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ARTICLE

β-Carotene Isomerization Kinetics during Thermal Treatments of Carrot Puree

Lien Lemmens, Kristel De Vleeschouwer, Katlijn R. N. Moelants, Ines J. P. Colle, Ann M. Van Loey, and Marc E. Hendrickx*

Laboratory of Food Technology and Leuven Food Science and Nutrition Research Centre (LFoRCe), Department of Microbial and Molecular Systems (M^2S), Katholieke Universiteit Leuven, Kasteelpark Arenberg 22, 3001 Leuven, Belgium

The effect of thermal processing on the stability of β-carotene in carrot puree was investigated in a broad temperature range (80−150 °C). Heat induced changes in the stability of β-carotene resulting in the conversion into its cis-isomers until an equilibrium state was reached after prolonged heating. By using nonlinear one-step regression analysis, the overall isomerization of *all-trans*-β-carotene and the formation of individual cis-isomers could be modeled with a fractional conversion model. The Arrhenius equation was used to describe the temperature dependence of the reaction rate constants. As indicated by the low activation energies for all compounds (11 kJ mol^{-1}), the isomerization rate constants showed little sensitivity toward the treatment temperature. The temperature dependence of the equilibrium concentration values after prolonged heating (C_f) varied for the different compounds, but in all cases, a linear relation between the C_f values and the treatment temperature could be noted. Although isomerization was observed as a result of thermal processing, it could be concluded that during industrially relevant heating processes, the retention of *all-trans*-β-carotene in plain carrot puree was relatively high, which is most likely due to the presence of the protecting food matrix.

KEYWORDS: β-Carotene; carrot; isomerization; kinetics; thermal processing

INTRODUCTION

Carrots are known to be a worldwide distributed good source of carotenoids, in particular, β-carotene (*1*). Because multiple health-promoting properties have been ascribed to β-carotene (*2*), assuring an adequate intake of β-carotene from the diet is essential for human health. In this context, Olson (*3*) highlighted the provitamin A function of β-carotene: β-carotene can be converted into vitamin A, which is crucial for normal growth and development. Its antioxidative properties, which are thought to be related to a reduced risk for some cancers and cardiovascular diseases, also contribute to the health-promoting effect of the consumption of β-carotene (*4*).

In carrots, β-carotene is deposited in crystalline form in the chromoplasts and the carotene crystals are stabilized by other components such as (lipo)proteins (*5*). Due to its crystalline nature, the stability of β-carotene in raw carrots is rather high. However, the stability can be affected during heat treatments; that is, β-carotene can partly be dissolved in cellular lipids, implying a higher susceptibility of β-carotene toward degradation (*6*). Moreover, during heat treatments, the membranes surrounding the crystals [i.e., the typical membranous structures of the chromoplasts (*7*) and the plant cell membrane] and the plant cell wall are further disrupted, which might have an effect on the stability of intracellular components such as β-carotene.

*Address correspondence to this author at Kasteelpark 22, Postbox 2457, 3001 Leuven, Belgium (telephone +32 16 32 15 85; fax +32 16 32 19 60; e-mail marc.hendrickx@biw.kuleuven.be).

In the literature, data describing the effect of thermal treatments, in the time/temperature range at which preservation processes such as pasteurization and sterilization are performed, on the stability of β-carotene in food products (in this case carrots) are limited. Marx et al. (*8*) studied the effect of pasteurization and sterilization treatments on the isomerization of β-carotene in carrot juice. It could be concluded that pasteurization as well as mild temperature sterilization caused only limited β-carotene isomerization. However, at temperatures at which significant disruption of the food matrix occurred (> 120 °C), increased β-carotene isomerization was observed. Chen et al. (*9*) reported similar observations. Next to Marx et al. (*8*), Nguyen et al. (*10*) emphasized the importance of the physical state of β-carotene in determining its susceptibility toward isomerization: boiling of tomatoes resulted in thermal isomerization of β-carotene, whereas lycopene, which has a different structure and, even more important, is synthesized and stored in different locations in the cell, was retained in its all-trans-configuration.

From a kinetic point of view, useful information can be gathered from studies performed on model systems. Although literature data on isomerization of carotenoids during storage, drying, and exposure to light are available, we will further limit this introduction to studies on the thermal isomerization of carotenoids. In model systems, thermal isomerization experiments on carotenoids have been reported at low temperature (< 100 °C) (*11*−*14*) and at higher temperature (150 °C) (*11*). All authors concluded that a reversible first-order model could be used to describe the isomerization of carotenoids in model systems, indicating that an equilibrium state is reached after a certain time interval.

