Kinetics of all-*Trans*-β-Carotene Degradation on Heating with and without Phenylalanine

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All-*trans*-β-carotene was heated in liquid paraffin at 210°C for 15 min in the presence and the absence of phenylalanine to assess the effect of the amino acid on the rate of degradation of all-*trans*-β-carotene. The curve that represents all-*trans*-β-carotene degradation in both model systems is formed of two distinct parts that correspond, respectively, to the propagation and termination phases of an autocatalytic reaction. The reaction over 1–15 min followed first-order reaction kinetics in both systems, and the rate constant obtained was 2.8 times lower in the presence of phenylalanine. The kinetic behavior and the rate constant for color loss were similar to those for all-*trans*-β-carotene degradation for each model system.

KEY WORDS: all-*trans*-β-carotene, all-*trans*-β-carotene degradation, color, kinetics, phenylalanine.

β-Carotene, a vitamin A precursor, occurs naturally in foods and is also used extensively as a food coloring material in a number of processed foods, including soups, margarine and flour confectionery (1).

The oxidative degradation of β-carotene is considered to be an autocatalytic free-radical chain reaction but reportedly follows first-order reaction kinetics (2–4). β-Carotene is fairly unstable at elevated temperatures, and literature data (5) show that 92% of β-carotene is degraded on heating in glycerol at 210°C for 15 min. Although the stability of β-carotene at ambient temperatures can be improved by the use of synthetic and natural antioxidants (6), at elevated temperatures the protection offered is relatively weak and depends, at least partly, on the thermal stability of the antioxidant (7). Although there have been few studies on β-carotene–amino acid model systems, some amino acids improve the stability of β-carotene at ambient temperatures (8,9). The present study was undertaken to investigate how the kinetics of all-*trans*-β-carotene loss at 210°C is affected by the presence of phenylalanine. The model system and the temperature and time were chosen to simulate deep-fat frying of foods.

MATERIALS AND METHODS

Materials. Light liquid paraffin, GPR, *L*-phenylalanine (chromatographically homogeneous) and analytical-grade solvents were obtained from BDH Chemicals Ltd. (Poole, United Kingdom). High-performance liquid chromatography (HPLC)-grade solvents were obtained from Rathburn Chemicals Ltd. (Walkerburn, United Kingdom). All-*trans*-β-carotene was obtained from Roche Products Ltd. (Basel, Switzerland). Its purity was ≈95% as assessed by HPLC, ultraviolet (UV) visible, infrared (IR), nuclear magnetic resonance and mass spectrometry (10).

Preparation and heating of model systems. All-*trans*-β-carotene (0.5 g) and phenylalanine (4 g) were added to liquid paraffin (200 mL) at 210°C, and the mixture was maintained at 210°C with mechanical agitation (Model System M). A model system containing only all-*trans*-β-carotene was also prepared (Model System B). Model systems were heated in an open glass flask and no attempt was made to exclude air. Aliquots of the reaction mixture (1 mL) were withdrawn at predetermined time intervals, cooled immediately under cold tap water and analyzed by HPLC.

HPLC. After cooling, HPLC was carried out based on the method of Quackenbush (11), with a 25 cm × 4.6 mm i.d. Vydac 201TP54 column, 5 μm particle size, fitted with a (5 cm × 4.6 mm i.d.) guard column packed with the same stationary phase (The Separations Group, Hesperia, CA). Elution was with methanol for 5 min, followed by methanol/chloroform (94:6) for 20 min. The solvent flow rate was 1 mL/min^{-1}. The sample was diluted appropriately in hexane, and 5-μL aliquots were injected.

Two HPLC systems were used. The first was comprised of a Perkin-Elmer binary LC pump, model 250 (Perkin-Elmer, Beaconsfield, United Kingdom) with a Rheodyne injection valve (model 7125) linked to a Spectroflow 757 absorbance detector at 460 nm (Kratos Analytical, Manchester, United Kingdom) and an HP3396A integrator (Hewlett Packard Ltd., Bracknell, United Kingdom). The second system was comprised of a Philips quaternary (Model PU4100M) pump linked to a Philips PU4120 diode array detector with scanning over the range 190–390 or 390–700 nm (Philips Scientific, Cambridge, United Kingdom), and a Dell Systems 310 computer (Dell Computer Corporation, Bracknell, United Kingdom) loaded with PU6003 diode array software (Philips Scientific). UV-visible absorption spectra were reconstructed from the diode array data. All analyses were performed in duplicate on two replicate model systems.

