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Carrot β-Carotene Degradation and Isomerization Kinetics during Thermal Processing in the Presence of Oil

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ABSTRACT: The effect of thermal processing (85−130 °C) on the stability and isomerization of β-carotene in both an olive oil/carrot emulsion and an olive oil phase enriched with carrot β-carotene was studied. During processing, degradation of total β-carotene took place. Initially, total β-carotene concentration decreased quickly, after which a plateau value was reached, which was dependent on the applied temperature. In the oil/carrot emulsion, the total β-carotene concentration could be modeled by a fractional conversion model. The temperature dependence of the degradation rate constants was described by the activation energy and was estimated to be 45.0 kJ/mol. In the enriched oil phase, less degradation took place and the results could not be modeled. Besides degradation, β-carotene isomerization was studied. In both matrices, a fractional conversion model could be used to model total isomerization and formation of 13-*Z*- and 15-*Z*-β-carotene. β-Carotene isomerization was similar in both the oil/carrot emulsion and enriched oil phase as the simultaneously estimated kinetic parameters (isomerization reaction rate constant and activation energy) of both matrices did not differ significantly. The activation energies of isomerization were estimated to be 70.5 and 75.0 kJ/mol in the oil/carrot emulsion and enriched oil phase, respectively.

KEYWORDS: *β-carotene, carrot, olive oil, degradation, isomerization, thermal processing, kinetics*

INTRODUCTION

The presence of fruits and vegetables is crucial in a healthy human diet because of their high nutritional value. In the specific case of carrots, the most important nutrient is β-carotene, which is a lipid-soluble carotenoid.[1] β-Carotene consists of a polyene system with 11 conjugated double bonds and a β-ring at each end of the chain. As a result of this specific chemical structure, β-carotene has some health-related properties.[2] First, it can act as a precursor of vitamin A, which plays an important role in vision, cell differentiation, organ development, etc. Furthermore, β-carotene has antioxidant activity and may consequently offer protection against cancer and cardiovascular diseases.[3,4] During processing, however, alterations in the chemical structure are possible as a result of isomerization and degradation.[5] In nature, β-carotene is mainly present as *all-E*-β-carotene, which is thermodynamically the most stable form. Nevertheless, some *Z*-isomers, which are not sterically hindered, are easily formed and are relatively thermodynamically stable.[2] In processed carrot products, 9-*Z*-, 13-*Z*-, and 15-*Z*-β-carotene are the most common *Z*-isomers.[6] Unfortunately, the *Z*-isomers have a decreased provitamin A activity and an altered antioxidant activity.[5] Moreover, a lower bioavailability of 9-*Z*- and 13-*Z*-β-carotene compared to the *all-E* form was shown in some studies.[7,8] Next to isomerization, also degradation reactions of *all-E*-β-carotene as well as of *Z*-β-carotene result in loss of the health-related properties.[9]

Therefore, kinetic studies of β-carotene degradation and isomerization are important in the context of predicting β-carotene changes during processing in order to develop safe food products with a high nutritional value. Thermal processing, including pasteurization and sterilization, is the most commonly used method for food preservation. In a number of kinetic studies, β-carotene degradation during thermal processing has been described by a first-order reaction, but kinetic parameters differed greatly between different studies. Activation energies ranging from 20 to 171 kJ/mol have been reported, depending on the β-carotene source, the reaction medium, and the processing conditions.[10−12] Less information is available on the kinetics of β-carotene isomerization, although formation of *Z*-isomers from *all-E*-β-carotene has repeatedly been shown during thermal processing.[13−15]

Next to high temperature, the presence of lipids is another important factor for β-carotene isomerization and degradation and its health benefits. On the one hand, the positive effect of lipids on β-carotene bioavailability has been suggested.[16] On the other hand, oxidation of fatty acids results in the formation of free radicals, which may react with β-carotene.[17] In this way, the presence of lipids may also influence β-carotene degradation and isomerization reactions and related kinetics. As lipids are often added during processing, investigation of the effect of lipids on β-carotene degradation and isomerization kinetics is necessary.

Until now, the kinetics of thermal β-carotene degradation in the presence of oil have been described only in pure oils and not in real carrot systems. Furthermore, detailed kinetic studies of thermal β-carotene isomerization in the presence of oil (in pure oils as well as in real carrot systems) are completely lacking.

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The objective of the present study was to investigate degradation and isomerization kinetics of β-carotene in the presence of oil during thermal treatments. Moreover, it was examined if the kinetics of β-carotene degradation and isomerization are different when β-carotene is present in a matrix and still needs to be transferred to the oil phase in comparison with β-carotene that is already dissolved in an oil phase. Therefore, β-carotene degradation and isomerization were studied in an oil/carrot emulsion (in which carrot cells are still present), on the one hand, and in an oil phase enriched with carrot β-carotene (in which no carrot cells are present), on the other hand.

MATERIALS AND METHODS

Sample Preparation. Fresh carrots (*Daucus carota* cv. Nerac) were purchased in a local shop in Belgium and were stored at 4 °C. Carrots were peeled, cut into pieces, and blended with water (1:1) for 1 min in a kitchen blender.

To prepare a stable olive oil/carrot emulsion, 5% (w/w) extra virgin olive oil was added to the carrot puree and the mixture was further high-pressure homogenized (Panda 2K, Gea Niro Soavi, Mechelen, Belgium) at 10 MPa.