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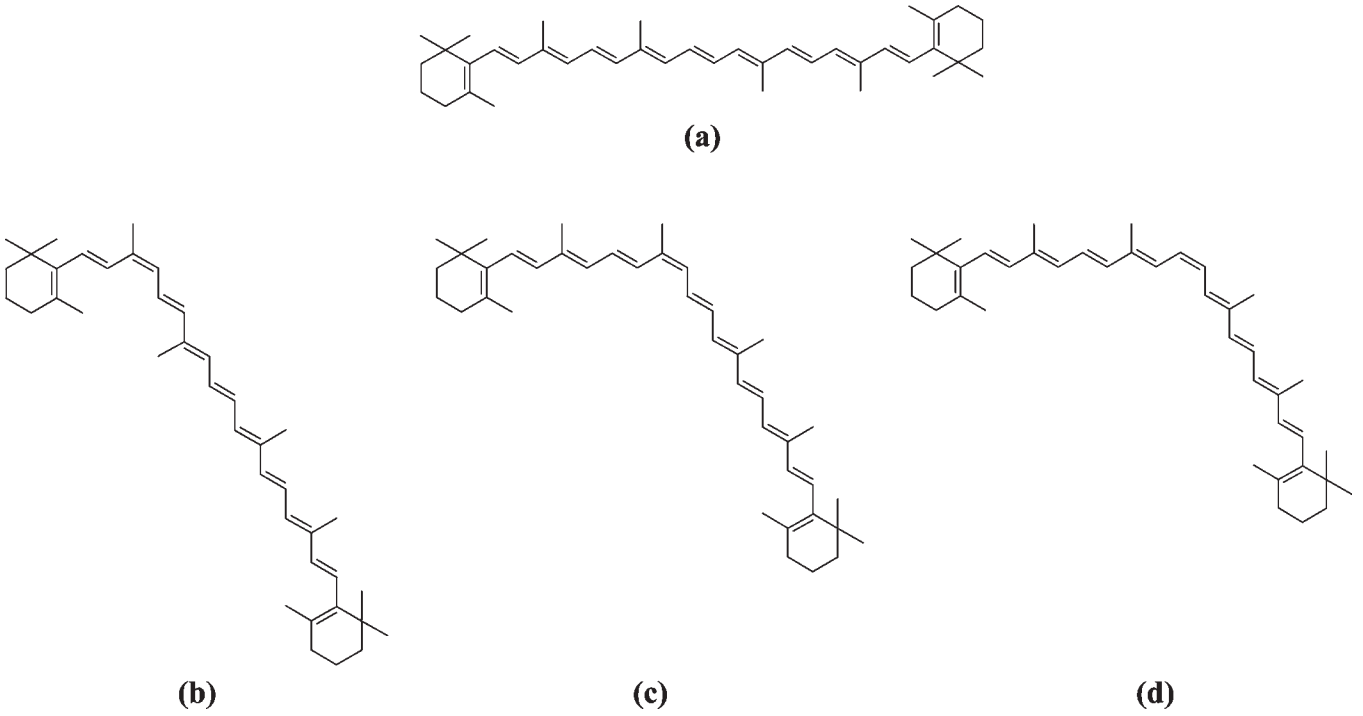

Figure 1. Chemical structures of the different reference compounds used for identification and quantification: (**a**) *all-trans*-β-carotene; (**b**) 9-*cis*-β-carotene; (**c**) 13-*cis*-β-carotene; (**d**) 15-*cis*-β-carotene.

The relative proportion of the formed cis-isomers seemed to be dependent on the treatment temperature (*12*, *13*), which could be explained by differences in activation energies and reaction energies (*12*, *15*). Studies describing the kinetics of carotenoid degradation due to isomerization in fruit- and vegetable-based products are rather limited, although this information would be very useful and industrially relevant for predicting nutritional changes during fruit and vegetable processing. Ahmed et al. (*16*), Dutta et al. (*17*), and Sharma et al. (*18*) investigated the thermal degradation kinetics of carotenoids in relation to the thermal color degradation kinetics for, respectively, papaya, pumpkin, and watermelon. The carotenoid content was determined spectrophotometrically, and hence degradation was assumed to be mainly through oxidation. In the temperature range studied (pasteurization), carotenoid degradation could be modeled by a first-order reaction. A similar conclusion was made by Dhuique-Mayer et al. (*19*) for pasteurized citrus juice, but in this study, HPLC analysis was used to determine the *all-trans*-β-carotene concentration. This implies that the observed losses of *all-trans*-β-carotene could be due to oxidation and isomerization reactions. In these studies, the proposed kinetic models described only the overall degradation of the carotenoids; the modeling of the formation of the different cis-isomers or oxidation products was not considered.

The objective of this research was to investigate the effect of thermal treatments of a carrot matrix (carrot puree) on the degradation of β-carotene due to isomerization (the formation of its cis-isomers) in order to identify models that describe these interconversion reactions in real food systems, in this case carrots. A broad range of processing intensities, including pasteurization and sterilization intensities, was applied in this study, facilitating the estimation of the impact of both industrially relevant processes on the nutritional quality of carrots.

MATERIALS AND METHODS

Reference Compounds. The standards used for identification and quantification, that is, *all-trans*-, 9-*cis*-, 13-*cis*-, and 15-*cis*-β-carotene, were obtained from CaroteNature (Lupsingen, Switzerland). The chemical structures of the different reference compounds are given in **Figure 1**.

Experimental Setup. Carrots (*Daucus carota* cv. Nerac) were mixed two times for 20 s (Büchi Mixer B-400, Switzerland) to obtain a homogeneous carrot puree. All heat treatments were performed in closed reactor tubes (inox, external diameter = 12 mm, internal diameter = 5 mm, length = 100 mm, custom-made). To minimize the contact with oxygen, the reactor tubes were filled completely with carrot puree (mass carrot puree = 1 g) so that the volume of the headspace is negligible. After the heat treatment, the samples were cooled in an ice bath, removed from the reactor tubes, frozen in liquid nitrogen, and stored at −80 °C in vacuum-sealed plastic bags until analysis of the β-carotene content.

To determine the relevant temperature range for the actual kinetic study, the thermal stability of β-carotene in carrot puree was screened. Therefore, carrot puree, enclosed in the reactor tubes, was heated in an oil bath at temperatures ranging from 80 to 150 °C for a fixed time interval of 20 min. Analyses of *all-trans*-β-carotene and the cis-isomers of β-carotene revealed which temperature range was relevant for β-carotene isomerization.

For the kinetic experiments, carrot puree, enclosed in the reactor tubes, was heated in an oil bath at temperatures ranging from 80 to 150 °C for time intervals ranging from 0 to 120 min. For each temperature/time condition, three reactor tubes were treated and the content of the three tubes was mingled for the subsequent analysis of β-carotene. During the thermal treatments, the complete time/temperature profile of the carrot puree was registered.

