclusion

1. Elevated temperatures (upto 150 °C) can be used viably to extract *β*-Carotene in edible oil. Despite thermal degradation of *β*-Carotene at high temperatures, the net rates of extraction observed in this study were found to be significantly higher or comparable with the rates observed in earlier studies using energy intensive technologies such as pulsed electric field, microwave and electrohydrodynamic in combination with ultrasound.
2. A model developed to determine the transient concentration of *β-Carotene* in sunflower oil, which accounts for thermal degradation occurring in the solid and extract phases, gave a good fit with the experimental data. This kinetic model can potentially be used to design and size extractors.

3. *β*-Carotene enriched sunflower can be used as a frying medium to enrich the nutritional 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.

553

554 ## Nomenclature

A	Pre-exponential factor, s^{-1},	p	Total number of predicted results from 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_L	Concentration of *b*-Carotene in the extract, kg m^{-3}	R	Universal gas constant, 8.314 $J\ mol^{-1}\ K^{-1}$
C_s	*β-Carotene* concentration in the solid phase at any time, kg betalain (kg dry solid)$^{-1}$	RSS	Residual sum of square
C_{si}	Initial concentration of *b*-Carotene that is extractable, kg m^{-3}.	SSE	Sum of squared error
c_t	Concentrations of *β-Carotene* at any time t, µg/ml	THF	Tetrahydrofuran
E_a	Activation Energy, Eqn 6, J mol^{-1}	t	Time, s
k_1	First order rate constant for *b*-Carotene degradation in the solid phase, s^{-1}	t^*	The time when C_L peaks, s
K_2	First order rate constant for *b*-Carotene degradation in the extract phase, s^{-1}	TSS	Total sum of square

k_m	First order rate constant for exhaustion of the given *β-Carotene* from the solid phase, s^{-1}	T	Extraction and degradation temperature, °C
k	First order isothermal degradation rate constant for *b*-Carotene, s^{-1}	V	Volume of the solvent, m^3
$(k_2)_{exp}$	Experimentally determined first order rate constant for *β-Carotene* 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^{-1})	y_{exp}	Experimental results
M_s	instantaneous rate of transfer of *b*-Carotene to the liquid phase, kg s^{-1}	y_{model}	Predicted results from model
n	Number of observations for each experiment		

555

556

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Conflicts of Interest

There are none to declare

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

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 lipid-containing 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

Chumnanpaisont, N., Niamnuy, C., & Devahastin, S. (2014). Mathematical model for continuous and intermittent microwave-assisted extraction of bioactive compound from plant material: Extraction of β-carotene from carrot peels. *Chemical Engineering Science*, *116*, 442–451. 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). Micronutrient status and dietary intake of iron, Vitamin A, iodine, folate and zinc in women of reproductive age and pregnant women in Ethiopia, Kenya, Nigeria and South Africa: A systematic review of data from 2005 to 2015. *Nutrients*, *9*(10). https://doi.org/10.3390/nu9101096

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 ultrasound-assisted 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 extractions of carotenoids from daucus carota L.-Supercritical carbon dioxide and enzyme-assisted methods. In *Molecules* (Vol. 24, Issue 23). MDPI AG. https://doi.org/10.3390/molecules24234339

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

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 β-Carotene 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

652 Sachindra, N. M., & Mahendrakar, N. S. (2005). Process optimization for extraction of carotenoids
653 from shrimp waste with vegetable oils. *Bioresource Technology*, *96*(10), 1195–1200.
654 https://doi.org/10.1016/j.biortech.2004.09.018

655 Saini, R. K., & Keum, Y. S. (2018). Carotenoid extraction methods: A review of recent
656 developments. *Food Chemistry*, *240*(April 2017), 90–103.
657 https://doi.org/10.1016/j.foodchem.2017.07.099

658 Salehi, L., & Taghian Dinani, S. (2020). Application of electrohydrodynamic-ultrasonic procedure
659 for extraction of β-carotene from carrot pomace. *Journal of Food Measurement and*
660 *Characterization*, *14*(6), 3031–3039. https://doi.org/10.1007/s11694-020-00542-w

661 Stewart, W. E., Caracotsios, M., & Sørensen, J. P. (1992). Parameter estimation from
662 multiresponse data. AIChe Journal, 38, 641–650.

663 Strati, I. F., & Oreopoulou, V. (2011). Process optimisation for recovery of carotenoids from
664 tomato waste. *Food Chemistry*, *129*(3), 747–752.
665 https://doi.org/10.1016/j.foodchem.2011.05.015

666 Stupar, A., Šeregelj, V., Ribeiro, B. D., Pezo, L., Cvetanović, A., Mišan, A., & Marrucho, I. (2021).
667 Recovery of β-carotene from pumpkin using switchable natural deep eutectic solvents. In
668 *Ultrasonics Sonochemistry* (Vol. 76). https://doi.org/10.1016/j.ultsonch.2021.105638

669 Sun, M., & Temelli, F. (2006). Supercritical carbon dioxide extraction of carotenoids from carrot
670 using canola oil as a continuous co-solvent. *Journal of Supercritical Fluids*, *37*(3), 397–408.
671 https://doi.org/10.1016/j.supflu.2006.01.008

672 Sun, Y., Ma, G., Ye, X., Kakuda, Y., & Meng, R. (2010). Stability of all-trans-β-carotene under
673 ultrasound treatment in a model system: Effects of different factors, kinetics and newly
674 formed compounds. *Ultrasonics Sonochemistry*, *17*(4), 654–661.
675 https://doi.org/10.1016/j.ultsonch.2009.12.005