The peak area corresponding to all-*trans*-β-carotene was obtained for each chromatogram and was converted to mg of all-*trans*-β-carotene by means of a standard curve with $r = 0.995$ (10).

Isomers of β-carotene were identified by a comparison of their HPLC retention times and UV-visible absorption characteristics with published data for the authentic compounds (10).

RESULTS AND DISCUSSION

Figure 1 shows the HPLC chromatograms obtained from the two model systems after heating for 1 min. Under the HPLC conditions employed, all-*trans*-β-carotene was separated from its 13-*cis* and 9-*cis* isomers, the main *cis* isomers formed on heating all-*trans*-β-carotene (11). Peaks 3, 4 and 5 were identified as all-*trans*-β-carotene, 13-*cis*-β-carotene and 9-*cis*-β-carotene, respectively (12,13). The 9-*cis* and 13-*cis* isomers of β-carotene are known thermal degradation products of the all-*trans* compound (12).

Despite the use of 500 mg all-*trans*-β-carotene in all experiments, the area of the HPLC peak due to the starting material at t_0 (time zero), corresponded to 296 ± 8 mg, which represented only about 60% of the amount used (10). The most likely reason for a low recovery of all-*trans*-β-carotene is the definition of t_0 as the time by which all the all-*trans*-β-carotene had been added to the liquid

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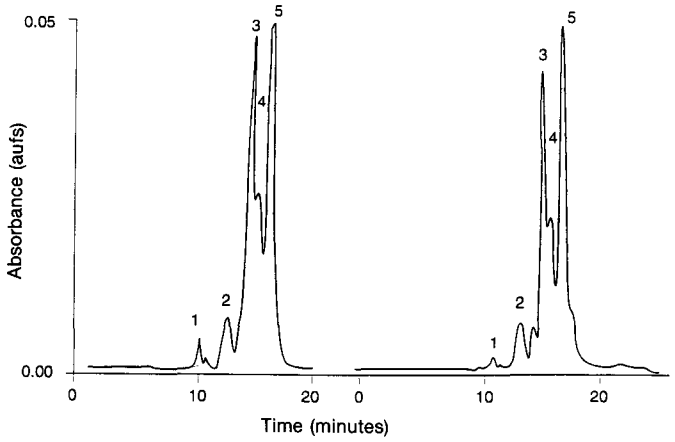

FIG. 1. High-performance liquid chromatography chromatograms obtained for Model Systems B and M at 460 nm after 1 min heating. A, Model System M; B, Model System B.

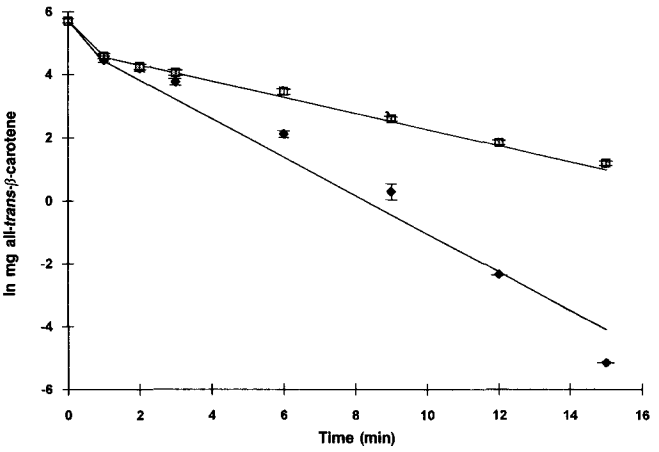

FIG. 3. Degradation of all-*trans*-β-carotene in Model Systems B (◆) and M (□) showing first-order reaction kinetics between 1–15 min heating at 210°C.

paraffin, and not the time at which addition started. (Addition of the starting material took approximately 10 s.)

All-*trans*-β-carotene degradation took place rapidly on heating at 210°C (see Fig. 2). A loss of 98% occurred after 6 min heating in Model System B and compares with reported losses of 91% after 5 min heating in the presence of nitrogen at the same temperature (5). Although oxygen plays an important role in the rate of β-carotene loss at 100°C (10), at higher temperatures the oxygen concentration becomes less important than the effect of heat (14). In addition, Onyewu *et al.* (5) measured total β-carotene, not only all-*trans*-β-carotene, and therefore the percent loss figure would be expected to be lower in the study reported here. The difference in the isomers measured, combined with the slight differences in the model system, probably account for the fairly small differences in β-carotene loss reported by Onyewu *et al.* (5) and in the current study.