Extraction and Quantification of *all-trans*-β-Carotene and the Cis-Isomers of β-Carotene. β-Carotene was extracted from the carrot puree according to the extraction procedure of Sadler et al. (*20*) with some modifications as described by Lemmens et al. (*21*). Briefly, defrosted carrot puree (1 g) was homogenized with 50 mL of extraction solvent, containing 50% hexane, 25% ethanol, 25% acetone, and 0.1% butylated hydroxytoluene. To enhance the separation between the water layer and the organic layer in a later phase of the extraction procedure, $CaCl_2$ was added in a ratio 1:1 ($CaCl_2$/carrot puree). The mixture was stirred at 4 °C during 20 min, and afterward 15 mL of reagent grade water was added followed by another stirring step of 10 min at 4 °C. The organic layer, which could be separated from the water layer and which contains β-carotene, was filtered and analyzed by RP-HPLC. To obtain a good separation of *all-trans*-β-carotene and its cis-isomers, an HPLC system (Agilent Technologies 1200 Series, Diegem, Belgium) equipped with a C_{30}-column

(thermostated at 25 °C) (5 μm × 250 mm × 4.6 mm, YMC Europe, Dinslaken, Germany) and a diode array detector was used. A linear gradient, starting from the initial conditions (81% methanol, 15% methyl *tert*-butyl ether, 4% reagent grade water) was built up in 20 min to the end conditions (41% methanol, 55% methyl *tert*-butyl ether, 4% reagent grade water) at a flow rate of 1 mL/min. Identification and quantification were performed at 450 nm, at which the maximal absorbance of *all-trans*-β-carotene was found. For each sample, the extraction of β-carotene was performed in duplicate. Reference compounds were used to establish calibration curves for quantifying each of the individual compounds. To avoid degradation due to contact with light, the procedure was carried out in a room with dimmed red light using dark glassware.

Data Analysis. As already mentioned, first-order kinetics for the isomerization of carotenoids in model sytems have been reported. On the basis of these earlier observations and knowledge that isomerization reactions are commonly known as reversible and hence equilibrium reactions (*22*), a fractional conversion model was proposed to describe the overall isomerization of β-carotene and the formation of the individual cis-isomers in a real food system, in this case carrot puree. In this type of model, it is assumed that the reaction follows first-order kinetics until a plateau value is reached. In this specific case, this plateau value is reached when an equilibrium in the conversion of β-carotene into its cis-isomers is achieved. Generally, a fractional conversion model can be described by a differential equation (eq 1), and this equation can be integrated for a fixed k value (time independent), resulting in eq 2

$$dC = (C - C_f)k\,dt \quad (1)$$

$$C = C_f + (C_0 - C_f)\exp(-kt) \quad (2)$$

where C represents the β-carotene concentration, C_f the β-carotene concentration in the equilibrium state, C_0 the initial β-carotene concentration (μg of β-carotene/g of carrot puree), t the reaction time (min), and k the reaction rate constant (min^{-1}).

As is common for chemical reactions, the Arrhenius equation was used to describe the temperature dependence of the reaction rate constants. This temperature dependence can be quantified by using the activation energy as shown in eq 3 with k the reaction rate constant (min^{-1}) at temperature T (K), k_{ref} the reaction rate constant (min^{-1}) at reference temperature T_{ref} (K), E_A the activation energy (J/mol), and R the universal gas constant (8.314 J/K·mol).

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

Because it is expected that the concentrations of the different compounds in the equilibrium state are also dependent on the treatment temperature, an equation describing the dependency of the C_f values on the temperature was calculated by linear regression for each compound on the basis of the estimation of the C_f values using the technique of two-step regression analysis. This knowledge was applied for further data analysis using nonlinear one-step regression analysis (SAS, v9.1, Cary, NC). The quality of fit was evaluated graphically by means of the parity plot. Normality of the residues was checked by inspecting the normal probability plot.

RESULTS AND DISCUSSION

Identification and Separation of *all-trans*-β-Carotene and the Cis-Isomers of β-Carotene. In **Figure 2** is given a typical chromatogram for the separation of the carotenoids extracted from carrot puree. By applying gradient elution, a good separation of *all-trans*-β-carotene (peak 1), *all-trans*-α-carotene (peak 2), 9-*cis*-β-carotene (peak 3), 13-*cis*-β-carotene (peak 4), and 15-*cis*-β-carotene (peak 5) was obtained. Peak identification was performed on the basis of a comparison with the retention times of the standards and DAD spectra of the individual compounds. Because the contribution of other unknown peaks is limited in the chromatogram in **Figure 2** (except for an unknown compound (peak 6), but this peak area seemed to be independent of the treatment time and temperature), the three isomers were considered to be the main isomeric configurations of *all-trans*-β-carotene that are formed during thermal treatments of carrot puree.

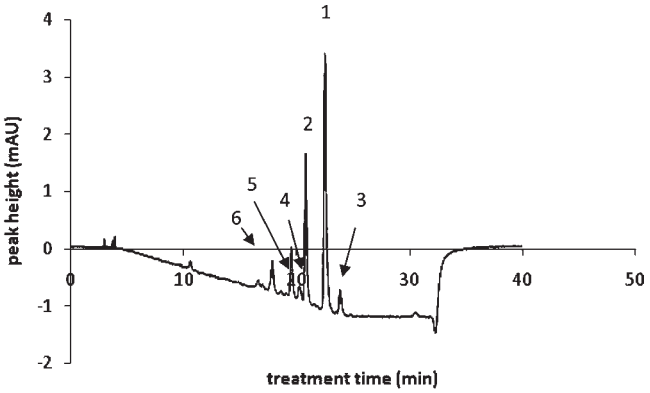

Figure 2. Typical chromatogram for the separation of *all-trans*-β-carotene (peak 1), *all-trans*-α-carotene (peak 2), 9-*cis*-β-carotene (peak 3), 13-*cis*-β-carotene (peak 4), 15-*cis*-β-carotene (peak 5), and an unknown compound (peak 6) in carrot puree (DAD detection at 450 nm).