676 Syamila, M., Gedi, M. A., Briars, R., Ayed, C., & Gray, D. A. (2019). Effect of temperature,
677 oxygen and light on the degradation of β-carotene, lutein and α-tocopherol in spray-dried
678 spinach juice powder during storage. *Food Chemistry*, *284*(September 2018), 188–197.
679 https://doi.org/10.1016/j.foodchem.2019.01.055

680 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, 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. 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: 144-158. 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

697
698

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)	$C_{si} \times 10^{-4}$ (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)
1	0.350	90	7.52±0.16	---	8.0±0.71	17.60	0.94	0.94	10.83
		115	7.61±0.19	---	8.0±0.77	14.07	0.95	0.95	9.68
		135	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
2	0.750	90	4.98±0.09	---	10.0±0.83	5.92	0.96	0.95	6.28
		115	6.27±0.11	---	16.0±1.09	90.82	0.99	0.99	2.46
		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
3	1.400	90	4.68±0.02	---	6.0±0.21	0.99	0.99	0.99	2.57
		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

699
700 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})

704 SSE – 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

708

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

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 monocaprylocaprate+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
(Hiranvarachat & 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

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

709

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

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	56.65	7.6
150	0.0002	57.75	0.99		
160	0.0004	28.87	0.96		
180	0.0006	19.25	0.96		
200	0.0009	12.83	0.98		
220	0.0022	5.25	0.97		

Note: the model parameters were deduced by plotting average value of triplicate experimental dataset (n=3).

k – First order isothermal degradation rate constant of *β-Carotene* (s^{-1})

$t_{1/2}$- Half life time for degradation of *β-Carotene* (min)

R^2- Co-efficient of determination

E_a – Activation energy of degradation for *β-Carotene* ($kJ/mole^{-1}$)

A - Pre-exponential Factor, (s^{-1})

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

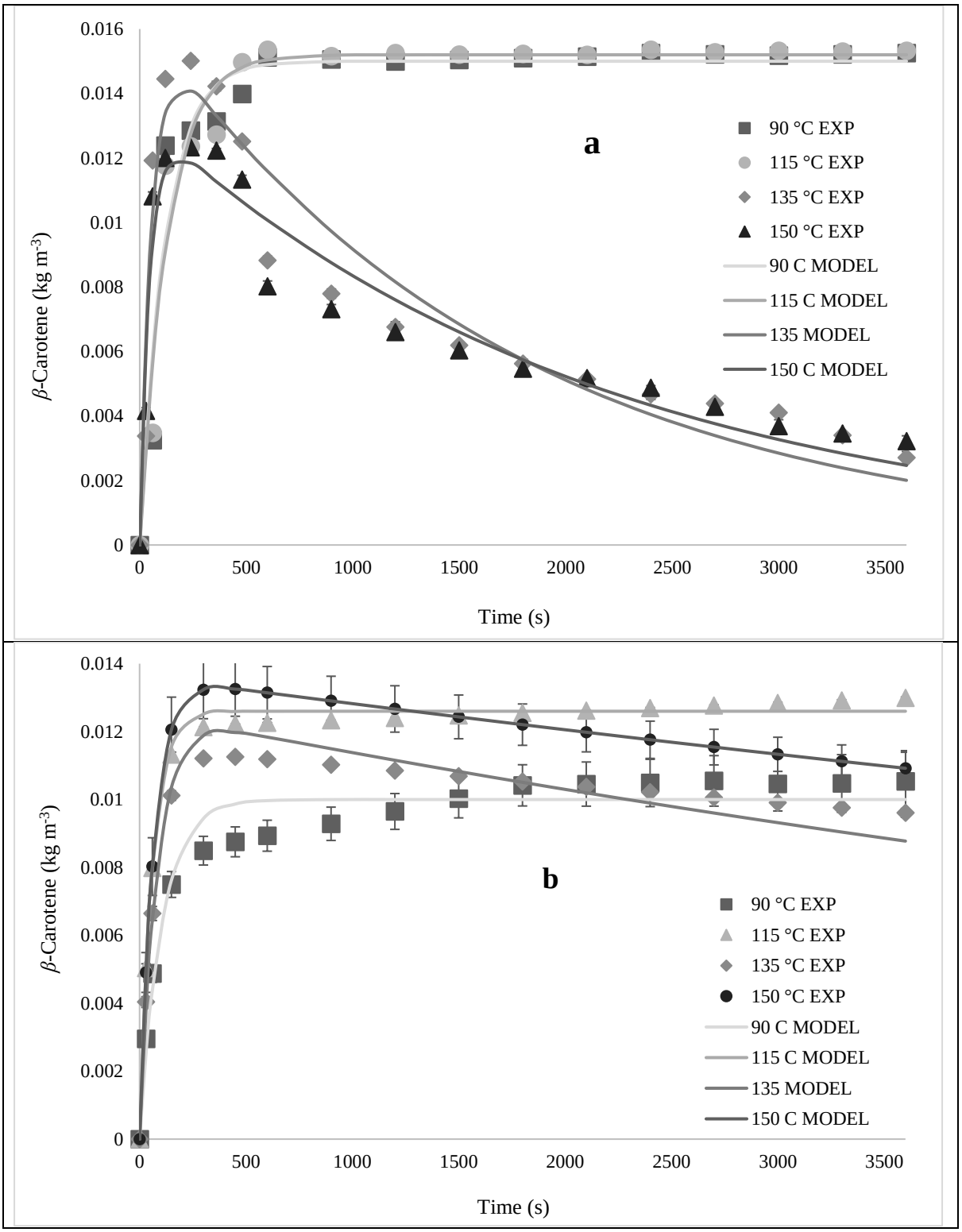
Reaction rate constants (min^{-1})	Temperatures (°C)				Activation Energy (kJ mol^{-1})	Goodness of fit	
	160	180	200	220		RSS	n
k_1	0.079±0.001	0.188±0.002	0.415±0.002	0.860±0.006	70.51		
k_2	0.148±0.001	0.330±0.002	0.689±0.005	1.356±0.010	65.52		
k_3	0.166±0.001	0.232±0.001	0.316±0.001	0.418±0.003	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: the model parameters were predicted by using average value of triplicate experimental dataset (n=3).

