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Formation of apocarotenals and epoxycarotenoids from β-carotene by chemical reactions and by autoxidation in model systems and processed foods

Evelyn B. Rodriguez [a], Delia B. Rodriguez-Amaya [b,*]

[a] *Institute of Chemistry, University of the Philippines at Los Baños, College, Laguna 4031, Philippines*
[b] *Departamento de Ciência de Alimentos, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, C.P. 6121, 13083-862 Campinas, SP, Brazil*

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Abstract

It is generally accepted that oxidation of carotenoids begins with epoxidation and cleavage to apocarotenals. However, systematic studies to demonstrate occurrence of these reactions are lacking. In this study, the products formed by epoxidation with *m*-chloroperbenzoic acid (MCPBA), oxidative cleavage with $KMnO_4$ and autoxidation in model systems, in the presence and absence of light, at ambient condition were identified. The presence of oxidation products was also verified in processed products. β-carotene-5,6-epoxide, β-carotene-5,8-epoxide, β-carotene-5,6,5′,6′-diepoxide, β-carotene-5,6,5′,8′-diepoxide and β-carotene 5,8,5′,8′-diepoxide were formed by the reaction of β-carotene with MCPBA. The oxidation products with $KMnO_4$ were identified as β-apo-8′-carotenal, β-apo-10′-carotenal, β-apo-12′-carotenal, β-apo-14′-carotenal and β-apo-15-carotenal, along with semi-β-carotenone and monohydroxy-β-carotene-5,8-epoxide. Except for β-carotene 5,6,5′,6′-diepoxide, these products were detected in the model systems. Some of these products were also found in mango juice, acerola juice and dried apricot. Increased *Z*-isomerization was also observed and *Z*-isomers of the oxidation products were detected.

Keywords: Carotenoids; Oxidative degradation; Epoxycarotenoids; Apocarotenals; β-Carotene; Oxidation

1. Introduction

Because of their role as natural pigments, vitamin A precursors and bioactive phytochemicals with protective effects against degenerative diseases, carotenoids have been the subject of a plethora of scientific articles, especially on the human health implications. Yet there are some basic aspects about these compounds which have not been adequately addressed. For example, the highly unsaturated carotenoid molecules are prone to oxidative degradation, and losses of carotenoids during the processing and storage of food have been reported in numerous papers. However, unlike lipid oxidation for which detailed information on the different reactions that constitute the process, the volatile and non-volatile decomposition products and the mechanistic pathways have been presented and discussed in the literature, knowledge on carotenoid oxidation is needed, even for β-carotene which is the most studied of all carotenoids. Better understanding of the reactions and the underlying mechanisms of the oxidative degradation of carotenoids is needed not only in avoiding losses of these important compounds during processing and storage of foods, but also in evaluating the implications in in vivo biological processes. A recent paper, for example, reported that cleavage products of β-carotene generated in vitro under oxidative conditions increased oxidative stress in isolated rat liver mitochondria by impairing mitochondrial function (Siems et al., 2002). On the other hand, 2,6-cyclolycopene-1,5-diol, an oxidation product of lycopene, was

* Corresponding author. Tel.: +55 19 3788 4013; fax: +55 19 3788 2153.
E-mail address: delia@fea.unicamp.br (D.B. Rodriguez-Amaya).

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found to marginally increase the expression of connexin 43 in 10T1/2 cells and in human keratinocytes, leading the authors to suggest that the conversion of dietary carotenoids to compounds that can increase gap junctional communication might play a role in the protective action of carotenoid-rich foods (King et al., 1997).

It is generally accepted that autoxidation of carotenoids is a free radical process, commencing with epoxidation and cleavage to apocarotenals. However, more studies to demonstrate the occurrence of these reactions are needed. Investigations in different food systems and conditions, taking advantage of refinements in analytical methodology, should be carried out.

Of the few studies, in which the epoxycarotenoids and/or the apocarotenals formed from β-carotene were identified, this carotenoid was dissolved in toluene (El-Tinay & Chichester, 1970; Handelman, van Kuijk, Chatterjee, & Krinsky, 1991) and glycerol (Onyewu, Ho, & Daun, 1986) or in solid state in sealed tubes or ampoules (Marty & Berset, 1986, 1990), conditions very different from those encountered in food. Marty and Berset (1986, 1988, 1990) did simulate extrusion cooking, Kanasawud and Crouzet (1990) suspended β-carotene in water by sonication and Ouyang, Daun, Chang, and Ho (1980) simulated commercial deodorization of bleached red palm oil to which 1% β-carotene was added. The experiments were carried out by Ouyang et al. (1980) and Onyewu et al. (1986) at 210 °C for 4 h, by Marty and Berset (1986, 1988, 1990) at 180 °C for 2 h or extrusion with the last heating zone at 180 °C, by Kanasawud and Crouzet (1990) at 97 °C for 3 h, by El-Tinay and Chichester (1970) and Handelman et al. (1991) at 60 °C, thus representing thermal degradation. Handelman et al. (1991) also employed a temperature of 37 °C. In a more recent paper (Henry et al., 2000), β-carotene, β-cryptoxanthin and lycopene were adsorbed on a C18 solid phase and exposed to continuous flow of water saturated with oxygen or ozone at 30 °C. The effect of light was not evaluated in these investigations.

Marty and Berset (1986, 1988, 1990) studied both the epoxycarotenoids and the apocarotenals; Ouyang et al. (1980), Onyewu et al. (1986) and Handelman et al. (1991) investigated only the apocarotenals; and El-Tinay and Chichester (1970) and Kanasawud and Crouzet (1990) investigated only the epoxycarotenoids.

Insights into the mechanism of carotenoid oxidation can be derived from model systems, which are more easily controlled than foods and the formation of initial, intermediate and final products can be more easily monitored. Extrapolation to foods, however, needs to be done with caution since simple model systems may not reflect the nature and complexity of the multi-component food matrix and the interactions that can occur. The model systems should mimic the conditions in foods as much as possible.