A plot of ln mg all-*trans*-β-carotene against time was formed of two distinct parts (see Fig. 3) for both model systems. An initial rapid depletion of all-*trans*-β-carotene took place within the first minute of reaction, and the slope of the curve is correspondingly steep and similar for both model systems. The two data points obtained over the 0–1 min heating interval are insufficient to determine the rate of reaction.

From one minute onward, the degradation proceeded at a slower rate for both systems, and the data obtained from 1–15 min, i.e., with exclusion of the datum at 0 min, best fit first-order reaction kinetics (see Table 1). A marked difference in the rate of degradation of all-*trans*-β-carotene between 1–15 min heating was observed for the two model systems, and the rate constant for Model System M, estimated from the linear regression data, is 2.8 times lower than that for Model System B (see Table 1). Thus, it appears that all-*trans*-β-carotene degradation is retarded in the presence of phenylalanine under the experimental conditions.

Previous studies of the kinetics of β-carotene degradation have been performed mainly at room temperature or at temperatures up to about 100°C. It is generally accepted that β-carotene degrades by oxidation at such temperatures. The reaction is likely to be an autocatalytic free-radical chain reaction (15) that involves induction, propagation and termination phases, Thus, it would be expected to give a sigmoidal curve on plotting β-carotene loss against time, and this has been observed in some

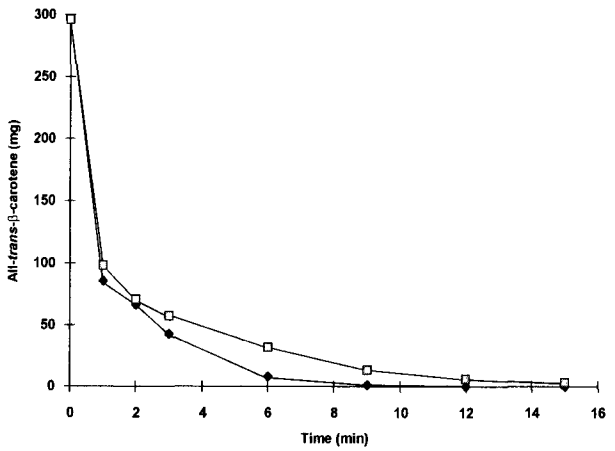

FIG. 2. Degradation of all-*trans*-β-carotene over 15 min heating in Model System B (◆) and Model System M (□).

TABLE 1

Rate Constants Estimated from the Regression Analysis Data[a]

	r^{2b}	k (min^{-1})[c]
Model system B		
ln mg all-*trans*-β-carotene	0.981	0.679 ± 0.109
ln color[d]	0.978	0.547 ± 0.094
Model System M		
ln mg all-*trans*-β-carotene	0.998	0.243 ± 0.013
ln color[d]	0.997	0.245 ± 0.016

[a]The results are the averages of two replicate experiments (coefficient of variation about 8%), data were collected over 1–15 min heating, and seven data points were obtained.
[b]r^2 = Coefficient of determination.
[c]Rate constant determined at the 95% confidence limits.
[d]Color was equated to the total high-performance liquid chromatography peak area at 460 nm (the λ_{max} of β-carotene).

cases (16,17). Despite the generally accepted mechanism, most reports state that β-carotene degradation obeys first-order or pseudo first-order reaction kinetics, following an induction period that decreases in length as the temperature increases (2–4). The fact that the length of the induction period decreases as temperature increases may explain why some researchers, using temperatures of 60–100°C, did not observe this phase of the reaction (18,19). Also, if collection of data stops at a relatively early stage, the termination phase may not be clearly observed.