Screening of the Thermal Stability of *all-trans*-β-Carotene in Carrot Puree. For a fixed treatment time of 20 min, a decrease in the concentration of *all-trans*-β-carotene was observed at temperatures >100 °C. At lower temperatures, the concentration of *all-trans*-β-carotene remained apparently constant, although this might be a net effect of some (limited) degradation together with an increased extractability. Due to thermal treatments of carrot puree, the carrot matrix and the cellular structure are further disrupted and, hence, the extractability, that is, the release of *all-trans*-β-carotene during the extraction procedure, is affected (*23*). With regard to the formation of 9-*cis*-, 13-*cis*-, and 15-*cis*-β-carotene, an increase in the individual isomer concentration could be noted at temperatures >100 °C, which is in agreement with the corresponding decrease in the *all-trans*-β-carotene concentration. It should, however, be kept in mind that for a fixed treatment time of 20 min, pronounced isomerization occurs only at high temperatures (≥130 °C). The results of the screening study gave an indication for the temperature/time range for the actual kinetic study. The kinetic experiment was performed in a broad temperature range (80−150 °C), and to detect clear concentration changes of *all-trans*-β-carotene and the cis-isomers, longer treatment times (especially for the lower temperatures) and shorter treatment times (especially for the high temperatures) were included.

Data Transformation. The data obtained show that for most of the time/temperature combinations, the total β-carotene content, that is, in this study the sum of *all-trans*-β-carotene, 9-*cis*-, 13-*cis*-, and 15-*cis*-β-carotene, was higher for the treated carrot puree than for the raw carrot puree, and it was very difficult to detect a logic trend in the evolution of the total β-carotene content for the different treatment times and temperatures. As already explained, this might be due to the fact that thermal treatments of carrot puree induce changes in the extractability of β-carotene. To eliminate the effect of the changing extractability with temperature and time, it is suggested to "transform" the data. The concentrations of all compounds at each point of time were considered relatively to the total β-carotene content at that moment, implying that all concentrations are expressed as a fraction of the total β-carotene content at a certain moment in

time. The data transformation is visualized in the following scheme (C = concentration).

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

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

However, it should be kept in mind that some simplifying assumptions were made. For example, the formation of isomerization products is limited to the formation of 9-*cis*-, 13-*cis*-, and 15-*cis*-β-carotene. In the literature, Rodriguez-Amaya et al. (*23*) reported that these isomers are the principal cis-isomers of β-carotene and, moreover, our chromatographic data support this hypothesis. Furthermore, the oxidative degradation of β-carotene is not taken into consideration with this data transformation and, hence, the reaction kinetics are limited to thermal isomerization kinetics. This assumption seems to be plausible; because the reaction is performed in closed reactor tubes with a limited/negligible headspace volume, no additional compounds, which might be intermediate oxidation products, are eluted during the chromatographic separation and even at the lowest treatment temperature, where matrix disruption and hence extractability changes might be limited and oxidative degradation might take place, the total β-carotene retention is still above 90% after prolonged heating. To conclude, it can be stated that the suggested data transformation effectively filtered out the effect of changing extractability of β-carotene during thermal treatments of carrot puree (data not shown). The transformed data were used for further kinetic data analysis to study specifically the effect of thermal treatments of carrot puree on the overall isomerization of *all-trans*-β-carotene and the formation of individual cis-isomers.

Kinetic Data Analysis: Two-Step Regression Analysis. The degradation of *all-trans*-β-carotene due to isomerization, the formation of the total cis-isomers, and the individual formation of 9-*cis*-, 13-*cis*-, and 15-*cis*-β-carotene were studied. A two-step regression approach was used for which the kinetic parameters [reaction rate constants (k) and equilibrium concentration values (C_f)] were estimated at different treatment temperatures in a first step, followed by estimating the temperature dependence of these parameters in a second step. It could be concluded that for all compounds, except for 15-*cis*-β-carotene, and for all treatment temperatures, the concentration evolved to an equilibrium after prolonged heating, and this equilibrium concentration was temperature dependent. The use of a fractional conversion model to fit the experimental data was shown to be appropriate in this case study, except for 15-*cis*-β-carotene. It has been shown by Guo et al. (*15*) that a low energy of the rotational barrier is associated with the formation of 15-*cis*-β-carotene, explaining why this compound can already be detected at lower treatment temperatures. However, the stability of 15-*cis*-β-carotene seems to be low: from the moment it is formed, the reverse reaction, that is, the isomerization of 15-*cis*-β-carotene to *all-trans*-β-carotene, takes place with a high efficiency (*24*). This implies that the amount of 15-*cis*-β-carotene that can be detected in the equilibrium state is limited and that it is even more difficult to perceive changes in the concentration of 15-*cis*-β-carotene with changing treatment temperature and time (*12*). Hence, the data of the present study cannot be used to describe the complex situation of 15-*cis*-β-carotene formation in carrot puree with a kinetic model.

Table 1. Temperature Dependence of C_f Values for the Different Compounds Estimated by Linear Regression (Valid in a Temperature Range from 80 to 150 °C) and the Correlation Coefficients for the Corresponding Parity Plots

	temperature dependence of C_f values	R^2, parity plot
all-trans-β-carotene	$C_f = -0.38T + 104.57$	0.94
sum of cis-isomers	$C_f = 0.37T - 3.97$	0.93
9-*cis*-β-carotene	$C_f = 0.43T - 31.48$	0.98
13-*cis*-β-carotene	$C_f = 0.02T + 2.41$	0.83
15-*cis*-β-carotene	nd[a]	nd

[a] nd, not determined.

The Arrhenius equation (eq 3) could be used to describe the temperature dependence of the reaction rate constants. The equilibrium concentration values change linearly with increasing temperature (the equations are given in **Table 1**). The temperature dependence of the equilibrium concentration values clearly differs for the different compounds. As an indication for the quality of fit, the correlation coefficients for the corresponding parity plots are included in **Table 1**.

The knowledge obtained from this two-step regression approach was used as a starting point for further data analysis using nonlinear one-step regression analysis.