RSS – Sum of square of the residuals ($RSS = \sum_{i=1}^{n}([X_{optipred}] - [X_{exp}])$), where n is the number of data points, $[X_{exp}]$ the experimental result, and $[X_{optipred}]$ the optimized simulated result.

n – no. of datapoints model was evaluated.

727 # Figures


a
90 °C EXP
115 °C EXP
135 °C EXP
150 °C EXP
90 C MODEL
115 C MODEL
135 MODEL
150 C MODEL
β-Carotene (kg m-3)
Time (s)
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
0
500
1000
1500
2000
2500
3000
3500
b
90 °C EXP
115 °C EXP
135 °C EXP
150 °C EXP
90 C MODEL
115 C MODEL
135 MODEL
150 C MODEL
β-Carotene (kg m-3)
Time (s)
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
0
500
1000
1500
2000
2500
3000
3500

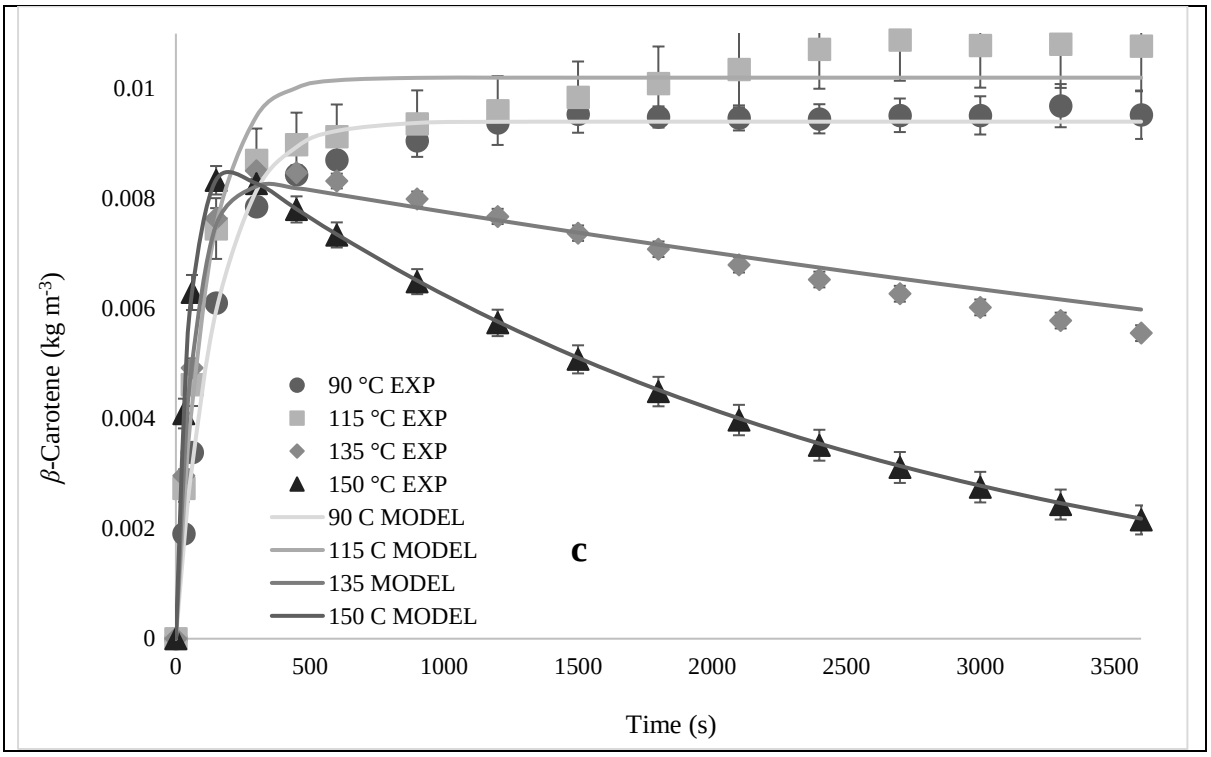


Figure 1: Extraction of *β-Carotene* from carrot powders at 90, 115, 135 and 150 °C into sunflower oil, Solid loading =20 kg/m^3, a) Particle size – 0.35 mm, b) Particle size – 0.75 mm, (c) Particle size – 1.40 mm. The points indicate experimental values of the concentration and the solid line represents 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).