To obtain an oil phase enriched with carrot β-carotene, β-carotene was transferred from the carrot puree to the oil phase. Carrot puree was first high-pressure homogenized (Panda 2K, Gea Niro Soavi) at a higher pressure (100 MPa) to maximally break the carrot cells, thereby enhancing the release of β-carotene from the cells. Afterward, extra virgin olive oil (16% w/w) was added to the homogenized carrot puree, and the mixture was incubated at room temperature (21 °C) for 5 h while rotating end-over-end. Finally, the oil phase enriched with carrot β-carotene was separated by centrifugation for 15 min at 18900*g* and 4 °C.

Thermal Treatments. Thermal treatments were carried out in a temperature-controlled oil bath. Closed stainless steel reactor tubes (external diameter = 12 mm, internal diameter = 5 mm, length = 100 mm) were completely filled with oil/carrot emulsion or enriched oil to make the headspace negligible and minimize the contact with oxygen. The temperature profile in one tube was measured using a type T thermocouple connected to a thermocouple box (TR9216, Ellab, Hilleroed, Denmark) and a CMC-92 data acquisition system (Ellab, Hilleroed, Denmark). Before the actual heat treatment, the tubes were first equilibrated for 10 min at 40 °C to have a reproducible dynamic heating phase. After the heat treatment, the samples were cooled in ice water, removed from the reactor tubes, frozen in liquid nitrogen, and stored at −80 °C until β-carotene analysis. Samples were stored for maximally 5 days to prevent changes in β-carotene concentration due to storage.

Thermal treatments were performed at 85, 100, 115, and 130 °C. Treatment times between 0 and 120 min (85 °C) or between 0 and 60 min (other temperatures) were applied. The sample of 0 min equals the sample after equilibration at 40 °C.

β-Carotene Isomer Analysis. β-Carotene was extracted from the oil/carrot emulsion or enriched oil phase using an extraction procedure based on the method described by Sadler et al.[18] with some modifications. To 1 g of emulsion or oil phase were added 1 g of $CaCl_2 \cdot 2H_2O$ and 50 mL of extraction solution (50% hexane, 25% acetone, 25% ethanol, 0.1% BHT). The mixture was stirred for 20 min at 4 °C, and after the addition of 15 mL of reagent grade water, the mixture was stirred for another 10 min at 4 °C. The organic phase containing β-carotene was collected and filtered (Chromafil PET filters, 0.20 μm pore size, 25 mm diameter).

all-E-β-Carotene and its *Z*-isomers were separated using an HPLC system equipped with a reversed phase C_{30} column (5 μm × 250 mm × 4.6 mm, YMC Europe, Dinslaken, Germany) and a diode array detector (Agilent Technologies 1200 series, Diegem, Belgium). During analysis, the autosampler and column were kept at 4 and 25 °C, respectively. Linear gradient elution was applied. In 20 min, the gradient was built up from 81% MeOH, 15% methyl-*tert*-butyl ether, and 4% reagent grade water to 41% MeOH, 55% methyl-*tert*-butyl ether, and 4% reagent grade water at a flow rate of 1 mL/min. The absorbance of the different isomers was measured at 450 nm (= maximal absorbance of *all-E*-β-carotene). Identification of the β-carotene isomers was based on comparison of their retention times and spectral characteristics with those of standards of the different β-carotene isomers. The concentration of *all-E*-β-carotene and its *Z*-isomers was quantified using calibration curves of *all-E*-β-carotene, 15-*Z*-β-carotene, 13-*Z*-β-carotene, and 9-*Z*-β-carotene standards, respectively (CaroteNature, Lupsingen, Switzerland). During the whole procedure, light was excluded as much as possible to avoid β-carotene degradation due to contact with light. Extractions were performed in duplicate.

Data Analysis. In a previous study, isomerization of β-carotene in carrot puree has been described by a fractional conversion model, which is characterized by first-order kinetics until a plateau value is reached.[14] Similarly, the same model could be used to describe the thermal isomerization and degradation of β-carotene in an oil/carrot emulsion or in an enriched oil phase. The fractional conversion model can be described by the following differential equation (eq 1)

$$dC = -k(C - C_f)\, dt \tag{1}$$

or in integrated form as eq 2

$$C = C_f + (C_0 - C_f)\exp(-kt) \tag{2}$$

where C represents the β-carotene isomer content, C_f the β-carotene isomer content in equilibrium state, C_0 the initial β-carotene isomer content, k the reaction rate constant (min^{-1}), and t the reaction time (min).