Kinetic Data Analysis: One-Step Regression Analysis. As can be seen from time/temperature data (**Figure 3**), some time was required to attain the actual treatment temperature in the carrot puree. As a simplification, it could be assumed that the temperature of the samples, taken during this initial, dynamic temperature phase, is equal to the actual treatment temperature (assumption of isothermal conditions). Because the dynamic temperature phase is a substantial part of the whole treatment for some data points, it is more accurate to perform the data analysis on the basis of the actual time/temperature profile of the samples. Therefore, the temperature profile in the carrot puree was recorded during the heat treatment, and this information was included in the kinetic data analysis (using actual dynamic temperature conditions). To estimate the effect on the kinetic parameters due to the simplifying assumption of isothermal conditions, one-step regression analysis of the transformed data was carried out using both assumptions.

The differences in model fit as well as the differences in the estimation of the kinetic parameters, assuming isothermal conditions or using the actual dynamic temperature conditions, were very small, implying that the contribution of the dynamic heating phase to the isomerization reactions is limited. Nevertheless, including the dynamic temperature phase is more accurate and, hence, this strategy, that is, one-step regression analysis taking into consideration the actual temperature history of each sample, was used in further data analysis. During one-step regression analysis, the equations describing the temperature dependence of the reaction rate constants (Arrhenius equation, eq 3) and the temperature dependence of the equilibrium concentrations values (known from two-step regression analysis, **Table 1**) were substituted in eq 1. The result is given in eq 4

$$dC = (C - C_f(T))k_{ref} \exp\left[-\frac{E_A}{R}\left(\frac{1}{T(t)} - \frac{1}{T_{ref}}\right)\right] dt \qquad (4)$$

where C_f (T) represents the temperature dependence of the equilibrium concentration values as given in **Table 1** and $T(t)$ represents the time dependence of the temperature as shown in **Figure 3**.

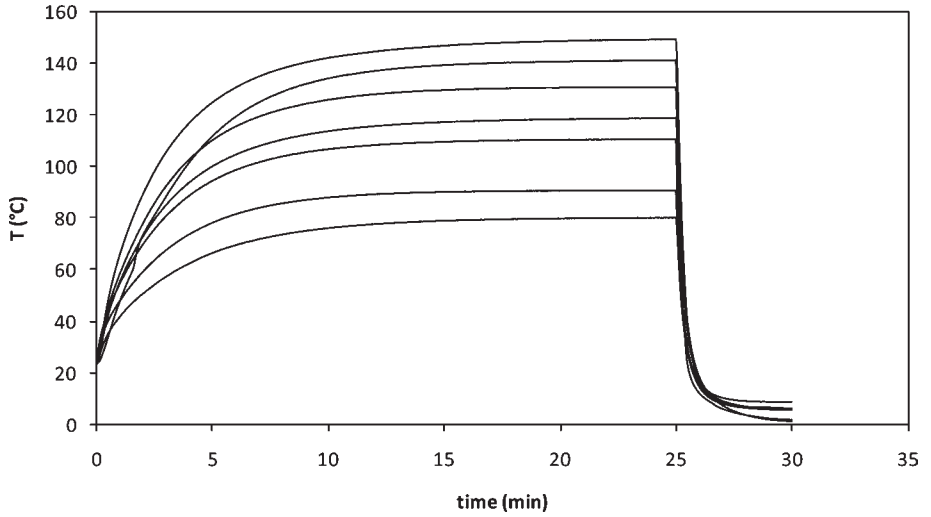

Figure 3. Typical temperature−time profiles of samples heated in closed reactor tubes at temperatures between 80 and 150 °C.