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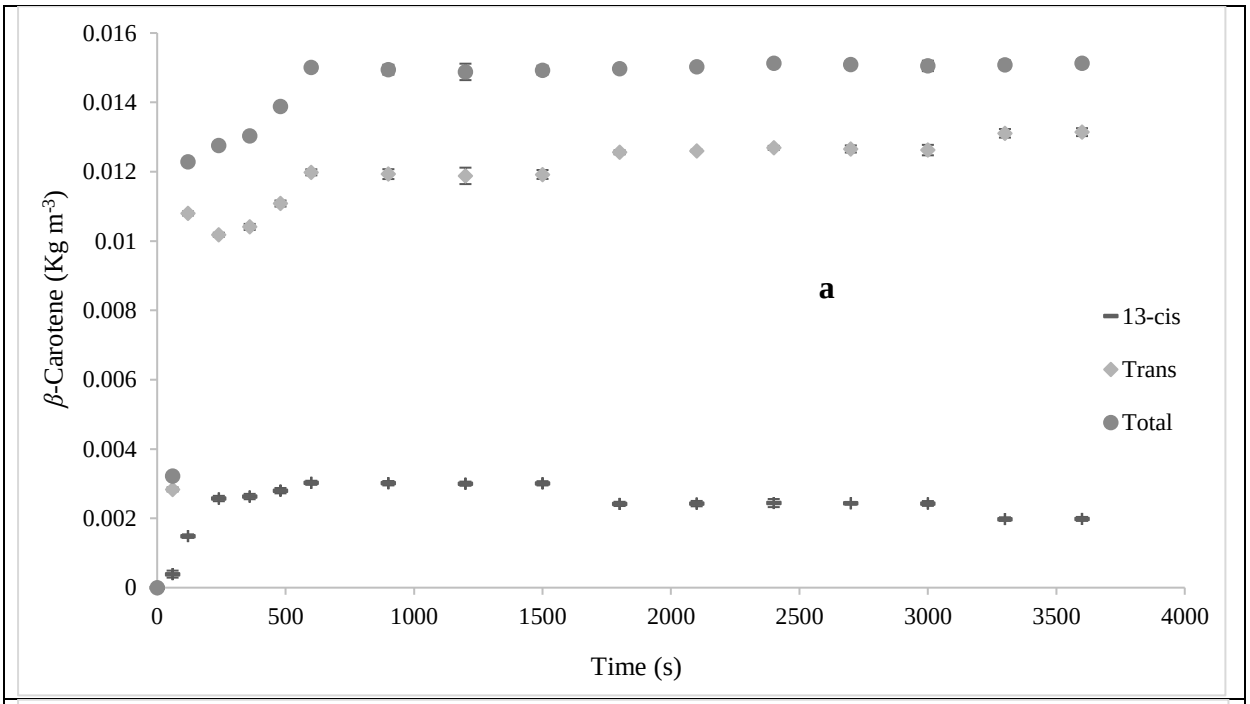
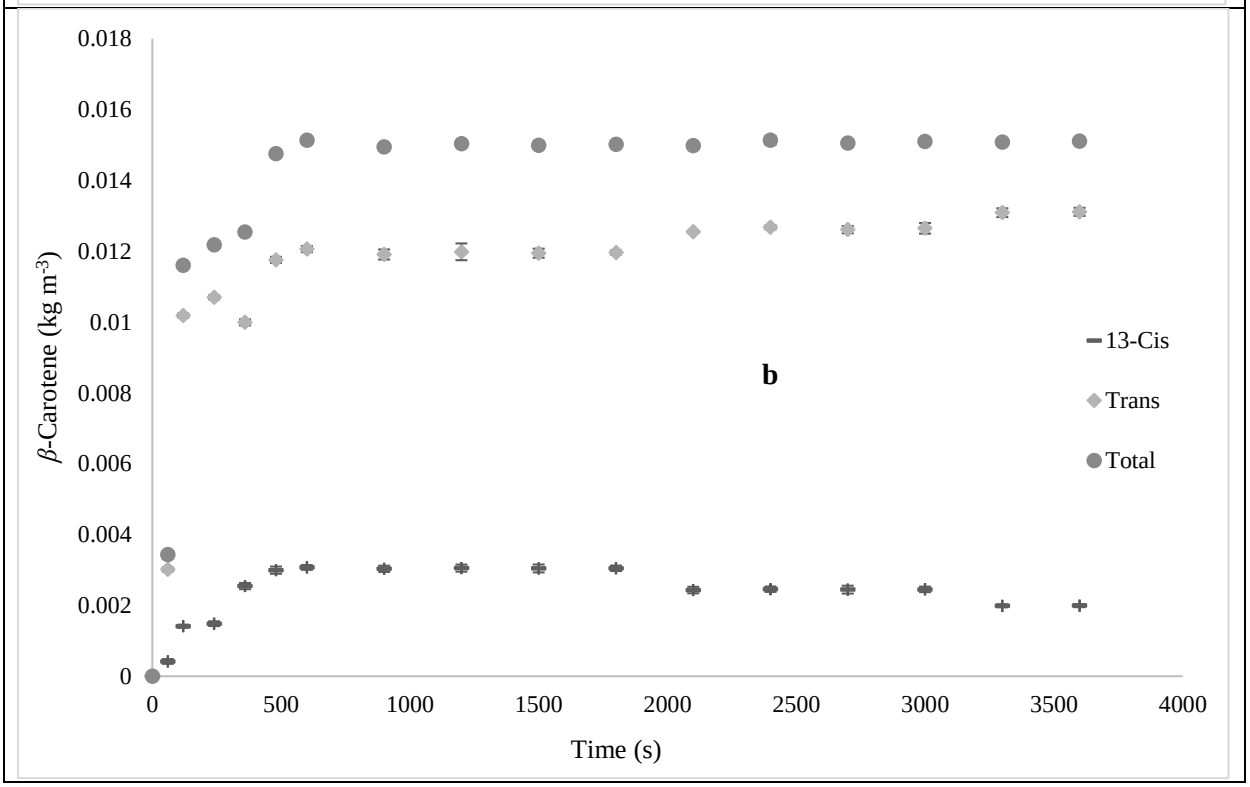

