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Effect of type of oxidation on beta-carotene loss and volatile products formation in model systems

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The effect of different types of oxidation on β-carotene loss and the formation of volatile products was investigated by autoxidation in a microcrystalline cellulose model system, which was compared with photosensitized and chemical oxidations in solution. Beta-carotene loss was faster during autoxidation at 80°C, followed by chemical oxidation, photosensitized oxidation and autoxidation at 20°C. Similar volatile degradation products were found for the different types of oxidation, however, their relative concentrations varied. Autoxidation at 20°C and chemical oxidation led to several volatile oxidation products, originating mainly from cleavage at bonds 7–8, 9–10 and 8–9 of the β-carotene molecule. However, autoxidation at 80°C and photosensitized oxidation led to more specific oxidation products: dihydroactinidiolide and β-ionone, respectively.

INTRODUCTION

The oxidation of β-carotene is of commercial importance. It is desirable in the production of tea and tobacco as it plays an important role in their flavor and aroma development (Wahlberg *et al.*, 1977; Yamanishi *et al.*, 1980; Enzell, 1981). In other foods such as dehydrated vegetables, however, oxidation of β-carotene can diminish the attractive color of foods, their nutritive value and flavor, leading to unacceptability and reduced storage life (Falconer *et al.*, 1964; Archer & Tannenbaum, 1979). Accurate monitoring of β-carotene in food systems is desirable as it would lead to improved information on the effects of storage on β-carotene loss, and to accurate prediction of vitamin content and of product acceptability.

Several attempts have been made to elucidate the mechanism for the oxidative degradation of β-carotene (Alekseev *et al.*, 1968; Gagarina *et al.*, 1970; Chou & Breene, 1972; Finkel'stein *et al.*, 1973, 1974; Arya *et al.*, 1979; Ramakrishnan & Francis, 1979; Goldman *et al.*, 1983). However, the data are contradictory and difficult to interpret because of failure in reporting or controlling important parameters and, in most of the investigations, the methodology for β-carotene determination was not appropriate.

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The objective of this study was to follow β-carotene oxidation in model systems and to associate volatile oxidative degradation products with different types of oxidation: autoxidation at 20 and 80°C, photosensitized and chemical oxidations.

MATERIALS AND METHODS

Synthetic all-*trans*-β-carotene (Sigma Chemicals, St Louis, MO) was purified by column chromatography using alumina as adsorbent. The purity was tested by HPLC. All solvents used for extraction were glass-distilled, and the solvents used for HPLC were UV spectroscopic grade.

The oxidation of β-carotene was carried out under the following conditions:

Autoxidation at 20°C

All-*trans*-β-carotene (40 mg) impregnated in 40 g of microcrystalline cellulose (Avicel; FMC Corporation, Philadelphia, PA) (Chou & Breene, 1972; Baloch *et al.*, 1977; Arya *et al.*, 1979; Ramakrishnan & Francis, 1979; Stefanovich & Karel, 1982) was placed in a 500 ml round-bottomed distillation flask covered with aluminum foil and oxidized under a 10 ml/min stream of pure oxygen (Michigan Welding Supply, Lansing, MI), at 20 ± 1°C.

Autoxidation at 80°C

The reaction conditions were the same as described above, but at a temperature of 80 ± 1°C.

Photosensitized oxidation

A solution of 100 mg of all-*trans*-β-carotene and 2 mg of rose bengal (Suyama *et al.*, 1983) in 100 ml of absolute ethanol was placed in a 500 ml round-bottomed distillation flask. The oxidation was carried out at 20 ± 1°C with oxygen bubbling at a rate of 10 ml/min, under a 20 W fluorescent lamp (soft white F20T12.SW, General Electric, Cleveland, OH) 5 cm away from the reaction vessel.

Chemical oxidation

Metachloroperbenzoic acid (Aldrich Chemicals, Milwaukee, WI) was added to all-*trans*-β-carotene (ratio of 0·2 mole/mole) in methylene chloride. The solution was introduced into a flask topped with calcium chloride guard and immersed in an ethanol bath at 0 ± 1°C with magnetic stirring (Marty & Berset, 1986).

Periodically, samples were withdrawn from each reaction vessel and analysed in duplicate for UV-visible spectra and β-carotene content. When the concentration of β-carotene reached half of its original level, some samples were analysed for the volatile degradation products.

Petroleum ether extract was obtained by vigorous vortexing of the sample with five 25 ml portions of acetone : petroleum ether (50 : 50), followed by centrifugation at 650 rpm for 2 min. The extract was washed free of acetone with water, filtered through anhydrous sodium sulphate, and concentrated to 10 ml.