The temperature dependence of the reaction rate constants is quantified by the activation energy E_a (J/mol) and is expressed by the Arrhenius equation according to eq 3, in which k is the reaction rate constant at temperature T (K), k_{ref} is the reaction rate constant at a reference temperature (T_{ref}) of 383 K (110 °C), and R is the universal gas constant (8.314 J/mol·K).

$$k = k_{ref}\exp\left[\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \tag{3}$$

For estimation of the kinetic parameters, a two-step regression method assuming isothermal conditions was applied at first in which the C_f values at all process temperatures were estimated using eq 2. Second, k_{ref} and E_a were estimated simultaneously using a one-step regression model. Therefore, the Arrhenius equation (eq 3) was substituted in the fractional conversion model (eq 1), resulting in eq 4. $C_{f(T)}$ represents a linear equation, describing the temperature dependence of the equilibrium concentration values, which were estimated from the two-step regression under isothermal conditions. Moreover, the actual dynamic temperature conditions were included in the kinetic data analysis to obtain a more accurate prediction.

$$dC = -(C - C_{f(T)})k_{ref}\exp\left[\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T_{(t)}}\right)\right] dt \tag{4}$$

To estimate the kinetic parameters, the sum of squares of errors was minimized using nonlinear regression (statistical software package SAS, version 9.3, Cary, NC, USA). The quality of fit was evaluated by visual inspection of residual plots and by plotting the experimental values against the predicted values (parity plot).

To evaluate the statistical confidence of the jointly estimated parameters, 90% joint confidence regions (JCR) were constructed using eq 5. The JCR takes into account the correlation between the simultaneously estimated parameters k_{ref} and E_a.

$$SSQ \le SSQ(\theta)\left\{1 + \frac{p}{m-p}F(p,\ m-p,\ 1-\varphi)\right\} \tag{5}$$

$SSQ(\theta)$ represents the error sum of squares associated with the least-squares estimate θ, p the number of parameters estimated

simultaneously, m the number of observations, and F the upper $1 - \varphi$ quantile for an F distribution with p and $m - p$ degrees of freedom.[19]

RESULTS AND DISCUSSION

β-Carotene Degradation. The total β-carotene concentration in the oil/carrot emulsion, expressed relative to the initial total concentration as a function of time for the different temperatures studied, is given as single data points in Figure 1.

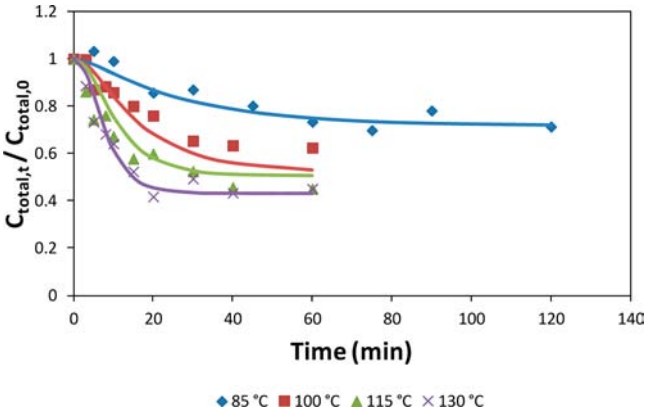

Figure 1. Relative total β-carotene concentration after thermal treatments of an olive oil/carrot emulsion, modeled by one-step regression using a fractional conversion model. The single data points represent the experimental values, whereas the full lines represent the values predicted by the kinetic model.

At all temperatures, the total concentration initially decreased, after which a plateau value was reached. β-Carotene degradation is mainly attributed to oxidation. Complete consumption of the oxygen in the closed reactor tubes used in this study probably protected β-carotene against further degradation. Degradation already took place at the lowest temperature studied but became more pronounced as the temperature increased. At 85 °C, for example, 71.3% of the initial total β-carotene concentration remained after 120 min. At 130 °C, on the other hand, the total concentration already decreased to this percentage after <8 min and decreased further to ±45.1% after 60 min. To estimate the kinetic parameters, one-step regression assuming a fractional conversion model and taking into account the dynamic temperature conditions was used. The model fits the experimental data fairly well, and a good correlation between the experimental and predicted values was observed. For total β-carotene degradation, a rate constant at 110 °C of 0.10 ± 0.01 min^{-1} and an activation energy of 45.0 ± 8.6 kJ/mol were found. Chen and Huang[20] reported a similar activation energy (39 kJ/mol) for total β-carotene degradation of an *all-E*-β-carotene standard dissolved in hexane. On the other hand, a broad range of activation energies ranging from 20 to 171 kJ/mol has been reported in the literature as mentioned in the Introduction. It should be mentioned that a lot of different matrices, reaction media, and heating conditions were used in the different studies, which makes comparison of the obtained kinetic parameters difficult. In the kinetic study of Lemmens et al.,[14] no oxidation was observed during thermal treatments of a carrot puree. The presence of oil, which is reported to act as a prooxidant,[21] can explain the observed differences between the carrot puree and the oil/carrot emulsion.

In pure olive oil enriched with β-carotene, however, less degradation was observed (results not shown). Below 115 °C, almost no total β-carotene degradation took place. Only at temperatures above 115 °C could similar profiles (i.e., initial degradation followed by a plateau) as in the oil/carrot emulsion be found, but the end concentrations in the enriched oil remained higher compared to the ones in the emulsion (73.9% compared to 45.1% at 115 °C and 61.3% compared to 45.1% at 130 °C). This might be explained by competition between fatty acids and β-carotene for oxidation by oxygen.[21] In enriched oil, the fatty acids that are present in excess might be primarily oxidized. In the emulsion, on the other hand, in which the fatty acid concentration is much lower, the β-carotene molecules will probably be more rapidly attacked. As β-carotene degradation in the enriched oil phase was taking place at only some of the temperatures that were studied, the results of degradation could not be modeled using a one-step regression approach.