0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
β-Carotene (Kg m-3)
a
13-cis
Trans
Total
0
500
1000
1500
2000
2500
3000
3500
4000
Time (s)


0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
β-Carotene (kg m-3)
b
13-Cis
Trans
Total
0
500
1000
1500
2000
2500
3000
3500
4000
Time (s)

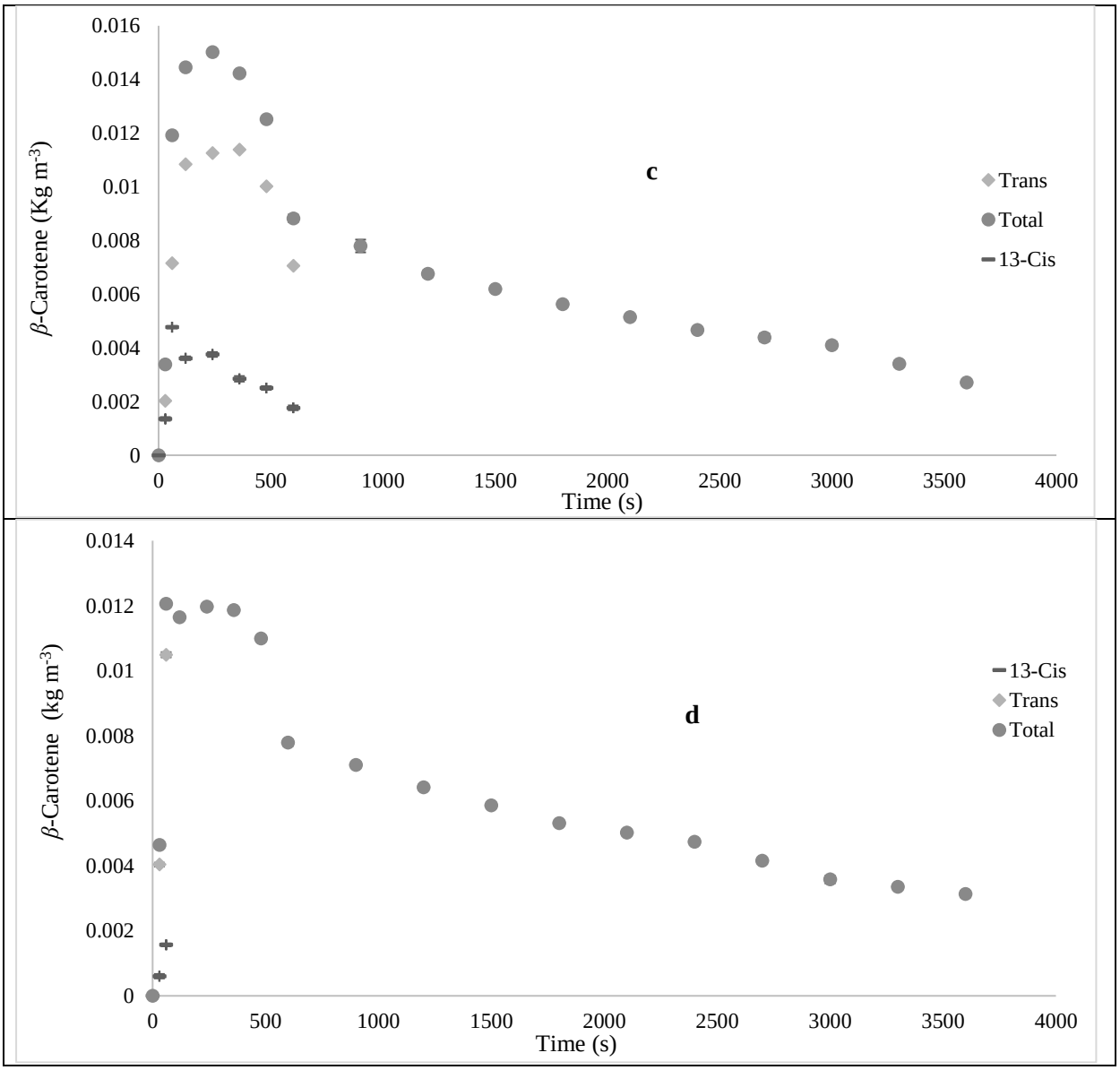


Figure 2: Composition of *β-Carotene* isomers, *trans* and 13-*cis*, in sunflower oil extract at different extraction temperatures (a) 90 °C, (b) 115 °C, (c) 135 and (d) 150 °C. It may be noted that 13-cis isomer was only observed in the initially stages of extraction at higher temperatures of 135, and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