In the present paper, the epoxycarotenoids and apocarotenals formed during the oxidation of β-carotene with atmospheric oxygen in low-moisture and aqueous model systems at ambient temperature were investigated. These systems simulated dehydrated foods and juices or purees, respectively, during storage. The presence of the oxidation products in processed foods (dried apricot, acerola juice and mango juice) was also investigated. Being intermediates with fast turn-over, epoxycarotenoids and apocarotenals formed by autoxidation do not accumulate and are usually found at very low levels, making their detection and identification very difficult. Thus, epoxidation by *m*-chloroperbenzoic acid (MCPBA) and oxidative cleavage by $KMnO_4$ were also carried out for comparative purposes, confirming the identifications.

2. Materials and methods

2.1. Materials

Synthetic β-carotene (95%, Sigma Chemical Company, St. Louis, MO) was recrystallized to yield (all-*E*)-β-carotene (98%) together with a *Z*-isomer (2%). The carotenoid was dissolved in minimum amount of dichloromethane and methanol was added dropwise until the solution became turbid. Crystallization was allowed to occur at room temperature and completed in an ice bath.

MCPBA (77%) was obtained from Aldrich (Milwaukee, WI) and $KMnO_4$ was obtained from Bayer (Tarrytown, NY).

Processed mango juice, acerola juice and dried apricots were bought from a supermarket in Campinas, São Paulo, Brazil. Food products with β-carotene as the predominating carotenoid were chosen.

2.2. Epoxidation of β-carotene by m-chloroperbenzoic acid

A saturated aqueous solution of $NaHCO_3$ was added to an ice-cooled solution of β-carotene in dichloromethane. To the resulting two-layered mixture, a solution of MCPBA (1.5 mol equiv.) in dichloromethane was added dropwise over 2 h with vigorous stirring. The reaction mixture was stirred for another 2 h after the complete addition of MCPBA, after which the β-carotene content was reduced to about 22% of the original amount as shown by high performance liquid chromatography (HPLC). The organic layer was separated from the reaction mixture and washed successively with a 20% solution of $Na_2S_2O_3$, saturated aqueous $NaHCO_3$ and water, and dried over anhydrous Na_2SO_4. An aliquot of the product mixture in dichloromethane was dried under a stream of nitrogen, taken up in acetone and subjected to HPLC, under the conditions described in Section 2.7.

2.3. Oxidative cleavage of β-carotene by potassium permanganate

A solution of β-carotene in dichloromethane containing several drops of water was treated with $KMnO_4$ (2.6 mol equiv.) at room temperature with stirring for

12 h, after which the content of β-carotene was about 20% of the original amount as revealed by HPLC. The reaction mixture was filtered to remove MnO_2 and the filtrate was washed several times with water (until the color of excess $KMnO_4$ was removed) and dried over anhydrous Na_2SO_4. An aliquot of the product mixture in dichloromethane was dried under a stream of nitrogen, taken up in acetone and subjected to HPLC, as described in Section 2.7.

2.4. Preparation and storage of the model systems

For the low-moisture model system, β-Carotene was impregnated into starch using the following procedure. Commercial potato starch (240 g) was placed in a 1 l-round bottom flask and a portion of a dichloromethane solution of β-carotene (12 mg in 500 ml of dichloromethane), enough to cover the starch, was added; the solvent was then evaporated in a rotary evaporator. The process was repeated until the β-carotene solution was completely added and uniformly dispersed in the starch. Residual solvent was then removed under a stream of nitrogen. The impregnated starch was packed in 12 polyethylene packets, each packet containing approximately 1 mg β-carotene in 20 g of starch. The packets after sealing were stored at room temperature, six being placed on a table continuously exposed to a ceiling 40-W standard fluorescent light (at an intensity of 318 lux), simulating lighting conditions in processing plants or supermarkets, and the remaining six kept in the dark. Formation of non-volatile degradation products was monitored by HPLC after 1, 2, 7, 14 and 21 days.

For the aqueous model system, a solution of β-carotene (20 mg) in dichloromethane was added to water (100 ml) and dispersed in water by adding 2–3 drops of Tween 20. Dichloromethane was removed under a stream of nitrogen and the resulting aqueous mixture was transferred into five 25-ml colorless serum bottles, each bottle containing approximately 4 mg β-carotene in 20 ml of water. The bottles were sealed with Teflon-coated septa and aluminum caps and exposed to fluorescent light at room temperature as previously described. Formation of non-volatile products was monitored by HPLC after 15, 21 and 36 days.

2.5. Extraction of carotenoids

The carotenoids from the food products (30 g for mango juice and 20 g for acerola juice and dried apricot) and starch (20 g) were extracted three times with cold acetone (about 100 ml each time) and partitioned into petroleum ether (50 ml) with the aid of water in a separatory funnel. The water-dispersed carotenoids were extracted three times with 100 ml of dichloromethane each time. The petroleum ether and dichloromethane solutions of carotenoids were dried under a stream of nitrogen, taken up in acetone and injected into the HPL chromatograph.

2.6. Identification of the oxidation products

Identification of the oxidation products was carried out by the combined use of chromatographic behavior (in HPLC and TLC), visible absorption spectra obtained by a photodiode array detector, chemical tests and mass spectra. Because oxidation and isomerization of carotenoids could occur during tedious and time-consuming separation and isolation procedures, resulting in products that could be mistaken for those produced by the chemical reactions and autoxidation that were being investigated, especially at the very low levels at which these products were found, identification was carried out with the total extracts using the photodiode array and mass detectors and chemical reactions.

To express the spectral fine structure, the %III/II was calculated. This is the ratio of the height of the longest-wavelength absorption peak, designated III, and that of the middle absorption peak, designated II, taking the minimum between the two peaks as baseline, multiplied by 100 (Britton, 1995). The *Z*-isomers were identified by λ_{maxs} lower than those of the all-*E*-carotenoids and by the presence of the "*cis*" peak at about 142 nm below the longest-wavelength absorption maximum of the all-*E*-form. The location of the *Z*-double bond was indicated by the $\%A_B/A_{II}$, which is the ratio of the height of the "*cis*" peak, designated A_B, and that of the middle main absorption peak, designated A_{II}, multiplied by 100 (Britton, 1995). This ratio is an indicator of the intensity of the "*cis*" peak, which is greater as the *Z*-double bond is closer to the center of the molecule, being equal to 10, 45 and 56 for (9*Z*)-β-carotene, (13*Z*)-β-carotene and (15*Z*)-β-carotene, respectively, in a study in which the structures were confirmed by ^1H NMR (Mercadante, Steck, & Pfander, 1999).