β-Carotene Isomerization. Next to total β-carotene degradation, β-carotene isomerization during thermal treatments was studied. In analogy with Lemmens et al.,[14] the data were transformed to rule out the problem of changing β-carotene extractability as a function of treatment time and temperature. For each temperature−time point, the contribution of β-carotene isomers to the total β-carotene concentration was calculated as follows:

$$\frac{C_{\text{isomer } \beta\text{-carotene},\ t}}{C_{(\beta\text{-carotene}+Z\text{-isomers}),\ t}}$$

It was assumed that isomerization was limited to the formation of 13-*Z*-, 15-*Z*-, and 9-*Z*-β-carotene, which are the three *Z*-isomers that could be identified in the study. This assumption is acceptable as it is described in the literature that these three isomers are the main *Z*-isomers of β-carotene.[6]

In Figure 2A, the contribution of the sum of β-carotene *Z*-isomers in the oil/carrot emulsion, as a function of treatment time and for the different temperatures studied, is given as single data points. Isomerization took already place at rather low temperature (85 °C), and more isomers were formed as time or temperature increased. After a certain time, a constant value was reached. As isomerization is known to be a reversible reaction,[22] the isomerization reaction from *all-E*-β-carotene to *Z*-isomers is in equilibrium with the reverse reaction at the moment the plateau is reached. Similar to total β-carotene degradation, one-step regression assuming a fractional conversion model and taking into account the dynamic temperature conditions was used to model the experimental data. The fractional conversion model could fit the experimental data properly. The obtained kinetic parameters are reported in Table 1. As a consequence of expressing the data as contributions, the same kinetic parameters for the isomerization of *all-E*-β-carotene and for the formation of the sum of *Z*-isomers were found. In the kinetic study of Lemmens et al.,[14] in which carrot puree was thermally treated in the absence of oil, the reaction rate constant for thermal isomerization of *all-E*-β-carotene was much lower (0.035 min^{-1} compared to 0.099 min^{-1}). In other words, the presence of oil accelerates the isomerization of β-carotene in carrot puree. This may be explained by solubilization of β-carotene crystals in the oil droplets, which makes β-carotene more susceptible to isomerization. It has already been stated in the literature that solubilization of carotenoids is necessary to induce isomerization.[15] Furthermore, the solubilization of β-carotene probably also resulted in a higher activation energy (70.5 kJ/

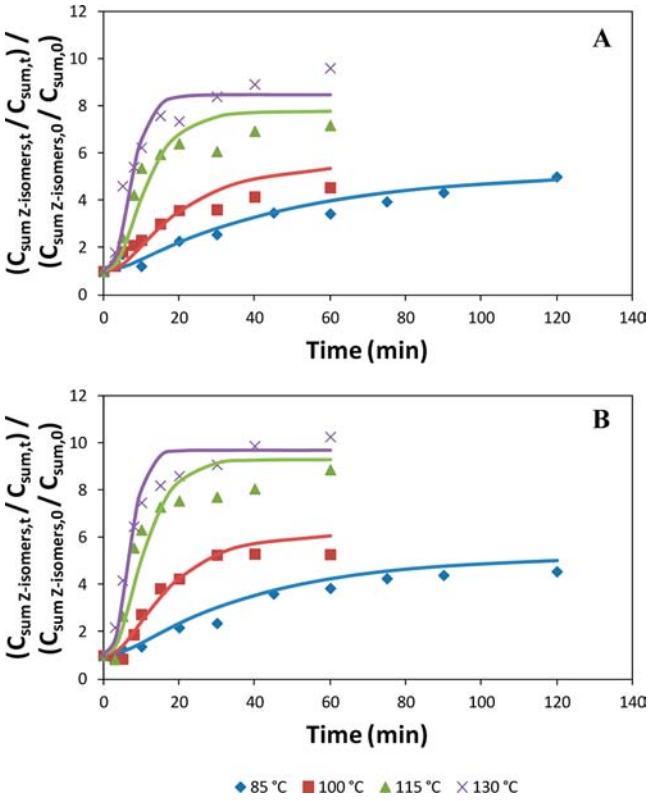

Figure 2. Formation of total β-carotene *Z*-isomers during thermal treatments of an olive oil/carrot emulsion (A) or olive oil phase enriched with carrot β-carotene (B), modeled by one-step regression using a fractional conversion model. The single data points represent the experimental values, whereas the full lines represent the values predicted by the kinetic model.