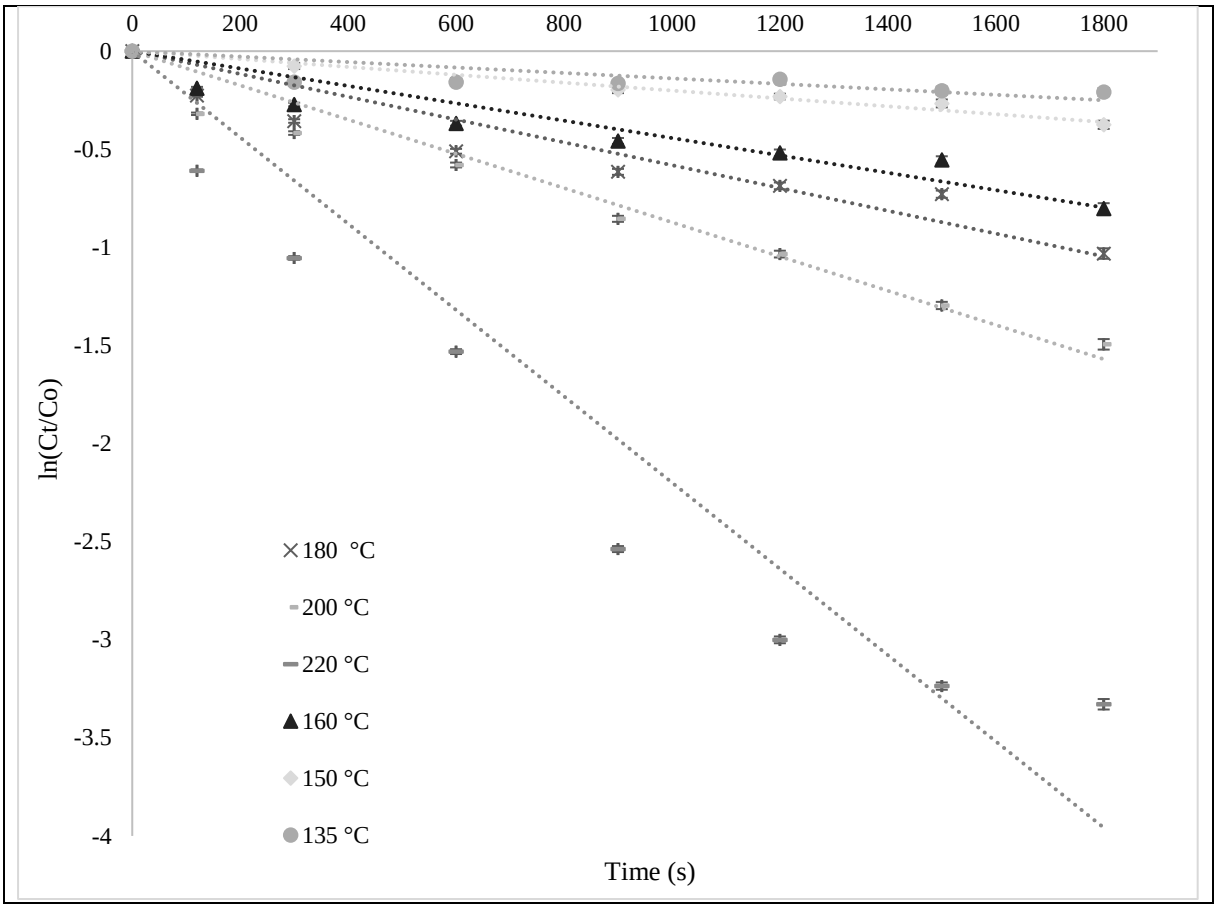


Figure 3: Degradation kinetics of *β-Carotene* in sunflower oil at different temperatures. Temperature range was selected to reflect normal frying and cooking conditions. Solid lines indicate first-order kinetic fit. The rate constant at different temperatures are reported in Table (3). Note: the model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

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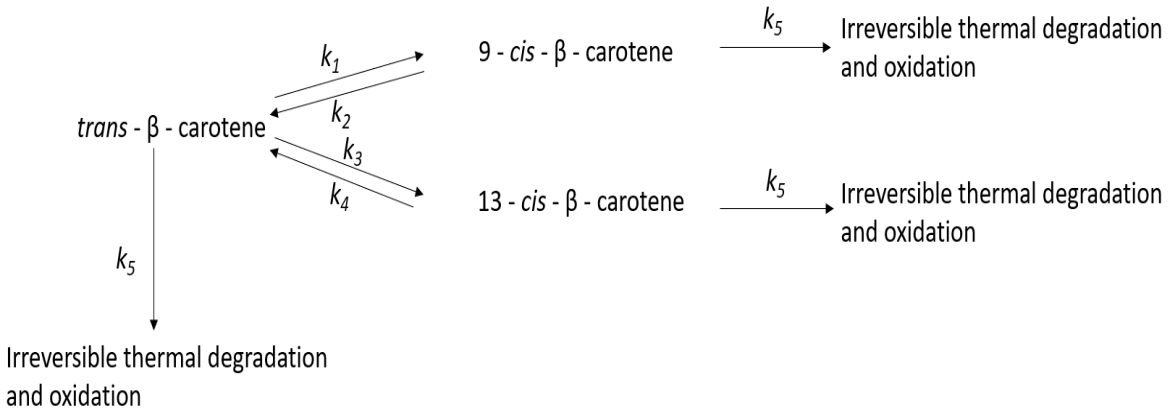

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736 Figure 4: Schematic representation of the reaction network which includes degradation and