Carotenoids with epoxy groups in the 5,6 or 5,6,5′,6′ positions were indicated by conversion to the furanoid derivatives using a few drops of ethanolic 0.1 N HCl. The conversion was indicated by the disappearance or decrease of the peaks of the original epoxycarotenoids and the appearance or increase of the peaks of the corresponding furanoids, the spectra of the 5,8-epoxide and 5,8,5′,8′-diepoxide showing a hypsochromic shift of 20–25 and 50 nm, respectively. Additionally, silica thin-layer (developed with 5% methanol in toluene) chromatograms exposed to HCl fumes gave blue-green and blue spots corresponding to monoepoxides and diepoxides, respectively, in contrast to the original yellow spots.

Carbonyl groups conjugated with the polyene chain were confirmed by reduction with $NaBH_4$. The reduction was indicated by the disappearance of the peaks with one-band spectra characteristic of carbonyl-containing carotenoids and the appearance of peaks with the three-maxima spectra characteristic of the resulting hydroxycarotenoids.

Although the chromophore was indicated by the visible absorption spectra, the presence of hydroxyl groups was based solely on the retention time, the dihydroxycarotenoids eluting at the beginning and the monohydroxy in

the middle of the chromatogram; thus, this identification was tentative.

2.7. HPLC instrumentation and conditions

The HPLC system consisted of a Waters separation module (Model 2690, Waters Corporation, Milford, MA) equipped with quaternary pump, autosampler injector, degasser, photodiode array (PDA Model 996) and electron impact mass (Waters Integrity System with Thermabeam interface) detectors, controlled by a Millenium workstation (Version 2010).

The HPLC separations were carried out on a monomeric C18 column (Spherisorb ODS2, 3 μm, 4.6 mm × 150 mm, Waters Corp., Milford, MA). The mobile phase consisted of acetonitrile (with 0.05% triethylamine), methanol and ethyl acetate, used at a flow rate of 0.5 ml/min. A concave gradient (curve 10) was employed, consisting of an isocratic elution with 95:5 (v/v) acetonitrile/methanol for the first 10 min, and a gradual change to 60:20:20 (v/v/v) acetonitrile/methanol/ethyl acetate during the next 10 min; this composition was maintained for the rest of the run. The column was re-equilibrated for 15 min under the initial isocratic condition. Detection with PDA was at the wave-

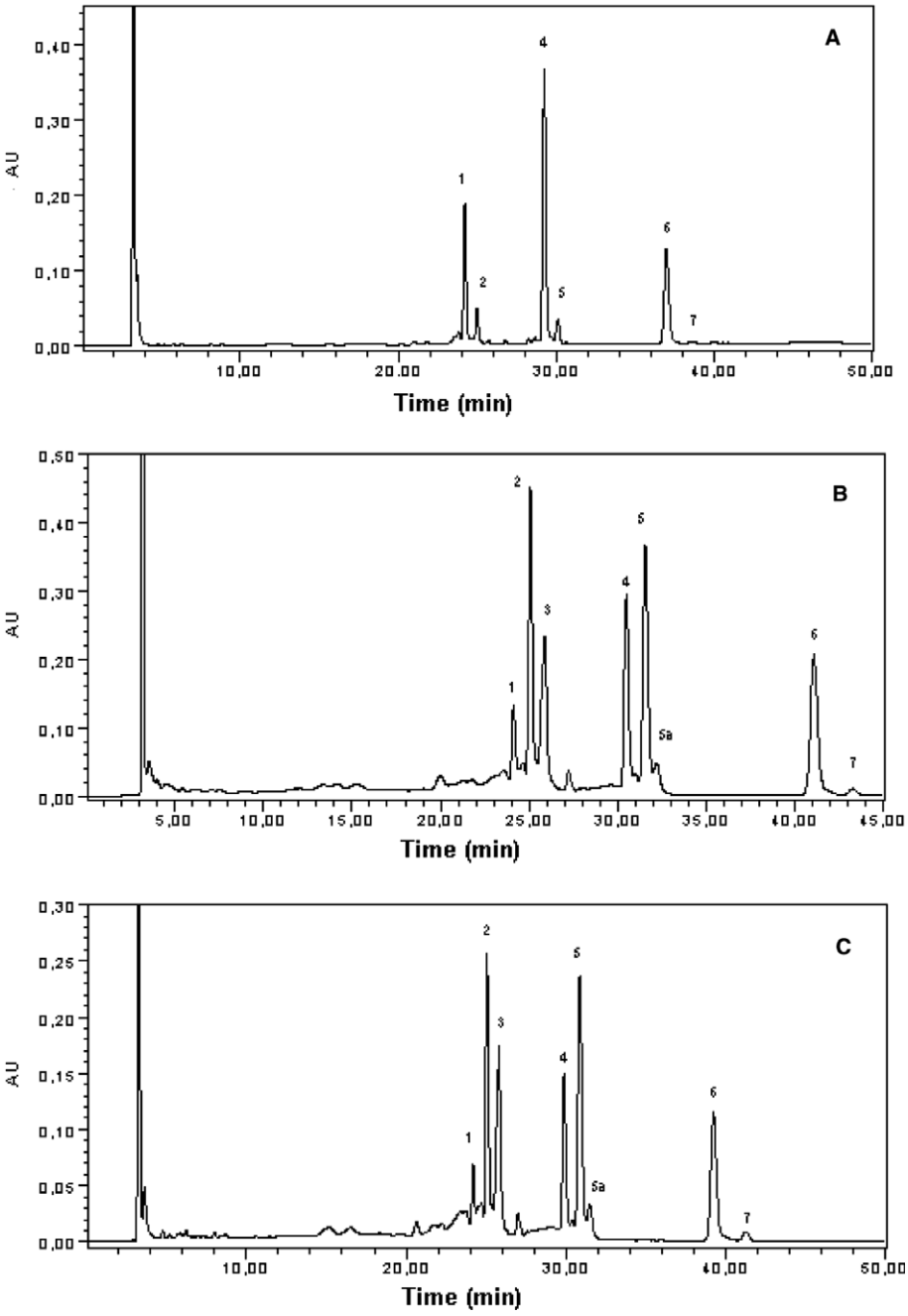

Fig. 1. HPLC profiles of product mixtures from reactions of β-carotene with MCPBA: (A) with $NaHCO_3$, (B) without $NaHCO_3$, (C) with $NaHCO_3$ followed by treatment with dilute HCl. HPLC conditions are described in the text and peak identification is given in Table 1.

lengths of maximum absorption (max plot); the absorption spectra were recorded from 300 to 550 nm. For HPLC-MS, the expansion region and nebulizer temperatures were 80 and 90 °C, respectively. The ionizing voltage was 70 eV and the temperature of the ion source was 210 °C. The mass spectra were acquired from m/z 150 to 650.