UV-visible absorbance spectra of the samples in petroleum ether were performed on a Perkin-Elmer Lambda 4B UV/Vis C099–1301 spectrophotometer (Perkin-Elmer, Norwalk, Conn., USA) from 300 to 600 nm.

Quantitative determination of β-carotene was performed on an M6000A Waters Associates High Pressure Liquid Chromatograph (HPLC) (Waters Associates, Milford, MA, USA) attached to a Waters model 450 variable wavelength detector operating at 450 nm. The column used was a 10 micra Waters Micro-Porasil (30 cm × 0·39 cm). A gradient of acetone in hexane (0·5, 2, 5, 8 and 15% per 12 min each) was used at a flow rate of 1·5 ml/min.

Volatile degradation products

The volatiles formed during the oxidation of β-carotene were collected at 60°C for 90 min by a Tenax GC trap system (Tenax, Hewlett Packard, Palo Alto, CA, USA) under a 457 mm Hg vacuum, separated on a Hewlett-Packard (HP) 5890 gas chromatograph (GC) with a flame ionization detector (FID) and a 30 m × 0·25 mm Heliflex Bonded FSOT Superox polyethylene glycol capillary column (Heliflex, Hewlett Packard, Palo Alto, CA, USA) (column temperature: 30°C/5 min, then 2°C/min to 180°C and held at 180°C for 10 min, injector and detector temperature: 200 and 275°C, respectively), and identified by comparing retention index and GC/mass spectrometry (MS) spectra with that of standards as described by Glória *et al.* (1991). As a control, volatiles were also collected for purified all-*trans*-β-carotene in order to evaluate the formation of volatiles due to the trapping system.

RESULTS AND DISCUSSION

UV-visible spectra

Figure 1 shows the change in the UV-visible absorbance spectra of the petroleum ether extract due to the degradation of β-carotene during the different oxidation treatments. In every type of oxidation investigated, there was a significant increase in the apparent absorbance throughout the entire spectra before the absorbance finally decreased. This same behavior was observed by Hunter & Krakenberger (1946) during oxidation of β-carotene in peanut oil, and by Seely & Meyer (1971) during oxidation of β-carotene photosensitized by hypericin.

In every type of oxidation, no significant shift in wavelength of the main absorbance band (450 nm), or change in the overall shape of the spectra was observed; however, a small extent of *trans–cis* isomerization was observed based on the presence of a low-intensity *cis* peak at 340 nm.

The increase in the intensity of the absorption spectra observed at the earlier stages of every treatment could be due to the higher molar extinction coefficients of the oxidation products compared to that of all-*trans*-β-carotene (Foppen, 1971).

Beta-carotene loss

The HPLC method developed was able to separate β-carotene from its degradation products and was therefore used to follow the loss of β-carotene in the model system. According to the HPLC pattern observed for samples oxidized by the different methods, β-carotene was not the only compound absorbing at 450 nm. Figure 2 shows the contribution of all-*trans*-β-carotene (elution time—3 min) and of its degradation products (elution time—4·00, 6·30, 7·12, 11·00, 11·50 and 13·05 min) to the absorption at 450 nm, at the time of maximum absorbance indicated in Fig. 1. These compounds showed polarity very similar to that of β-carotene; however, they were not identified.

Marty & Berset (1986, 1988) identified several compounds from the degradation of all-*trans*-β-carotene, among them, mono- or poly-*cis*-stereoisomers, diepoxide derivatives, apo-carotenal, a polyene ketone, a dihydroxide derivative and a monohydroxide diepoxide derivative. All of these compounds had polarities similar to that of β-carotene and absorbed at 450 nm.