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

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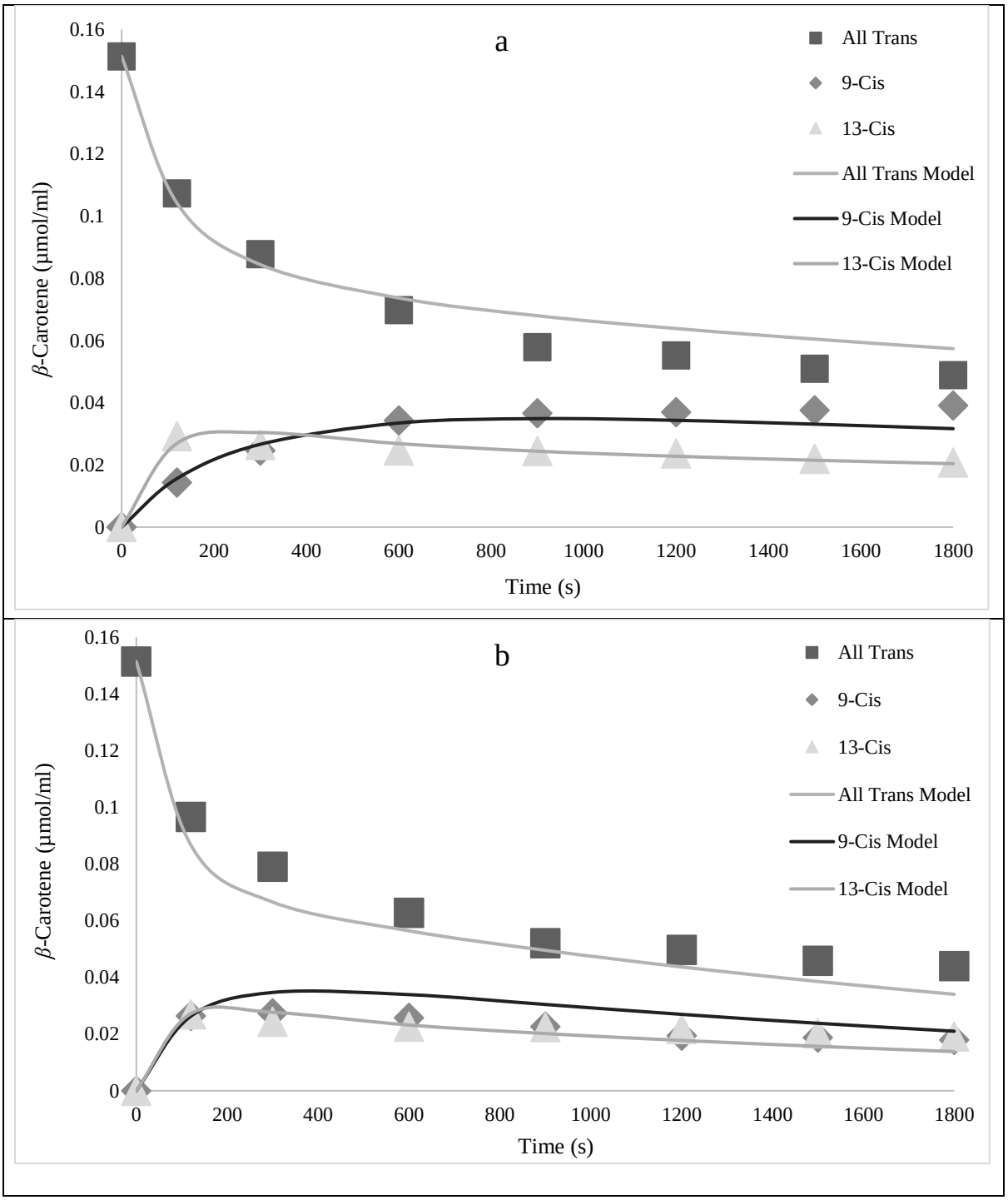
a
All Trans
9-Cis
13-Cis
All Trans Model
9-Cis Model
13-Cis Model
β-Carotene (µmol/ml)
Time (s)
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
200
400
600
800
1000
1200
1400
1600
1800
b
All Trans
9-Cis
13-Cis
All Trans Model
9-Cis Model
13-Cis Model
β-Carotene (µmol/ml)
Time (s)