Precautionary measures to prevent artifact formation during analysis were rigorously followed, including completion of the analysis within the shortest possible time; exclusion of oxygen; protection from light; avoiding high temperatures; avoiding contact with acid and use of high purity solvents, free from harmful impurities (Britton, 1991; Davies, 1976; Rodriguez-Amaya, 1999; Schiedt & Liaaen-Jensen, 1995).

3. Results and discussion

3.1. β-Carotene epoxides formed with *m*-cloroperbenzoic acids

MCPBA is a reagent known to effect epoxidation of carbon–carbon double bonds. Treatment of β-carotene with 1.5 equivalents of MCPBA, in the presence of saturated $NaHCO_3$ for 4 h at ice bath temperature, resulted in the formation of a number of monoepoxides and diepoxides. The epoxides in order of elution were identified as β-carotene-5,6,5′,6′-diepoxide (**1**), β-carotene-5,6,5′,8′-diepoxide (**2**), β-carotene-5,6-epoxide (**4**), β-carotene-5,8-epoxide (**5**), with the 5,6-epoxide (**4**) and 5,6,5′,6′-diepoxide (**1**) as the major products (Fig. 1A). Peaks of the unreacted all-*E*-β-carotene (**6**) and *Z*-β-carotene (**7**) could also be seen in the chromatogram. The semi-systematic names and identifying characteristics of these carotenoids are presented in Table 1.

Sodium bicarbonate was added to the reaction mixture to mop out the *m*-chlorobenzoic acid being released from MCPBA; this was necessary to ensure the formation of unrearranged epoxides as acid would catalyze 5,6- to 5,8-epoxide rearrangement. When the reaction was conducted without $NaHCO_3$, the major products were the rearranged 5,6,5′,8′-diepoxide (**2**) and 5,8-monoepoxide (**5**), and in

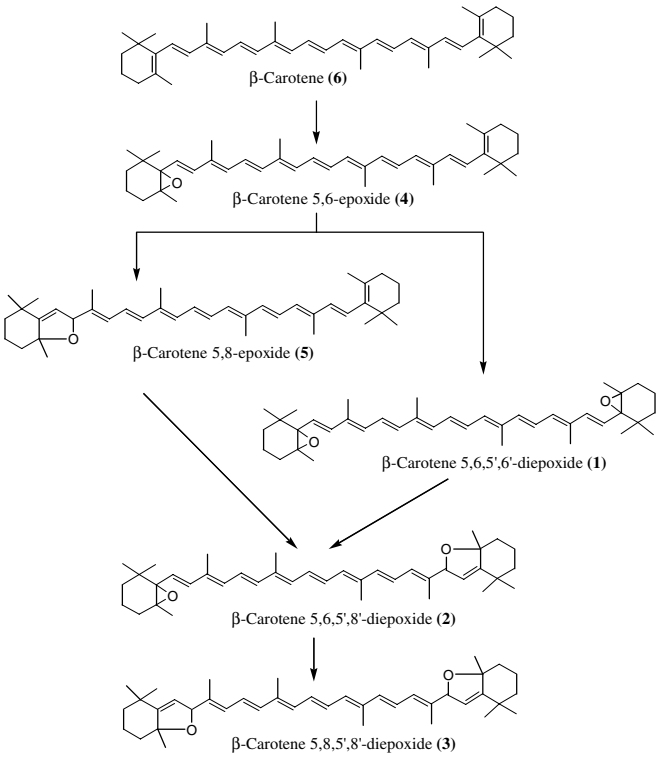

Fig. 2. Formation of epoxycarotenoids from β-carotene with MCPBA.

Table 1
Characteristics of β-carotene epoxides formed with *m*-chloroperbenzoic acid and the unreacted starting material

Peak	Compound	t_R (min)	λ_{max}[a] %III/II (%A_B/A_{II})	Molecular ion formula	Characteristic fragments
1	(all-*E*)-5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene	24.1	418, 443, 472 %III/II = 88	568, $C_{40}H_{56}O_2$	(M-80), (M-146), 205, 165
2	(all-*E*)-5,6,5′,8′-diepoxy-5,6,5′,8′-tetrahydro-β,β-carotene	25.1	401, 424, 450 %III/II = 91	568, $C_{40}H_{56}O_2$	(M-80), (M-146), 205, 165
3	(all-*E*)-5,8,5′,8′-diepoxy-5,8,5′,8′-tetrahydro-β,β-carotene	25.9	381, 403, 427 %III/II = 102	–	–
4	(all-*E*)-5,6-epoxy-5,6-dihydro-β,β-carotene	30.5	(422), 449, 477 %III/II = 57	552, $C_{40}H_{56}O$	(M-80), (M-146), 205, 165
4a	(13*Z*)-5,6-epoxy-5,6-dihydro-β,β-carotene	31.0	334, 439, 475 %A_B/A_{II} = 32	–	–
5	(all-*E*)-5,8-epoxy-5,8-dihydro-β,β-carotene	31.6	(407), 430, 456 %III/II = 58	552, $C_{40}H_{56}O$	(M-80), (M-146), 205, 165
5a	(13*Z*)-5,8-epoxy-5,8-dihydro-β,β-carotene	32.2	317, (395) 425, 449 %A_B/A_{II} = 33	–	–
6	(all-*E*)-β,β-carotene	41.1	(428), 455, 482 %III/II = 22	536, $C_{40}H_{56}$	(M-92), (M-106), (M-137)
7	(13*Z*)-β,β-carotene	43.3	341, (421) 448, (473) %A_B/A_{II} = 46		–

[a] In the mobile phase.

addition 5,8,5′,8′-diepoxide (**3**) was obtained (Fig. 1B). A product mixture obtained from a reaction employing $NaHCO_3$ also yielded the rearranged epoxides as the major products upon treatment with dilute aqueous HCl for a few minutes (Fig. 1C). The probable reaction pathway for the formation of the various epoxides is shown in Fig. 2.