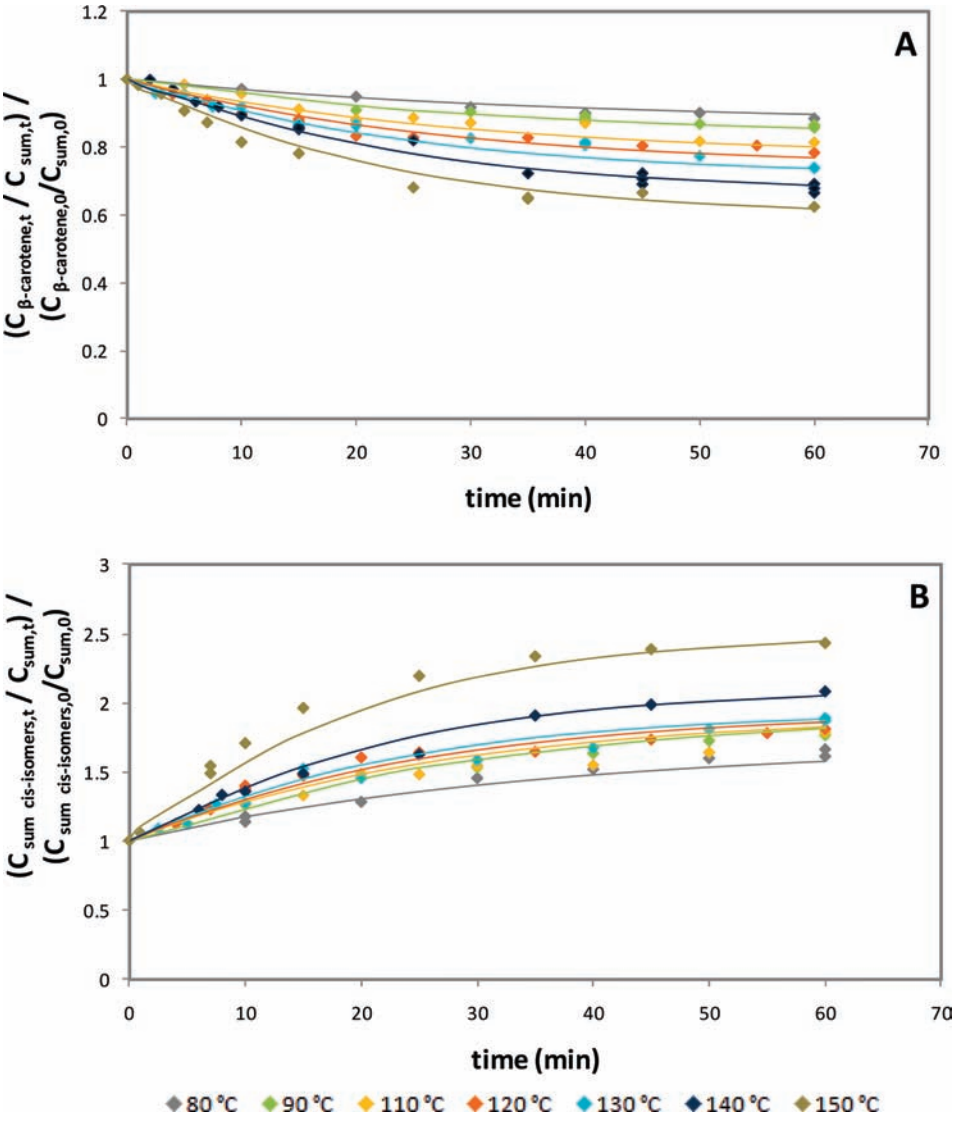

Figure 4. Degradation of *all-trans*-β-carotene due to isomerization (**A**) and formation of the total cis-isomers of β-carotene (**B**) during thermal treatments of carrot puree modeled by a fractional conversion model. The full lines represent the concentration values predicted by the kinetic model, whereas the experimental data are represented by the symbols.

In **Figure 4**, the model fit, assuming a fractional conversion model, for the degradation of *all-trans*-β-carotene due to isomerization and the formation of the total cis-isomers is shown by the full lines. All concentrations are expressed relative to the

Table 2. Overview of the Kinetic Parameters ± Standard Deviation (Based on 95% Confidence Interval) (T_{ref} = 110 °C) and the Correlation Coefficients of the Parity Plot and the Normal Probability Plot for the Modeling of the Degradation of *all-trans*-β-Carotene Due to Isomerization and the Formation of 9-*cis*-, 13-*cis*-, and 15-*cis*-β-Carotene during Thermal Treatments of Carrot Puree

	β-carotene degradation	total isomer formation	9-*cis*-β-carotene formation	13-*cis*-β-carotene formation	15-*cis*-β-carotene formation
k_{ref} ($\times 10^{-3}$ min^{-1})	35.2 ± 0.089	38.3 ± 1.2	23.0 ± 1.1	66.4 ± 3.5	nd[a]
E_A (kJ mol^{-1})	10.5 ± 1.3	11.5 ± 1.7	14.1 ± 3.1	11.7 ± 2.0	nd
R^2, parity plot	0.97	0.97	0.97	0.96	nd
R^2, normal probability plot	0.99	0.98	0.91	0.95	nd

[a] nd, not determined.

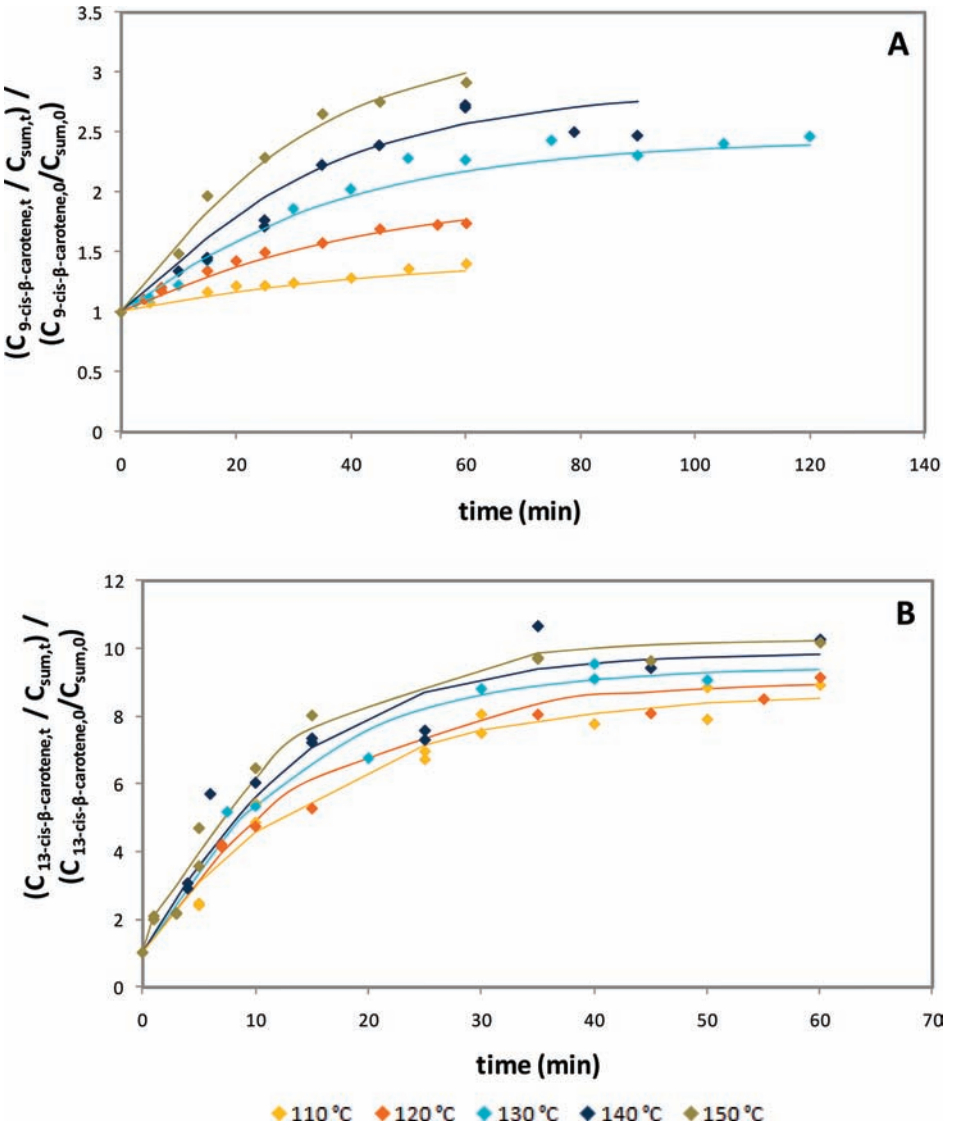

Figure 5. Formation of 9-*cis*-β-carotene (**A**) and 13-*cis*-β-carotene (**B**) during thermal treatments of carrot puree modeled by a fractional conversion model. The full lines represent the concentration values predicted by the kinetic model, whereas the experimental data are represented by the symbols.