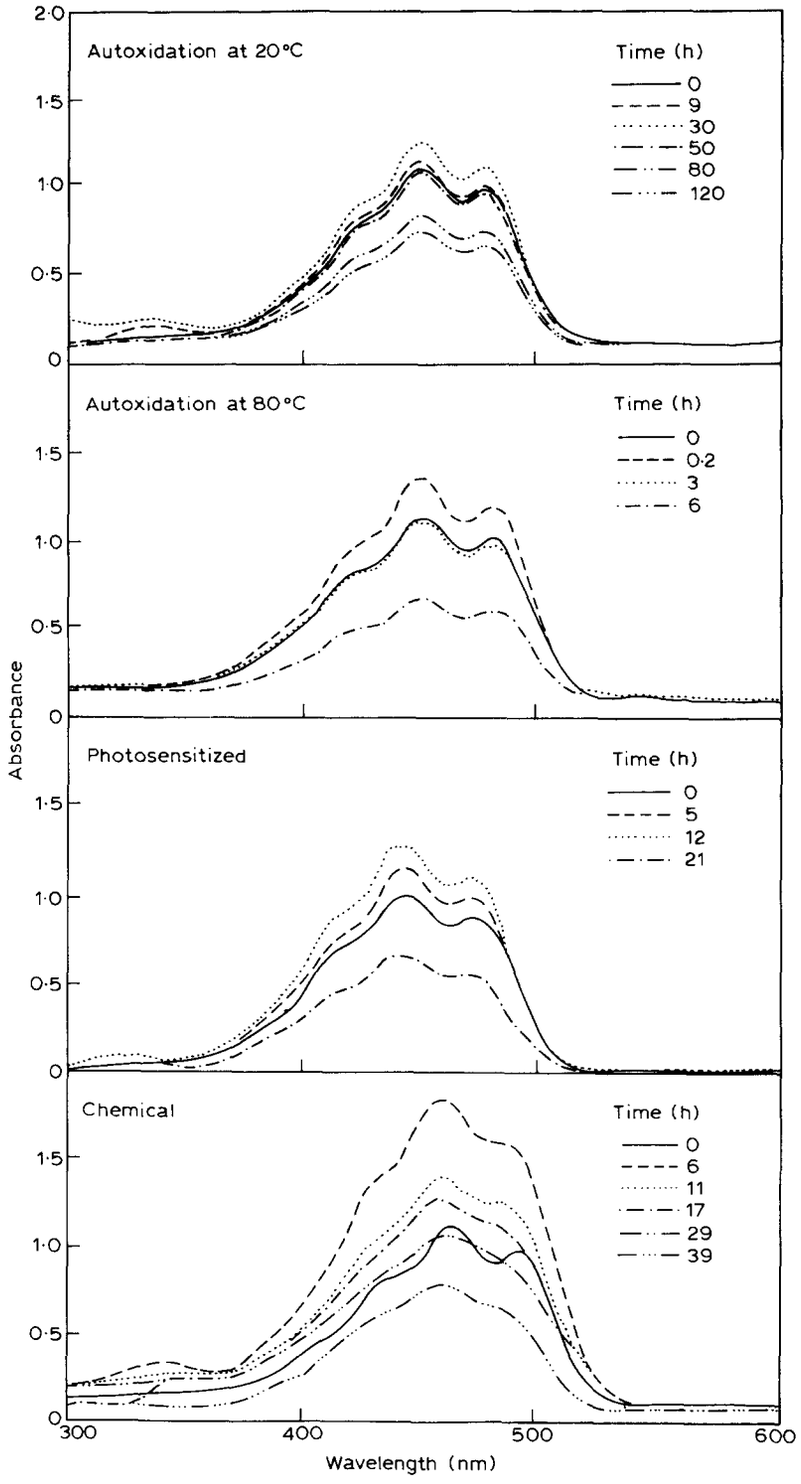

Fig. 1. UV-visible absorbance spectra of β-carotene during autoxidation at 20 and 80°C, and photosensitized and chemical oxidation.

According to Panalaks & Murray (1970) and Sweeney & Marsh (1970) isomers and oxidation products from all-*trans*-β-carotene have lower vitamin-A activity.

Figure 3 shows all-*trans*-β-carotene concentration as a function of time for the four oxidation methods. In contrast to Fig. 1, the HPLC method showed that β-carotene concentration decreased as oxidation proceeded, giving a better measurement of its loss.

The rate of β-carotene loss was higher during autoxidation at 80°C, followed by chemical oxidation, photosensitized oxidation and autoxidation at 20°C.

Effect of oxidation type on volatile degradation products

Table 1 summarizes the relative concentration of volatile products identified from oxidized β-carotene, collected when 50% of its original content had been lost and from the control. Similar degradation products were found for the different types of oxidation; however, their relative concentrations varied. For the control, β-cyclocitral and 2,2,6-trimethyl-cyclohexane-carboxaldehyde were present in significant amounts. These compounds could originate from oxygen attack on