Mass spectrometry would not differentiate between the 5,6- and the 5,8-epoxide group, but visible absorption spectrophotometry would (Britton, 1995; Enzell & Back, 1995). β-Carotene-5,6-epoxide (**4**, $\lambda_{max} = 449$ nm, molecular ion = 552 corresponding to $C_{40}H_{56}O$) exhibited λ_{max} 6 nm lower compared to β-carotene (**6**, $\lambda_{max} = 455$, molecular ion = 536 corresponding to $C_{40}H_{56}$), indicating the loss of a double bond in one of the β-rings. On the other hand, the λ_{max} of β-carotene-5,8-epoxide was 25 nm lower (**5**, $\lambda_{max} = 430$, molecular ion = 552 corresponding to $C_{40}H_{56}O$), commensurate with the loss of a double bond in the polyene chain, in addition to the loss of a β-ring double bond. The 5,6,5′,6′- (**1**, $\lambda_{max} = 443$ nm, molecular ion = 568 corresponding to $C_{40}H_{56}O_2$) 5,6,5′,8′- (**2**, $\lambda_{max} = 424$ nm, molecular ion = 568 corresponding to $C_{40}H_{56}O_2$) and the 5,8,5′,8′-diepoxides (**3**, $\lambda_{max} = 403$ nm) had the expected λ_{max}s, 12, 31 and 52 nm lower, respectively, than that of β-carotene, the first and the second epoxides being transformed into the 5,8,5′,8′-diepoxide on addition of dilute HCl. The β-carotene epoxides also exhibited characteristic fragments in the mass spectra (Table 1) (Enzell & Back, 1995).

Z-isomers of β-carotene epoxides were also detected, indicating that both *E*- and *Z*-β-carotenes were epoxidized.

3.2. β-Apocarotenals formed with $KMnO_4$

β-Apocarotenals were prepared from β-carotene by treatment with excess $KMnO_4$ in the presence of water for 12 h. Cold, dilute, neutral potassium permanganate is a reagent known to allow glycol formation from olefinic compounds. However, the reagent was found to cleave carbon–carbon double bonds of β-carotene, resulting in the formation of a series of apocarotenals (**I**–**V**); in addition, other oxidation products – epoxides (**4**, **5** and **VII**) and semi-β-carotenone (**VI**), were also formed (Fig. 3). The oxidation products in the order of elution were identified as β-apo-15-carotenal (**I**, $\lambda_{max} = 380$ nm, molecular ion = 284 corresponding to $C_{20}H_{28}O$), β-apo-14′-carotenal (**II**, $\lambda_{max} = 412$ nm, molecular ion = 310 corresponding to $C_{22}H_{30}O$), β-apo-12′-carotenal (**III**, $\lambda_{max} = 426$ nm, molecular ion = 350 corresponding to $C_{25}H_{34}O$), β-apo-10′-carotenal (**IV**, $\lambda_{max} = 449$ nm, molecular ion = 376 corresponding to $C_{27}H_{36}O$), β-apo-8′-carotenal (**V**, $\lambda_{max} = 460$ nm, molecular ion = 416 corresponding to $C_{30}H_{40}O$), semi-β-carotenone (**VI**, $\lambda_{max} = 469$ nm molecular ion = 568 corresponding to $C_{40}H_{56}O_2$), hydroxy β-carotene 5,8-epoxide (**VII**, $\lambda_{max} = 430$ nm), β-carotene 5,6-epoxide (**4**, $\lambda_{max} = 449$ nm) and β-carotene 5,8-epoxide (**5**, $\lambda_{max} = 430$ nm), unreacted (all-*E*)-β-carotene (**6**, $\lambda_{max} = 455$ nm), (9*Z*)-β-carotene (**6a**, $\lambda_{max} = 449$ nm) and (13*Z*)-β-carotene (**7**, $\lambda_{max} = 448$ nm). The semi-systematic names and characteristics of the oxidative cleavage products as well as the other associated oxidation products and the carotenoid starting material are presented in Table 2.

The reaction mechanism for the oxidative cleavage of β-carotene likely involves the isomerization of an *E*- to a *Z*-double bond so that *syn*-addition of the permanganate ion could take place to form a cyclic permanganate ester (a well-established reaction intermediate for this type of reaction). This was borne out by the formation of semi-β-carotenone (**VI**) as the major product – a product that resulted from the cleavage of the double bond in the β-ring, a double bond having a permanent *Z*-configuration. In addition, there was increased formation of *Z*-isomers (**6a** and **7**) from the (all-*E*)-β-carotene starting material. The positions of oxidative cleavage is illustrated in Fig. 4.

Aside from the characteristic single, broad absorption peaks at wavelengths commensurate with the number of conjugated double bonds and the molecular ions, the apocarotenals and semi-β-carotenone also gave the corresponding apocarotenols and semi-β-carotenol on reduction with

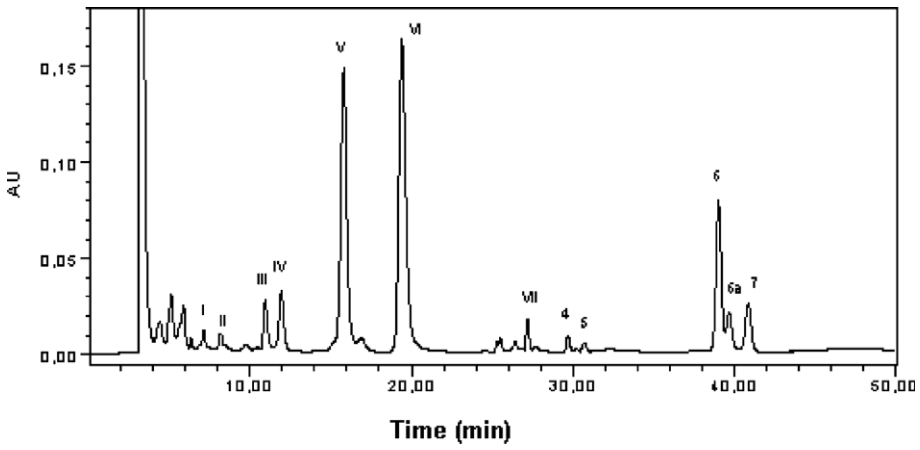

Fig. 3. HPLC profile of the product mixture from the reaction of β-carotene with $KMnO_4$. HPLC conditions are described in the text and peak identification is given in Table 2.