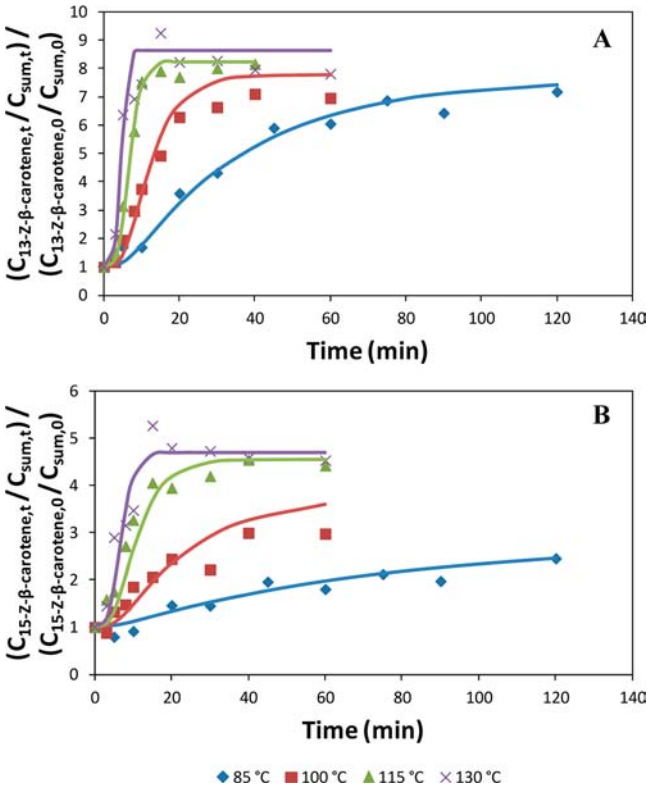

Figure 3. Formation of 13-*Z*-β-carotene (A) and 15-*Z*-β-carotene (B) during thermal treatments of an olive oil/carrot emulsion, modeled by one-step regression using a fractional conversion model. The single data points represent the experimental values, whereas the full lines represent the values predicted by the kinetic model.

mol) compared to the carrot puree without added oil (10.5 kJ/mol).

To study the isomerization of β-carotene during thermal treatments of an oil/carrot emulsion in more detail, the formation of 13-*Z*- and 15-*Z*-β-carotene was also modeled individually (see Figure 3). Here also, the fractional conversion model taking into account the dynamic temperature conditions could fit the data quite well, and the kinetic parameters are listed in Table 1. When isothermal conditions during the thermal treatments were assumed, the estimated kinetic parameters (not shown) were largely lower (up to 6 and 2.3 times for the reaction rate constants at reference temperature and activation energies, respectively) compared to the reported ones. This implies that the dynamic heating phase has a large contribution to the isomerization reactions. In the curves, this effect is clearly visible in the lag phase at the beginning of the treatments. After the initial lag phase, the isomers were quickly formed and reached an equilibrium. The formation of 13-*Z*-β-carotene proceeded more quickly compared to the formation of 15-*Z*-β-carotene, which is reflected in the higher k_{ref} value for the former. This observation can be explained by the lower rotational barrier for conversion of *all-E*-β-carotene to 13-*Z*- compared to 15-*Z*-β-carotene.[23] In the untreated emulsion, 9-*Z*-β-carotene was not detected, and as it was formed only at high temperatures (115 − 130 °C) and after long treatment times (60 min at 115 °C or 30 min at 130 °C), the formation of 9-*Z*-β-carotene could not be modeled.

In Table 2, the percentages of the individual isomers to the total β-carotene concentration are given for the untreated oil/carrot emulsion and for the oil/carrot emulsion after thermal treatments of 60 min at different temperatures. In untreated emulsion, on average 94.5% of all β-carotene was present in the *all-E* form, which is thermodynamically the most stable form. After a thermal treatment of 60 min, the percentage of *all-E*-β-carotene was decreased, with the decrease being larger at higher process temperatures. After 60 min at 130 °C, for example, only 51.1% of the total β-carotene concentration was still present as

Table 1. Kinetic Parameters ± Standard Error (T_{ref} = 110 °C) for the Modeling of the Thermal Isomerization of Different β-Carotene Isomers Estimated by One-Step Regression Analysis Using a Fractional Conversion Model

	olive oil/carrot emulsion		olive oil enriched with carrot β-carotene	
	E_a (kJ/mol)	k_{ref} (min^{-1})	E_a (kJ/mol)	k_{ref} (min^{-1})
isomerization of *all-E*-β-carotene	70.5 ± 7.4	0.099 ± 0.01	75.0 ± 7.3	0.14 ± 0.01
formation of sum of *Z*-isomers	70.5 ± 7.4	0.099 ± 0.01	75.0 ± 7.3	0.14 ± 0.01
formation of 13-*Z*-β-carotene	120 ± 8.6	0.39 ± 0.05	137 ± 6.7	0.48 ± 0.05
formation of 15-*Z*-β-carotene	98.4 ± 8.7	0.11 ± 0.01	69.5 ± 7.5	0.17 ± 0.02

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Table 2. Mean Concentration (± Standard Deviation), Expressed as Percentage of the Total β-Carotene Concentration, of Different β-Carotene Isomers Detected in Untreated Olive Oil/Carrot Emulsion and Untreated Olive Oil Enriched with Carrot β-Carotene and in Olive Oil/Carrot Emulsion and Olive Oil Enriched with Carrot β-Carotene after Thermal Treatments of 60 min at Different Process Temperatures