initial concentration. It can visually be detected that in both cases the kinetic model gives a good description of the experimental data points. Moreover, the equilibrium concentration values are decreasing or increasing with increasing treatment temperature for, respectively, the degradation of *all-trans*-β-carotene due to isomerization (**Figure 4A**) or the formation of the total cis-isomers (**Figure 4B**). The corresponding parity plots (see the Supporting Information), plotting the experimental concentrations versus the predicted concentrations, show a good quality of fit for both models. Besides, the normality of the residuals was checked by means of a normal probability plot (see the Supporting Information). The corresponding estimated kinetic parameters as well as the correlation coefficients for the parity plot and for the normal probability plot are listed for all compounds in **Table 2**.

To study the isomerization in more detail, the formation of 9-*cis*- and 13-*cis*-β-carotene during thermal treatments of carrot puree was modeled individually using a fractional conversion model. Due to their low initial concentrations and due to the fact that isomerization mainly occurs at temperatures higher than 100 °C (cfr. screening study), low temperatures were not included in this model. As explained earlier, the formation of 15-*cis*-β-carotene could not be modeled individually. In **Figure 5**, the model fit for the formation of 9-*cis*- and 13-*cis*-β-carotene is shown.

For both compounds, the kinetic model describes the experimental data points adequately. The goodness of fit is illustrated by the correlation coefficients for the corresponding parity plots in **Table 2**. For 9-*cis*- and 13-*cis*-β-carotene, the equilibrium concentration values increase with increasing temperature, although a large difference in temperature sensitivity can be observed: the equilibrium concentration of 9-*cis*-β-carotene strongly depends on the treatment temperature, whereas only small differences in the equilibrium concentration of 13-*cis*-β-carotene can be detected when the treatment temperature changed. This pronounced temperature dependence for the formation of 9-*cis*-β-carotene can be explained by its large rotational barrier, which makes the initial changes in the concentration of 9-*cis*-β-carotene smaller than the initial changes in the concentration of 13-*cis*-β-carotene (*12*, *15*). The formation of 13-*cis*-β-carotene requires the least energy (*15*). Guo et al. (*15*) also indicated that the rate of the reverse reaction, that is, the isomerization of the cis-isomers to *all-trans*-β-carotene, is the lowest for 9-*cis*-β-carotene, explaining why this compound could be detected in the highest concentration. In other studies, focusing on carrot juice, 13-*cis*-β-carotene was observed to be the most abundant isomer formed during thermal processing (*8*, *9*), whereas 15-*cis*-β-carotene and 9-*cis*-β-carotene were present in similar but lower concentrations (*9*) or only 9-*cis*-β-carotene could be quantified and detected in intensely treated samples (*8*).

Table 2 summarizes all results of the kinetic data analysis. Because all concentration changes are expressed as fractions of the total β-carotene content, it is obvious that a good correspondence for the degradation of *all-trans*-β-carotene due to isomerization and the formation of the total cis-isomers could be observed: the reaction rate constant at reference temperature and the activation energy are of the same order of magnitude. Although at reference temperature, the reaction rate constant for the formation of 9-*cis*-β-carotene is lower than the reaction rate constant for the formation of 13-*cis*-β-carotene, their activation energies are not significantly different. In the case of 9-*cis*-β-carotene, the C_f values are more temperature dependent than for 13-*cis*-β-carotene. These differences explain why the rate of 9-*cis*-β-carotene formation is lower and the attainment of the equilibrium state is slower as compared to 13-*cis*-β-carotene. Especially at higher temperatures, at which a high equilibrium concentration value for 9-*cis*-β-carotene was observed, more time is needed to actually reach this plateau value. All calculated activation energies, that is, for the overall isomerization of *all-trans*-β-carotene and for the isomerization of the individual compounds, are similar. In model systems, a higher temperature dependence of the reaction rate constants for isomerization was observed (*13*,*14*), but this might be explained by the absence of the protecting food matrix and, hence, the higher impact of a temperature increase on the isomerization rate constants. Generally, a higher thermal stability of β-carotene in carrot puree compared to the thermal stability of β-carotene in model systems was observed. For all compounds, the parity plot, as a graphical representation of the goodness of fit, shows a high correlation coefficient, indicating that there is a good correspondence between the experimental values and the values predicted by the model.

Summarizing, it can be stated that for the isomerization of *all-trans*-β-carotene and the formation of 9-*cis*- and 13-*cis*-β-carotene during the heating of carrot puree, a fractional conversion model was shown to be appropriate to fit the experimental data in an adequate way. This implies that after prolonged heating, an equilibrium situation is reached. This seems to be plausible, as it is known that isomerization reactions are reversible reactions leading to the formation of a state where a balance between all conversion reactions of *all-trans*-β-carotene (in this case isomerization reactions) can be observed. Moreover, it might be that, due to the presence of the carrot matrix, β-carotene is more protected against isomerization; that is, the agents necessary for isomerization (e.g., radicals) might be depleted after prolonged heating, partially explaining the equilibrium situation that is reached.