Table 2
Characteristics of β-apocarotenals and other oxidation products from the reaction of β-carotene with $KMnO_4$

Peak	t_R (min)	Compound	λ_{max}[a]	Molecular ion formula	Characteristic fragments
I	7.1	(all-*E*)-15-apo-β-caroten-15-al	380	284, $C_{20}H_{28}O$	(M-29), (M-83), (M-137), (M-149), 205
II	8.2	(all-*E*)-14′-apo-β-caroten-14′-al	412	310, $C_{22}H_{30}O$	(M-29), (M-83), (M-137), (M-149), 205
III	10.9	(all-*E*)-12′-apo-β-caroten-12′-al	426	350, $C_{25}H_{34}O$	(M-29), (M-83), (M-137), (M-149), 205
IV	11.9	(all-*E*)-10′-apo-β-caroten-10′-al	449	376, $C_{27}H_{36}O$	(M-29), (M-83), (M-137), (M-149), 205
V	15.8	(all-*E*)-8′-apo-β-caroten-8′-al	460	416, $C_{30}H_{40}O$	(M-29), (M-83), (M-137), (M-149), 205
VI	19.4	(all-*E*)-5,6-seco-β,β-carotene-5,6-dione	469	568, $C_{40}H_{56}O_2$	(M-16), (M-43), (M-127), (M-137), (M-155)
VII	27.2	(all-*E*)-5,8-epoxy-5,8-dihydro-β,β-caroten-ol	(408), 431, 456		
4	29.7	(all-*E*)-5,6-epoxy-5,6-dihydro-β,β-carotene	(422), 449, 477		
5	30.7	(all-*E*)-5,8-epoxy-5,8-dihydro-β,β-carotene	(407), 430, 456		
6	39.0	(all-*E*)-β,β-carotene	(428), 455, 482		
6a	39.7	(9*Z*)-β,β-carotene	342, (422), 449, 476		
7	40.8	(13*Z*)-β,β-carotene	341, (421), 448, 473		

[a] In the mobile phase.

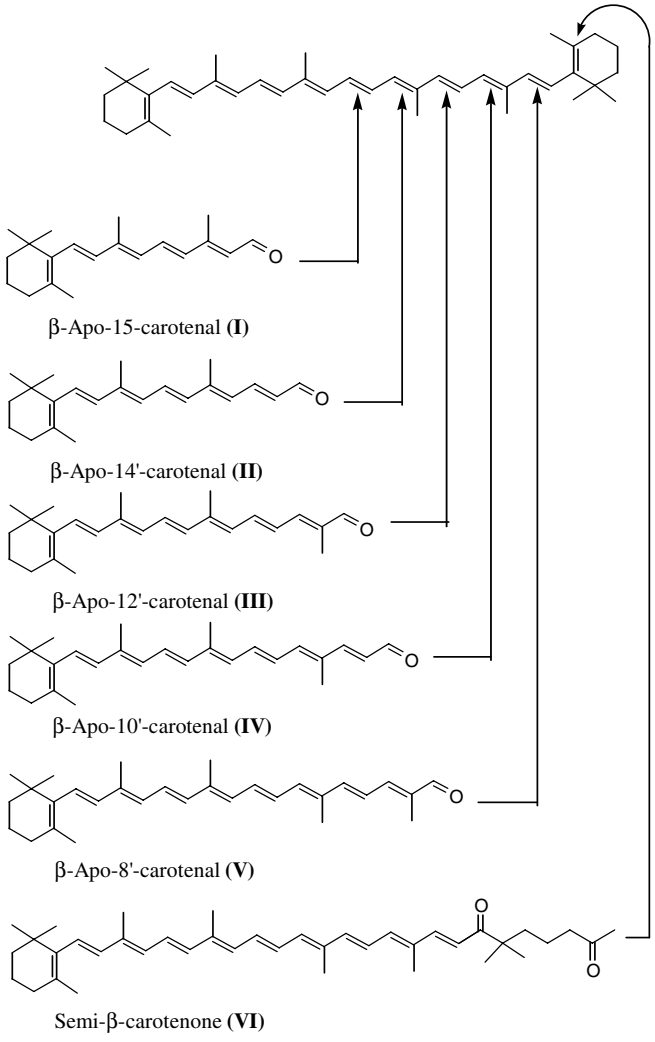

Fig. 4. Positions of cleavage of β-carotene by $KMnO_4$ and the corresponding products.

$NaBH_4$, manifested by the appearance of peaks with lower retention times and three-band absorption spectra. These compounds also showed characteristic mass fragments in the mass spectra (Table 2) (Enzell & Back, 1995).

3.3. Epoxycarotenoids and apocarotenals formed in the model systems

The HPLC profiles of the low-moisture model system exposed to light for 21 days and that kept in the dark for the same period did not show any significant difference (Fig. 5A and B). The aqueous model system exposed to light for 30 days also showed essentially the same HPLC profile, except for an enhanced *cis*-isomerization of the starting (all-*E*)-β-carotene (Fig. 5C). The minute amounts of the degradation products made identification difficult. This was done carefully, using the same criteria and in comparison with the products of the epoxidation with MCPBA and oxidative cleavage with $KMnO_4$. The occurrence of the degradation products in the model systems is summarized in Table 3.