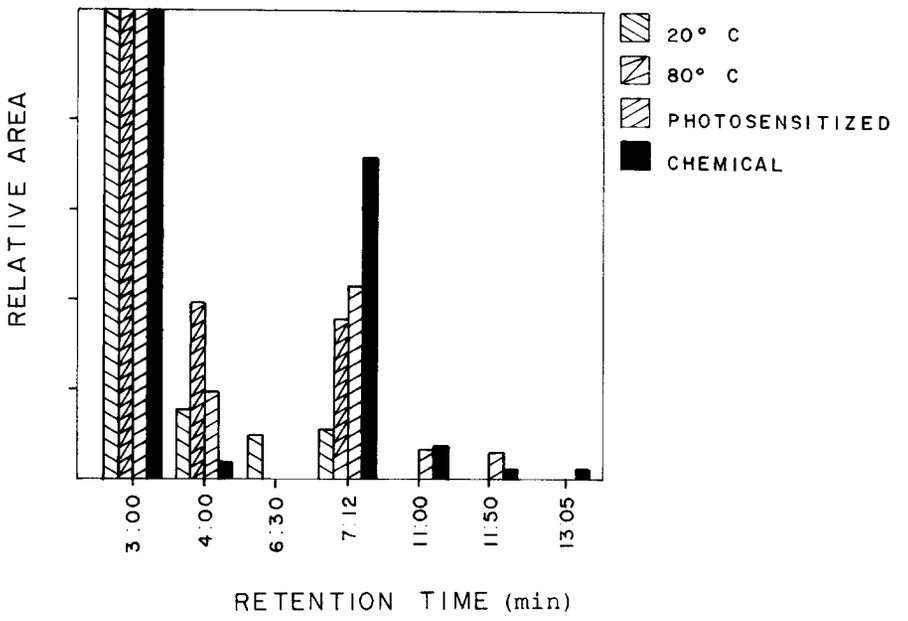

Fig. 2. Contribution of β-carotene (retention time—3 min) and its degradation products (elution time—4·00, 6·30, 7·12, 11·00, 11·50 and 13·05 min) to the absorbance at 450 nm, at the time of maximum absorbance (Fig. 1) during autoxidation at 20 and 80°C, and photosensitized and chemical oxidations of β-carotene. HPLC conditions described in the text.

bond 7–8 of the β-carotene molecule (Glória *et al.*, 1991) during sample preparation (Fig. 4).

When 50% of β-carotene had been lost during autoxidation at 20°C, 2,2,6-trimethyl-cyclohexane-carboxaldehyde (39%) and 5,6-epoxy-β-ionone (32%) were the major volatile oxidation products. These compounds originate from cleavage at bonds 7–8, and 9–10, respectively, of the β-carotene molecule (Fig. 4). Attack of oxygen on bond 8–9 was also observed, leading to the formation of dihydroactinidiolide (18%). Among these oxidation products, 5,6-epoxy-β-ionone has haylike and floral flavors and dihydroactinidiolide has flavor characteristics of black tea, hay, peach and gardenia (Glória *et al.*, 1991).

At 80°C, autoxidation of β-carotene at up to 50% of its original level, led mainly to dihydroactinidiolide (63%), indicating that bond 8–9 was the major site of oxygen attack. This volatile oxidation product has a flavor of black tea, hay, peach and gardenia.

During photosensitized and chemical oxidation of β-carotene, β-ionone was observed to be the main volatile degradation product contributing 43·5 and 33·7%, respectively, of the total volatile products formed, suggesting that bond 9–10 of the β-carotene molecule (Fig. 4) was the most susceptible site of cleavage. Beta-ionone has been reported to have violet, floral and haylike flavor (Glória *et al.*, 1991).

Products originating from cleavage at bond 7–8 were

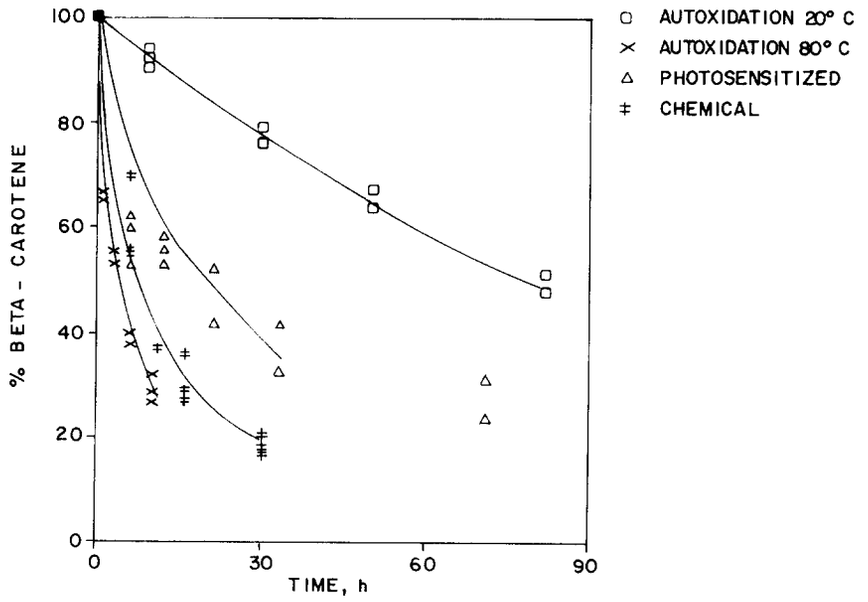

Fig. 3. Loss of β-carotene (measured by normal-phase HPLC) during autoxidation at 20 and 80°C, and photosensitized and chemical oxidations.