Implications for Industrially Relevant Processes. From a more practical point of view, the results of this kinetic study can be applied to industrially relevant processing conditions. Within the temperature/time frame studied, a typical pasteurization, sterilization, and UHT process could be identified and could be evaluated on the basis of the content of *all-trans*-β-carotene and the content of the total cis-isomers. Because carrots belong to the group of low-acid food products, the pasteurization value ($^{10\ °C}P_{90\ °C}$) was set at 10 min to obtain a stable pasteurized carrot puree for (limited) chilled storage (*25*). A typical pasteurization temperature of 90 °C was selected. A sterilization value ($^{10\ °C}F_{121\ °C}$) of 6 min was aimed for to obtain a shelf-stable carrot puree (*26*). The calculations were performed for a typical sterilization temperature of 120 °C. As a last point of comparison, an UHT process was simulated at 130 °C (*27*), resulting in the same sterilization value of 6 min. A very short processing time was necessary to achieve this, because in these types of processes, product heating is assumed to be isothermal. In **Figure 6** is depicted the effect of the different industrially relevant processes on the β-carotene content of carrot puree. Roughly speaking, these graphs indicate that for the three different preservation processes, the losses of *all-trans*-β-carotene in carrot puree are rather limited. However, because sterilization processes are performed in the temperature range at which isomerization of *all-trans*-β-carotene takes place, isomer formation is more pronounced and becomes more important than for pasteurization processes. Nevertheless, if an UHT process can be applied for the sterilization of carrot puree prior to packaging, it seems that an almost complete retention of *all-trans*-β-carotene may be expected. Generally, this kinetic study demonstrates that during industrial processing of plain carrot puree, only minor changes in the concentration of β-carotene due to isomerization will take place. Process optimization could even give a better control of the observed changes.

In conclusion, it can be stated that models describing the kinetics of changes occurring during thermal processing can be helpful to predict these changes and to optimize processing conditions specifically for one or more targets. In the case of nutrient destruction, it has recently been stressed that models describing the kinetics of carotenoid destruction and isomer formation in food matrices are scarcely found in the literature (*28*). Actually, this paper reports for the first time in a detailed way a model to describe the overall isomerization of *all-trans*-β-carotene and the formation of the individual cis-isomers in a real food system.

The overall isomerization of *all-trans*-β-carotene and the formation of the different cis-isomers during thermal processing (80−150 °C) of carrot puree could be modeled by using a fractional conversion model. This implies that heat causes interconversion reactions between *all-trans*-β-carotene and its cis-isomers until an equilibrium state is reached after prolonged heating. The concentration level of all compounds in the equilibrium state was dependent on the treatment temperature. For industrially relevant processing conditions, it could be concluded that, most likely due to food matrix protection, the losses of β-carotene and the concomitant formation of its cis-isomers are rather limited. Moreover, our previous investigations on the effect of thermal processing on the bioaccessibility of β-carotene in carrots (*21*) showed that a pronounced increase of the β-carotene bioaccessibility can be expected for the simulated processing conditions. Hence, from a nutritional point of view,

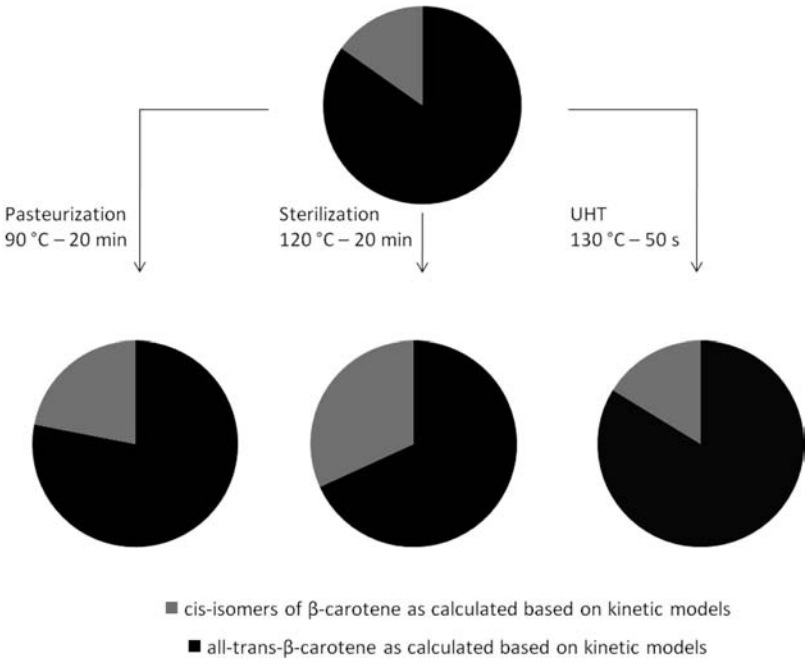

Figure 6. Comparison between the effect of different preservation processes, that is, a pasteurization process ($^{10\ °C}P_{90\ °C}$ = 10 min), a sterilization process ($^{10\ °C}F_{121\ °C}$ = 6 min), and an UHT process ($^{10\ °C}F_{121\ °C}$ = 6 min), on the β-carotene content in carrot puree.

an overall positive conclusion can be drawn from this research: the carrot matrix offers good protection for *all-trans*-β-carotene against thermal isomerization, whereas its bioaccessibility is enhanced during thermal processing. However, it should be stressed that these results are valid only for plain carrot puree and that the conclusions may change if other components are introduced into the system.

Supporting Information Available: Because the concentrations of the different compounds were used as relative values to filter out the effect of the changing extractability, some representative and exemplifying absolute concentrations of the different compounds are given in Table 3. As an example of the statistical analyses, the parity plots and normal probability plots are included. The parity plots in Figure 7 graphically represent the goodness of fit for the model describing the degradation of *all-trans*-β-carotene due to isomerization (Figure 7A) and for the model describing the formation of the total cis-isomers (Figure 7B). For both models, Figure 8 depicts the normal probability plots, checking the normality of the residuals. This material is available free of charge via the Internet at http://pubs.acs.org.

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