Notably, β-apo-15-carotenal, β-apo-14′-carotenal, β-apo-12′-carotenal and β-apo-10′-carotenal were all found in the low-moisture model system, both in the presence and absence of light, and in the aqueous model system exposed to light. β-Apo-8′-carotenal and semi-β-carotenone appeared in the aqueous model system but were not detected in the low-moisture model system. The fact that these products were not detected did not necessarily mean that they were not formed; they could have a fast turn over. In any case, β-apocarotenals and β-carotenone produced by oxidative cleavage with $KMnO_4$ were also formed in the model systems.

β-Carotene-5,6-epoxide and β-carotene-5,8-epoxide were detected in both model systems. β-Carotene-5,6,5′,8′-diepoxide and β-carotene-5,8,5′,8′-diepoxide were found only in the low-moisture model system kept in the dark. In any case, the same epoxycarotenoids appeared to be formed in the model systems as in the epoxidation with MCPBA. The fact that β-carotene-5,6,5′,6′-diepoxide was not detected in the model systems suggests that it had a fast turn over or that β-carotene-5,6-epoxide was formed first and then rearranged to β-carotene-5,8-epoxide. This was followed by the introduction of a 5,6-epoxide in the other β-ring, the rearrangement of which would form β-carotene-5,8,5′,8′-diepoxide.

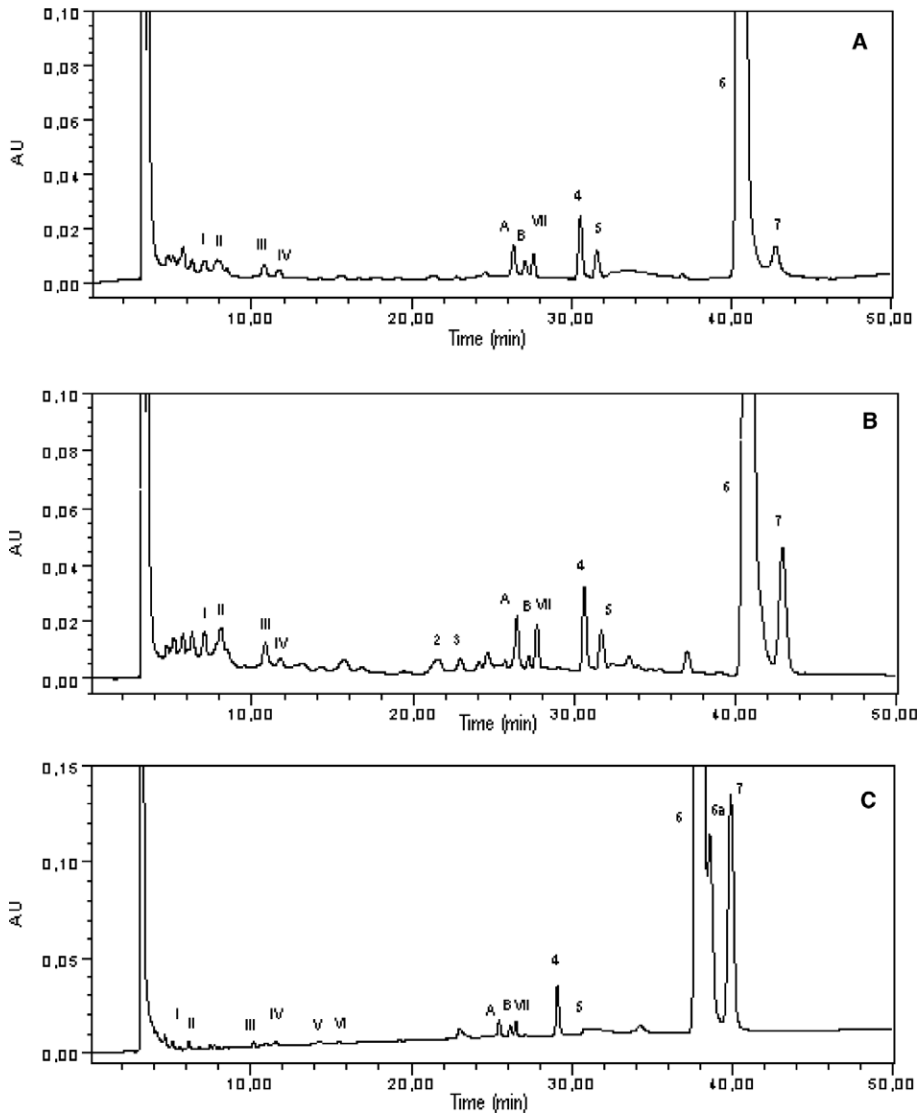

Fig. 5. HPLC profiles of: (A) low moisture (under light) and (B) in the dark, (C) aqueous (under light) model systems. HPLC conditions are described in the text.

The detection of monohydroxy-β-carotene and monohydroxy-β-carotene-5,8-epoxide and the increase in *Z*-β-carotene in both model systems indicate that hydroxylation and geometric isomerization accompanied epoxidation and cleavage to apocarotenals.

3.4. Epoxycarotenoids and apocarotenals detected in processed food

The identification of epoxycarotenoids and apocarotenals derived from β-carotene in the processed products was made difficult by the presence of carotenoids other than β-carotene, aside from the very low levels of these transient compounds. Nevertheless, two apocarotenal, several epoxycarotenoids, *Z*-isomers and hydroxylated products (Table 4) were detected, indicating that the epoxidation, cleavage to apocarotenals, geometric isomerization and hydroxylation demonstrated in the chemical reactions and autoxidation in the model systems also occurred in these products. In fact, the detection of β-carotene 5,6-epoxide is surprising, considering that the 5,6-epoxides of carotenoids are easily rearranged to the 5,8-epoxides during processing and storage, especially under acidic condition.