Table 1. Relative concentration of volatile compounds produced under different oxidation conditions

Bond[a]	Compound	Oxidation[b]				
		Control	Autoxidation		Photo	Chemical
			20°C	80°C		
6–7	α-Isophorone (1)	0	11	0	0	0
7–8	2,2,6-Trimethyl-cyclohexane-carboxaldehyde (2)	67	100	1	15	35
7–8	β-Cyclocitral (3)	100	16	0	9	48
7–8	Neral (4)	0	0	0	7	10
8–9	Dihydroactinidiolide (5)	0	45	100	46	65
9–10	β-Ionone (6)	15	13	7	100	100
9–10	5,6-Epoxy-β-ionone (7)	0	82	53	53	39

[a] Volatile products originate from cleavage at the C–C bond of the β-carotene molecule.
[b] Experimental details are given in the text. The peak area of highest intensity was designated 100%.

also predominant during chemical oxidation (31% of the total profile). Among them, neral has been observed to have a lemon flavor.

CONCLUSION

Non-volatile degradation products from oxidation of all-*trans*-β-carotene show polarities very similar to that of β-carotene and their separation could only be accomplished with normal-phase HPLC using a gradient elution system. These degradation products absorb in the same wavelength region as β-carotene, which could lead to overestimated vitamin-A values.

The degradation of β-carotene was faster during autoxidation at 80°C, followed by chemical and photosensitized oxidations and autoxidation at 20°C.

The same volatile compounds resulted from the different types of oxidation; however, their relative intensity varied, suggesting that at each oxidation condition, a specific site in the chain was more susceptible to cleavage.

Autoxidation at 20°C and chemical oxidation led to several volatile oxidation products, originating mainly from cleavage at bonds 7–8, 9–10 and 8–9 of the β-carotene molecule.

Autoxidation at 80°C and photosensitized oxidation led to more specific cleavage and, therefore, more specific volatile products: dihydroactinidiolide, originating from bond 8–9, was the major volatile oxidation product from autoxidation at 80°C and β-ionone, a product from cleavage at bond 9–10, from photosensitized oxidation.

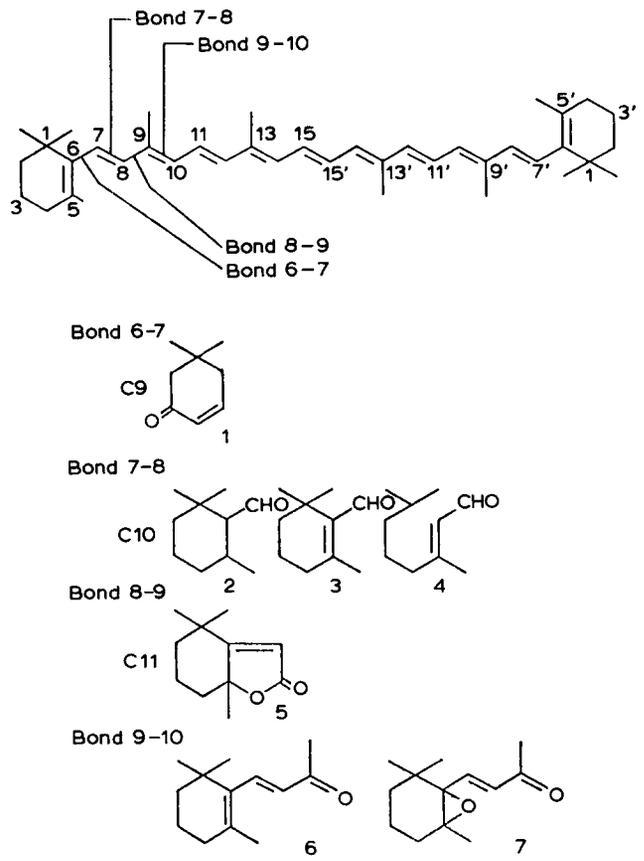

Fig. 4. Bond location of oxygen attack resulting in volatile degradation products from β-carotene (compounds numbered according to Table 1).

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