	untreated	85 °C	100 °C	115 °C	130 °C
Olive Oil/Carrot Emulsion					
all-E-β-carotene	94.5 ± 0.6	78.5 ± 1.4	72.9 ± 0.9	63.5 ± 1.1	51.1 ± 5.5
sum *Z*-isomers	5.5 ± 0.6	21.5 ± 1.4	27.1 ± 0.9	36.5 ± 1.1	48.9 ± 5.5
13-*Z*-β-carotene	2.5 ± 0.1	14.6 ± 0.8	18.0 ± 0.5	18.7 ± 0.1	19.6 ± 0.5
15-*Z*-β-carotene	3.0 ± 0.6	6.9 ± 0.6	9.1 ± 0.4	11.4 ± 0.2	11.7 ± 0.2
9-*Z*-β-carotene	nd[a]	nd	nd	6.4 ± 1.0	17.6 ± 4.8
Olive Oil Enriched with Carrot β-Carotene					
all-E-β-carotene	96.3 ± 0.5	83.3 ± 0.7	78.1 ± 0.4	72.0 ± 0.2	65.2 ± 0.2
sum *Z*-isomers	3.7 ± 0.5	16.7 ± 0.7	21.9 ± 0.4	28.0 ± 0.2	34.8 ± 0.2
13-*Z*-β-carotene	1.2 ± 0.2	10.6 ± 0.3	14.2 ± 0.5	14.3 ± 0.4	13.0 ± 0.2
15-*Z*-β-carotene	0.9	3.5 ± 0.3	5.2 ± 0.1	7.3 ± 0.1	6.4 ± 0.3
9-*Z*-β-carotene	1.6 ± 0.5	2.6 ± 0.6	2.5 ± 0.2	6.4 ± 0.3	15.4 ± 0.7

[a]nd, not detected.

all-E-β-carotene. Similarly, Colle et al.[24] found that lycopene in an olive oil/tomato emulsion is present as a 50:50 mixture of *all-E*-lycopene and *Z*-lycopene after a thermal treatment of 60 min at 130 °C. Logically, the decrease in the percentage of *all-E*-β-carotene in all samples was associated with a simultaneous increase in the percentage of total *Z*-isomers. In the untreated oil/carrot emulsion, 15-*Z*-β-carotene was present in the highest concentration followed by 13-*Z*-β-carotene. 9-*Z*-β-Carotene was not detected. Nevertheless, the concentration of all individual *Z*-isomers was very low (≤3% of total β-carotene concentration). After the thermal treatments, 13-*Z*-β-carotene was formed in the highest amount, followed by 15-*Z*- and 9-*Z*-β-carotene, except for the treatment at 130 °C, at which 9-*Z*-β-carotene became more important than 15-*Z*-β-carotene after long treatment times. Similarly, Imsic et al.[25] found that 13-*Z*-β-carotene was predominantly formed in carrots after boiling for 15 min, followed by 15-*Z*-β-carotene and with 9-*Z*-β-carotene being the least abundant isomer. After 60 min at 85 °C, 67.9% of all *Z*-isomers in the oil/carrot emulsion was present as 13-*Z*-β-carotene, whereas 15-*Z*-β-carotene accounted for 32.1% of the total *Z* concentration and 9-*Z*-β-carotene was still not formed. A similar distribution was reached after 60 min at 100 °C. At 115 °C, a decrease in the percentage of 13-*Z*-β-carotene to the total *Z* concentration and a simultaneous increase in the percentage of 9-*Z*-β-carotene to the total *Z* concentration was observed. A further increase in the percentage of 9-*Z*-β-carotene to 36.0% of the total *Z* concentration was observed at 130 °C, which was accompanied by decreases in the percentages of 13-*Z*- and 15-*Z*-β-carotene to 40.1 and 23.9% of the total *Z* concentration, respectively. In summary, it can be stated that 13-*Z*-β-carotene is the most important *Z*-isomer formed at lower temperatures, whereas 9-*Z*-β-carotene becomes more and more important at higher temperatures. In the study of Marx et al.,[15] it has also been shown that 9-*Z*-β-carotene is only formed at more severe treatment conditions.

In a previous study of Knockaert et al.,[26] the higher increase in the concentration of 13-*Z*-β-carotene compared to 15-*Z*- and 9-*Z*-β-carotene after a thermal pasteurization at 90 °C ($^{10\ °C}P_{90\ °C}$ = 10 min) of an olive oil/carrot emulsion has been shown. After the treatment, the formation of total *Z*-isomers and of 13-*Z*- and 15-*Z*-β-carotene individually was 19.0, 10.7, and 4.5% of the total β-carotene concentration, respectively. On the basis of the kinetic model of the present study, the formation of total *Z*-isomers and of 13-*Z*- and 15-*Z*-β-carotene individually is estimated to be 15.9, 9.9, and 4.7% of the total β-carotene concentration, respectively. The good agreement between the experimental and predicted values shows that the kinetic model can be used for adequate estimation of β-carotene isomerization during thermal processing of an olive oil/carrot emulsion.

For formation of total β-carotene *Z*-isomers (Figure 2B) and of 13-*Z*- and 15-*Z*-β-carotene (Figure 4) during thermal treatments of an enriched olive oil phase, similar graphs as for the olive oil/carrot emulsion were obtained. Furthermore, the same type of modeling could be used, and the estimated kinetic parameters are reported in Table 1. Qiu et al.[27] reported a slightly lower activation energy (59.0 kJ/mol) for the decay of β-carotene in soybean oil during thermal processing. In the study of Qiu et al.,[27] however, the combined effect of β-carotene isomerization and degradation was studied. Furthermore, another type of oil was used.