3.5. Comparison with previous work

The most detailed investigation of the initial degradation products of β-carotene was done by Marty and Berset (1986, 1988, 1990). Heating β-carotene in sealed ampoules led primarily to the formation of β-carotene-5,6-epoxide. β-Carotene-5,8-epoxide and β-carotene-5,6,5′,6′-diepoxide were also detected, along with very small amounts of *Z*-isomers of β-carotene-5,6-epoxide and β-carotene-5,8-epoxide (Marty & Berset, 1986). On extrusion cooking of β-carotene dispersed in corn starch, there was considerable decrease in the relative content of the 5,6-epoxide derivatives and an increase of 5,8-epoxide derivatives, with the

Table 3
Degradation products detected in the model systems

Compound (in order of elution)	Low moisture model system		Aqueous model system under light
	Under light	In dark	
(all-*E*)-15-apo-β-caroten-15-al (**I**)	(+)	(+)	(+)
(all-*E*)-14′-apo-β-caroten-14′-al (**II**)	(+)	(+)	(+)
(all-*E*)-12′-apo-β-caroten-12′-al (**III**)	(+)	(+)	(+)
(all-*E*)-10′-apo-β-caroten-10′-al (**IV**)	(+)	(+)	(+)
(all-*E*)-8′-apo-β-caroten-8′-al (**V**)	(−)	(−)	(+)
(all-*E*)-5,6-seco-β,β-carotene-5,6-dione (**VI**)	(−)	(−)	(+)
(all-*E*)-5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene (**1**)	(−)	(−)	(−)
(all-*E*)-5,6,5′8′-diepoxy-5,6,5′,8′-tetrahydro-β,β-carotene (**2**)	(−)	(+)	(−)
(all-*E*)-5,8,5′,8′-diepoxy-5,8,5′,8′-tetrahydro-β,β-carotene (**3**)	(−)	(+)	(−)
(all-*E*)-β,β-caroten-4-ol (**A**) (isocryptoxanthin)[a]	(+)	(+)	(+)
(all-*E*)-β,β-caroten-4-one[a] (**B**)	(+)	(+)	(+)
(all-*E*)-5,8-epoxy-5,8-dihydro-β,β-caroten-ol (**VII**)[a]	(+)	(+)	(+)
(all-*E*)-5,6-epoxy-5,6-dihydro-β,β-carotene (**4**)	(+)	(+)	(+)
(all-*E*)-5,8-epoxy-5,8-dihydro-β,β-carotene (**5**)	(+)	(+)	(+)
(all-*E*)-β,β-carotene (**6**)	(+)	(+)	(+)
(9*Z*)-β,β-carotene (**6a**)	(−)	(−)	(+)
(13*Z*)-β,β-carotene (**7**)	(+)	(+)	(+)

[a] Tentative identification.

Table 4
Degradation products detected in processed foods

Compound (in order of elution)	Mango juice	Acerola juice	Dried apricot
(all-*E*)-15-apo-β-caroten-15-al (**I**)	(+)	(−)	(−)
(all-*E*)-12-apo-β-caroten-12-al (**I**)	(+)	(−)	(−)
(all-*E*)-dihydroxy-β,β-carotene-5,8,5′,8′-diepoxide[a]	(+)	(+)	(−)
(9*Z*)-dihydroxy-β,β-carotene 5,8-epoxide[a]	(+)	(+)	(−)
(all-*E*)-β,β-carotene 5,8;5′,8′-diepoxide (**3**)	(+)	(−)	(−)
(all-*E*)-monohydroxy-β,β-carotene 5,8-epoxide (**VII**)[a]	(+)	(+)	(−)
(all *E*)-β,β-carotene 5,6-epoxide	(−)	(+)	(+)
(9*Z*-β,β-carotene 5,6-epoxide	(+)	(−)	(−)
(13*Z*-β,β-carotene 5,6-epoxide	(+)	(+)	(−)
(all *E*)-β,β-carotene 5,8-epoxide (**5**)	(+)	(+)	(+)
(9*Z*)-β,β-carotene (**6a**)	(+)	(+)	(+)
(13*Z*)-β,β-carotene (**7**)	(+)	(+)	(+)

[a] Tentative identification.

formation of β-carotene-5,6,5′,8′-diepoxide. In subsequent papers (Marty & Berset, 1988, 1990), β-carotene-5,8,5′,8′-diepoxide, β-apo-8′-carotenal, β-apo-10′-carotenal β-apo-12′-carotenal, β-apo-14′-carotenal, β-apo-15′-carotenal, β-carotene-4-one, β-carotene-3 or 4-ol-5,8,5′,8′-diepoxide and β-carotene-3,3′-diol were also identified. Since the authors showed that the hydroxyl groups were in allylic positions, this substituent should be in the 4- and 4′-positions. In these work, the degradation products were separated by alumina open column chromatography, alumina medium pressure liquid chromatography and silica high pressure liquid chromatography. Identification was based on the UV–vis, infrared and mass spectra (eletron impact or chemical ionization/desorption mode) and chemical reactions characteristic of epoxide, carbonyl, allyl hydroxyl functions. Aside from the oxidized products, (13,13′-di-*Z*)-β-carotene, (9,13′-di-*Z*)-β-carotene, (15*Z*)-β-carotene, (13*Z*)-β-carotene, (9,9′-di-*Z*)-β-carotene and (9*Z*)-β-carotene were also reported in the last paper (Marty & Berset, 1990).

Also investigating thermal degradation with β-carotene suspended in water by sonication, Kanasawud and Crouzet (1990) detected four degradation products separated and isolated by thin layer chromatography on aluminum oxide, the identification being based on the absorption spectra and exposure of the alumina plate to concentrated HCl after development. These were β-carotene-5,6-epoxide, β-carotene-5,8-epoxide, *Z*-β-carotene-5,6,5′,6′-diepoxide and β-carotene-5,8,5′,8′-diepoxide.

Ouyang et al. (1980) identified β-apo-15-carotenal, β-apo-14′-carotenal and β-apo-13-carotenone in palm oil during deodorization by infrared and electron impact mass spectrometry.

Henry et al. (2000) found that the degradation products formed from carotenoids during oxygen exposure were similar to those produced during ozonation. The major products from β-carotene, tentatively identified on the basis of their elution on the HPLC column, UV–vis spectra and electrospray LC-MS, were 13*Z*-, 9*Z*-, a di-*Z*-isomer, β-apo-13-carotenone, β-apo-14′-carotenal, β-carotene-5,8-epoxide and β-carotene-5,8-endoperoxide.

4. Conclusions

The results of the present study, carried out under ambient condition and in the presence or absence of light, along with those of previous work on thermal degradation, at different temperatures and duration, show that epoxidation and apocarotenal formation in the autoxidation of β-carotene occurs through the same route in different systems and conditions. Oxidation is accompanied by geometric isomerization, both *E*- and *Z*-isomers being subject to oxidation.

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