Many researchers prefer the simpler first-order model (which applies to the propagation stage of the autocatalytic reaction) over the autocatalytic model to describe β-carotene degradation in foods (4), because the general overall quality of food deteriorates to an unacceptable level before the termination stage is reached. Therefore, this stage is overlooked in order to simplify calculations. Some studies report zero-order kinetics for the degradation of β-carotene (18,19). This may be explained by the fact that the collection of data was stopped at a relatively early stage of the reaction. Unless the study is carried out for sufficient time and with sufficient experimental values, it can be hard to distinguish between zero- and first-order kinetic behavior (20). In the present study, an induction period was not observed because β-carotene degradation is rapid at 210°C, and any induction period would be difficult to monitor. The two parts of the curve depicted in Figure 3 may correspond to the propagation (0–1 min) and termination (1–15 min) phases of an autocatalytic reaction.

The kinetics of color loss in Model Systems B and M were studied to compare it with the pattern of all-*trans*-β-carotene loss. In fact, the kinetics of β-carotene degradation are usually studied by investigating the rates of color loss in the system, because this is most easily assessed by absorbance measurements at the λ_{max} of β-carotene (3,17). However, in such studies, discrimination between the all-*trans* and *cis* isomers of β-carotene is not possible. In the current study, color was equated with the total HPLC peak area at 460 nm (see Fig. 4), and a comparison of Figures 3 and 4 shows that the kinetics of all-*trans*-β-carotene degradation and color loss are similar.

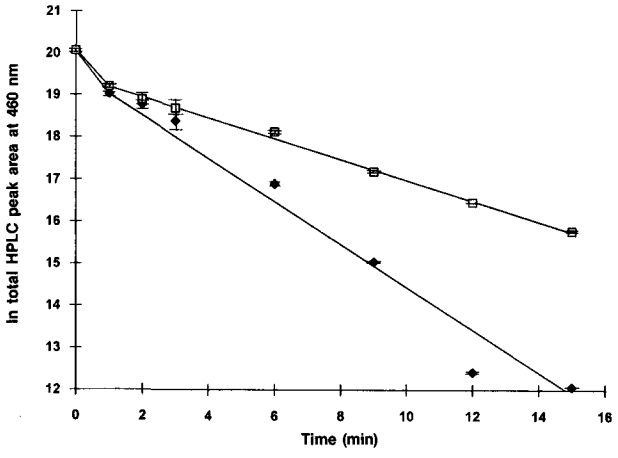

FIG. 4. Kinetics of color loss over 15 min heating at 210°C in Model Systems B (◆) and M (□). Color was equated to the total high-performance liquid chromatography (HPLC) peak area at 460 nm (the λ_{max} of β-carotene).

As for all-*trans*-β-carotene degradation, color loss takes place in two stages for both model systems. An initial rapid loss takes place between 0–1 min, followed by a lower rate of color loss between 1–15 min, which obeys first-order reaction kinetics. As seen from Table 1, the rate constants for color loss and all-*trans*-β-carotene degradation are similar, especially for Model System M. When ln total HPLC peak area at 460 nm was plotted against ln mg all-*trans*-β-carotene, a linear relationship was obtained over 0–15 min for Model System M, but only over 0–12 min for Model System B. The lower rate of color loss observed after 12 min heating relative to the rate of all-*trans*-β-carotene loss is most likely due to other degradation products contributing to absorption at 460 nm. In a study of the degradation of all-*trans*-β-carotene added to wheat flour prior to extrusion cooking, the color loss was always less than the loss of either all-*trans*-β-carotene alone or the combined loss of all-*trans*-β-carotene and its 9-*cis* and 13-*cis* isomers (21). It was postulated that the higher color retention was due to the formation of colored β-carotene thermal degradation products.

Color loss due to all-*trans*-β-carotene degradation during extrusion cooking has been reported to follow first-order reaction kinetics (21). The rate constant, calculated from the data given by Guzman-Tello and Cheftel (21), at 220°C is 0.87 min^{-1}, which is in line with the experimental rate constants for color loss obtained for Model System B over both 0–1 and 1–15 min (assuming color loss obeys first-order reaction kinetics over 0–1 min).

No studies have been reported in the literature on the effect of amino acids on β-carotene stability at temperatures comparable to those used in the current study. The protection afforded by phenylalanine appears to be of the same order of magnitude as that given by the antioxidant 3,5-di-*tert*-butylhydroxytoluene (BHT). BHT decreased the rate constant for β-carotene degradation by a factor of 1.5–1.8 when added to wheat flour prior to extrusion cooking at 160–220°C and at a moisture level of less than 13.7% (21). The mechanism by which phenylalanine may protect all-*trans*-β-carotene from thermal degradation will be reported in a subsequent paper.

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