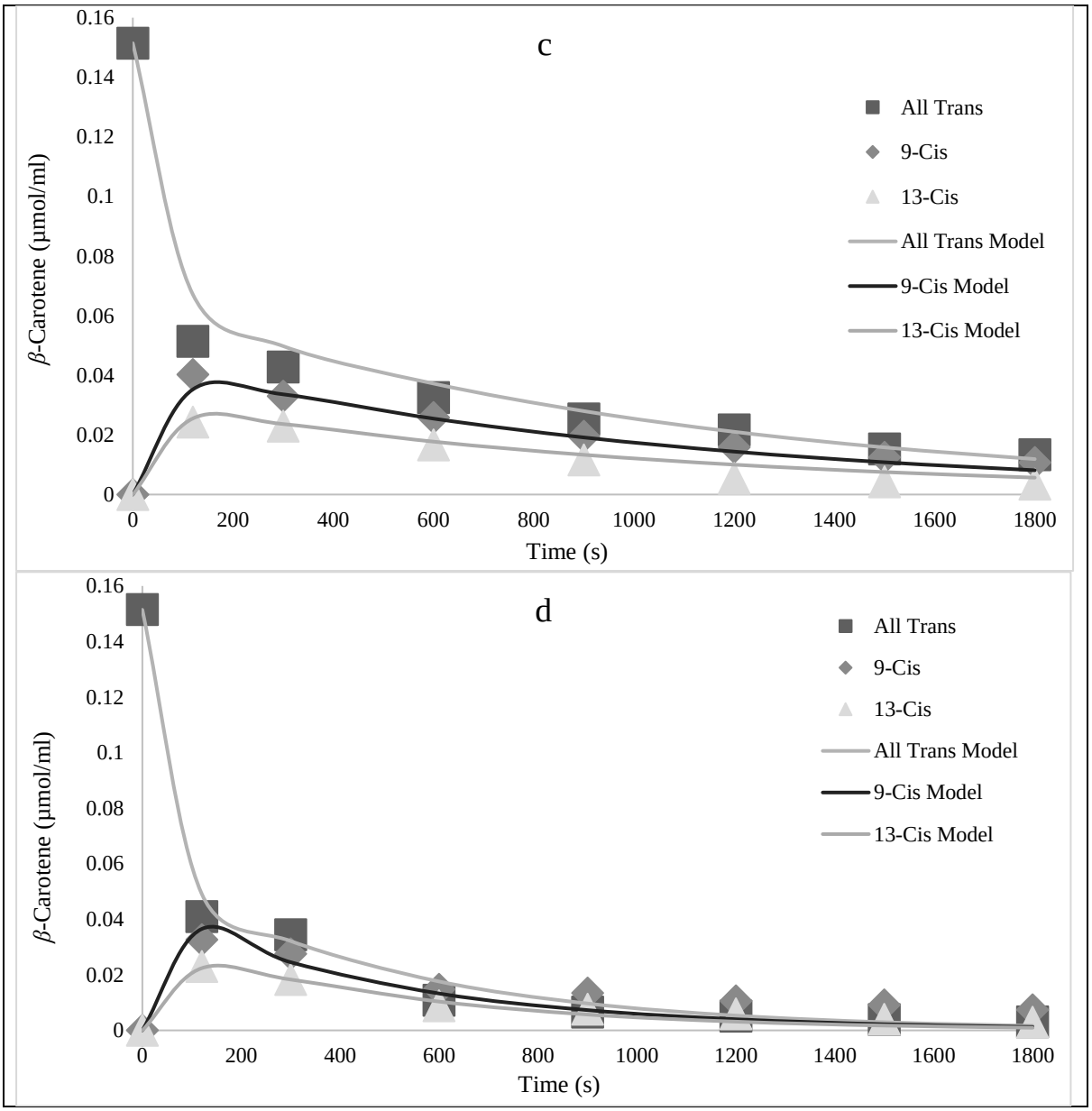

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Figure 5: Concentration of *β-Carotene* isomers during thermal degradation of trans-*β-Carotene* in sunflower oil at different temperatures (a) 160 °C, (b) 180 °C, (c) 200 °C, and (d) 220 °C. Solid lines passing through the experimental points are 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.

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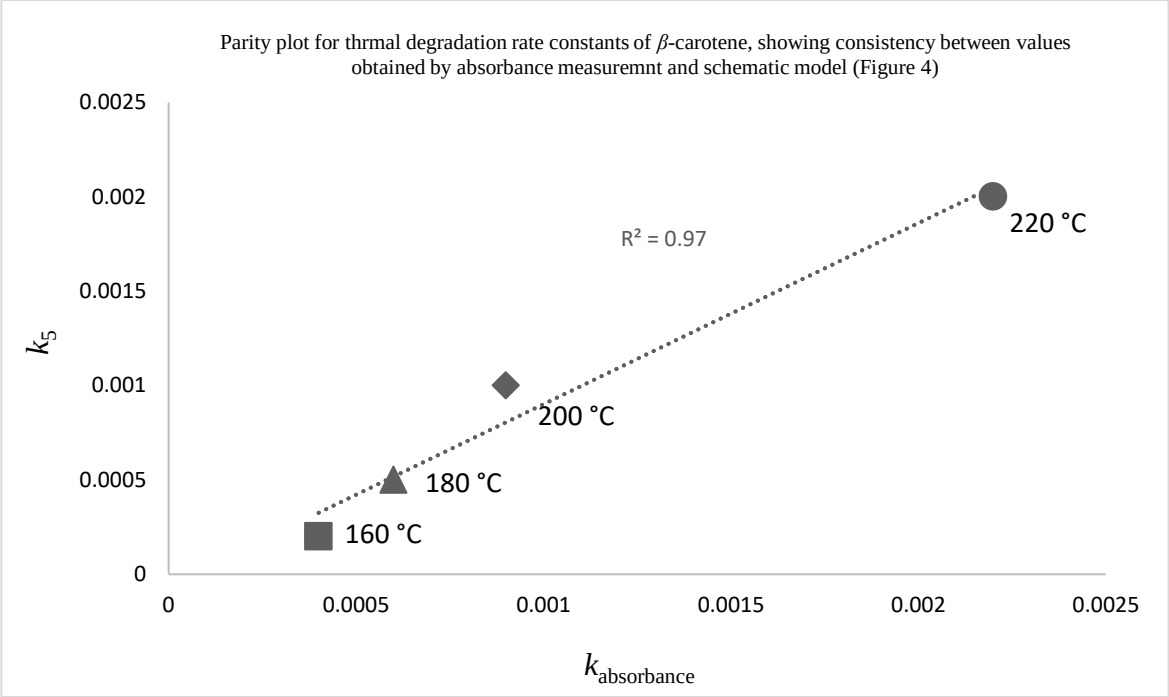

Figure 6: Parity plot showing consistency between the rate constant values obtained by absorbance measurement during *β-Carotene* degradation, and the value of k_5 estimated by Athena Visual Software.

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744 **supplemental Information**

Table 1S: Values of degradation rate constants in extract and solid phases for *β-Carotene* after fitting to eqn. (5) for Beta-carotene. 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

Note: the model parameters were predicted by using average value of triplicate experimental dataset (n=3).

k_1– Degradation rate constant in liquid phase (s^{-1})

k_2– Degradation rate constant in solid phase (s^{-1})

$(k_2)_{exp}$– Degradation rate constant in liquid phase experimental (s^{-1})

SSE – Sum of squared errors

R^2 – Co-efficient of determination

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

RMSE – Root mean squared error

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