In contrast to the oil/carrot emulsion, 9-*Z*-β-carotene was also detected in the untreated oil samples. However, formation of 9-*Z*-β-carotene during thermal treatments occurred only at a temperature of 115 °C or higher, and no equilibrium was reached within the time range that was studied. Therefore, the formation of 9-*Z*-β-carotene was not modeled. In the oil sample thermally treated at 130 °C, a decrease in the contribution of 13-*Z*- and 15-*Z*-β-carotene was observed after 60 min. Similarly, a small decrease in the contribution of 13-*Z*-β-carotene after 60 min at 115 °C was observed in the oil/carrot emulsion. As the decrease in the contribution of the isomers became significant only after a long treatment at a very high process temperature, more specifically at conditions that fall outside the relevant industrial process conditions, these data were removed from the data set. Consequently, the model and the kinetic parameters estimated in this study are valid only under the tested conditions. Further research, including higher temperatures and longer treatment times, is necessary to be able to model the degradation of 13-*Z*- and 15-*Z*-β-carotene during thermal treatments of an olive oil/carrot emulsion or enriched olive oil phase. In the study of Achir et al.,[11] an immediate increase in the concentration of 13-*Z*- and 9-*Z*-β-carotene, followed by a gradual decrease was observed during thermal treatments of enriched palm olein. The decrease in the concentration of the specific isomers was partly attributed to the back-isomerization

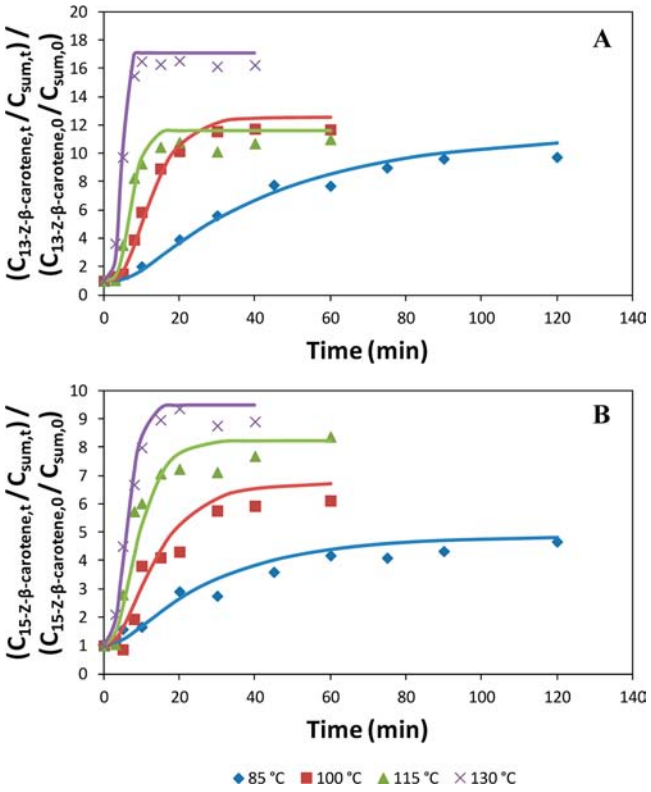

Figure 4. Formation of 13-*Z*-*β*-carotene (A) and 15-*Z*-*β*-carotene (B) during thermal treatments of an olive oil phase enriched with carrot *β*-carotene, modeled by one-step regression using a fractional conversion model. The single data points represent the experimental values, whereas the full lines represent the values predicted by the kinetic model.

to *all-E*-*β*-carotene and partly attributed to degradation by oxidation. As the results in the present study are expressed as contributions, only isomerization is taken into account. Therefore, the observed decrease will be related to back-isomerization to *all-E*-*β*-carotene and not to oxidation.

In Table 2, the percentages of the individual isomers to the total *β*-carotene concentration are also given for the untreated enriched oil phase and for the enriched oil phase after thermal treatments of 60 min at different temperatures. As the temperature increased, the percentage of *all-E*-*β*-carotene decreased, but the values were higher compared to the oil/carrot emulsion, and the difference became larger as the temperature increased. It can be hypothesized that the isomerization from the *Z*-isomers back to the *all-E* form occurred more rapidly in the pure oil phase compared to the oil/carrot emulsion and that this effect was more pronounced at higher temperatures. In contrast to the oil/carrot emulsion, 9-*Z*-*β*-carotene was also detected in the untreated oil phase and was present in the highest concentration, followed by 13-*Z*-*β*-carotene and 15-*Z*-*β*-carotene. However, similar trends in the distribution of the individual *Z*-isomers to the total *Z*-concentration were observed in the thermally treated enriched oil phase compared to the thermally treated oil/carrot emulsion. At lower process temperatures, 13-*Z*-*β*-carotene was the most important *Z*-isomer, followed by 15-*Z*- and 9-*Z*-*β*-carotene. Above 115 °C, 9-*Z*-*β*-carotene became more important and even became the most important *Z*-isomer after a treatment of 60 min at 130 °C. Similarly, Achir et al.[10] found that the proportion of 9-*Z*-*β*-carotene became equal to or higher than the proportion of 13-*Z*-*β*-carotene after thermal treatments of *β*-carotene in vegetable oils at temperatures above 120 °C and after long treatment times.

When the kinetic parameters for the oil/carrot emulsion and for the enriched oil phase are compared, it can be seen from Table 1 that the reaction rate constants are slightly higher in the oil phase compared to the oil/carrot emulsion, whereas the activation energy values are similar. However, to statistically compare the simultaneously estimated parameters obtained in the two different matrices, 90% JCR were constructed (see

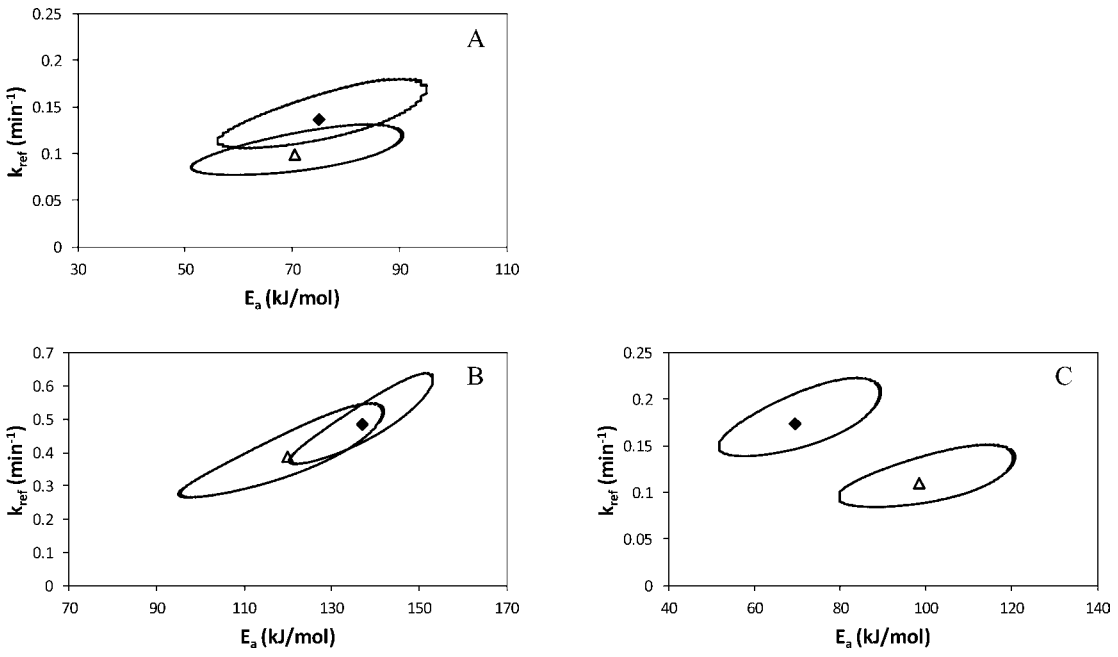

Figure 5. Joint confidence regions (90%) for *all-E*-*β*-carotene isomerization (A) and for formation of 13-*Z*-*β*-carotene (B) and 15-*Z*-*β*-carotene (C) in an olive oil/carrot emulsion (△) and in an olive oil phase enriched with carrot *β*-carotene (◆) during thermal treatments.

Figure 5). For *all-E-β*-carotene and 13-*Z-β*-carotene, there is overlap of the JCR of the oil phase, on the one hand, and the emulsion, on the other hand, so the combinations of k_{ref} and E_a are not significantly different in the two matrices. In other words, isomerization of *β*-carotene in an olive oil/carrot emulsion is not significantly different from isomerization in pure olive oil. As the kinetic parameters are of the same order of magnitude in both matrices, it can be hypothesized that diffusion of *β*-carotene to the oil phase during thermal treatments is not a rate-limiting step for isomerization. For 15-*Z-β*-carotene, on the other hand, formation seems to happen significantly more quickly in oil compared to the oil/carrot emulsion as there is no overlap between the JCR. For lycopene, similar results were found by Colle et al.:[28] activation energies for thermal isomerization of lycopene in an olive oil/tomato emulsion or an olive oil phase enriched with tomato lycopene did not differ significantly. Furthermore, higher reaction rate constants were observed in the enriched oil phase compared to the emulsion, but in contrast with the present study, the differences were significantly different.

For the first time, kinetic parameters of *β*-carotene degradation and isomerization during thermal processing of an oil/carrot emulsion and an oil phase enriched with carrot *β*-carotene were obtained. In conclusion, it can be stated that a fractional conversion model could be used to describe both total carrot *β*-carotene degradation and isomerization in the presence of oil during thermal processing between 85 and 130 °C. Using the obtained kinetic parameters, the impact of different thermal processes, both pasteurization and sterilization, on *β*-carotene degradation and isomerization of carrot products in the presence of oil might be evaluated, which is important for process design and optimization. The goodness of the obtained kinetic model was proven by a good agreement of experimentally determined values for the formation of *Z*-isomers after a thermal pasteurization of an olive oil/carrot emulsion with the simulated values. Furthermore, the importance of oil on total *β*-carotene degradation and isomerization during thermal processing was shown. First, *β*-carotene isomerization during thermal processing is strongly accelerated by the presence of oil as a result of solubilization of the *β*-carotene crystals in the oil phase. However, *β*-carotene isomerization in an oil/carrot emulsion is not significantly different from isomerization in a pure oil phase, indicating that the diffusion of *β*-carotene from the carrot matrix to the oil phase during thermal processing is not a rate-limiting step for isomerization. Second, oil that acts as a pro-oxidant also accelerates total *β*-carotene degradation, but the magnitude of the effect is dependent on the oil concentration.

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Notes

The authors declare no competing financial interest.

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