Food Chemistry 126 (2011) 927–934

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

A chemical study of β-carotene oxidation by ozone in an organic model system and the identification of the resulting products

Clícia Maria de Jesus Benevides [a,1], Márcia Cristina da Cunha Veloso [b], Pedro Afonso de Paula Pereira [a,*], Jailson Bittencourt de Andrade [a]

[a] Instituto de Quimica, Universidade Federal da Bahia, Campus Universitário de Ondina, 40, 0170-290 Salvador, BA, Brazil
[b] Instituto Federal de Educação, Ciência e Tecnologia – IFBA, Salvador, BA, Brazil

ARTICLE INFO

Article history:
Received 13 April 2010
Received in revised form 26 August 2010
Accepted 14 November 2010
Available online 23 November 2010

Keywords:
β-Carotene
Ozonolysis
Organic model system
Oxidation products
LC-MS

ABSTRACT

Carotenoids are present in many foods. Due to their polyenic chains, they undergo oxidation reactions which may give several compounds. Ozone, a powerful antimicrobial agent, is applied in the food industry due to its high reactivity and penetrability. This work presents a chemical study of the degradation of β-carotene in solutions, under the influence of ozone. The experiments were carried out at ozone concentrations ranging from 0.8 to 2.5 ppm and the β-carotene solutions were sampled and analysed from zero to seven hours of reaction. The oxidation products were collected in C18 cartridges coated with dinitrophenylhydrazine and the hydrazones formed were analysed by LC-MS. The oxidation reaction was found to follow a zero order kinetic model and the β-carotene decay ranged between 17.2% and 99.8%. Fourteen oxidation products were tentatively identified, amongst them eight which had not been cited yet in the literature as oxidation products of β-carotene.

1. Introduction

The carotenoids belong to one of the most important groups of natural pigments due to their high occurrence structural diversity and their diverse functions.

The basic chemical structure of the carotenoids consists of tetraterpenoids connected by opposite units at the centre of the molecule with a polyenic chain ranging from 3 to 15 conjugated double bonds. This structure is susceptible to a number of different modifications (cyclisation, migration of the double bonds and the addition of oxygenated functions, amongst others) and generates a great diversity of structures (Britton, 1995). These peculiar structural characteristics allow carotenoids to have a variety of different biological functions and chemical behaviours. In addition, due to the highly unsaturated polyenic chain, carotenoids are likely to suffer degradation reactions such as oxidation and hydrolysis, which modify their biological actions (Rodriguez & Rodriguez-Amaya, 2007). The oxidation of carotenoids is a complex process due to the formation of trace quantities of several compounds with a low molecular weight.

Ozone is an antimicrobial agent with several applications in the food industry, since its high oxidation power and penetrability increases the microbiological security of these products. In addition, ozone does not leave behind any toxic residues unlike other types of sanitisation agents (Greene, Few, & Serafini, 1993). However, ozone can also react with the organic matter present in foods, especially those rich in unsaturated compounds, such as carotenoid pigments, through a well known cycloaddition reaction which results in carbonyl compounds (CC) and Criegee's biradicals (Aschmann, Arey, & Atkinson, 2002; Nunes, Veloso, Pereira, de, & de Andrade, 2005). These highly energetic biradicals then undergo fragmentation and stabilisation processes, giving rise to more stable species such as carboxylic acids.

Despite the nutritional and biological functions of carotenoids, studies have demonstrated the deleterious effects of several of the oxidation products of these pigments. Aldehydes and epoxides, for example, may inhibit the respiration of mitochondrial isolates of rat livers (Siems et al., 2005, 2002), and may reduce the content of protein sulfhydryl groups and decrease the glutathione levels.

The number of studies investigating the oxidative degradation of carotenoids has increased in recent years. However, available data are still scarce and controverse, when compared to those regarding lipid oxidation (Rodriguez & Rodriguez-Amaya, 2007). Since foods and food derivatives constitute, in general, complex matrices, and the concentrations of the degradation products formed in these biological systems are, in many cases, too low in order they can be isolated and identified, the aim of this work was therefore to conduct a chemical study of the oxidation of β-carotene, when organic solutions of this compound were exposed

* Corresponding author.
E-mail address: pedroapp@ufba.br (P.A. de Paula Pereira).
[1] Present address: Departamento de Ciências da Vida – Universidade do Estado da Bahia, Salvador, BA, Brazil.

0308-8146 © 2010 Elsevier Ltd. Open access under the Elsevier OA license.
doi:10.1016/j.foodchem.2010.11.082

to ozone concentrations similar to those which is used in food sanitisation processes. The study emphasizes on the attempt to identify the oxidation products formed, which can also be possible products in food systems, as well as to propose their possible pathways.

2. Materials and methods

2.1. Reagents and standards

β-Carotene, β-ionone and glyoxal standards were obtained from Hoffman-La Roche, Inc (Nutley, NJ, US), with purities ranging from 95% to 97%. Purified water was obtained by distillation and treatment with a NANOpure Diamond system (Barnstead). Acetonitrile and methanol (HPLC grade) were purchased from Aldrich and were filtered through a 0.45 μm cellulose membrane before use. The other reagents (ethyl acetate, potassium iodide, carbon tetrachloride, dichloromethane, phosphoric acid and 2,4-dinitrophenylhydrazine) were of analytical grade and were obtained from Merck (Darmstadt, Germany).

2.2. Preparation of the β-carotene solution

The β-carotene solutions used in the organic solvent modeling-system (40 μg mL^{-1}) were prepared by weighing 1.2 mg of a solid standard in 0.5 mL of methylene chloride, followed by the addition of acetonitrile (ACN) up to 30 mL. The solutions were prepared immediately before each experiment and their purities were checked by injection in the LC-DAD-MS system.

2.3. Preparation of the 2,4-dinitrophenyl-hydrazine solution (DNPHi)

The solution of the derivatisation reagent DNPHi (0.4% w/v) was prepared in a $ACN/H_2O/H_3PO_4$ (60/39/1% v/v/v) mixture. The purity of the reagent was checked by injection in a LC-DAD system and, whenever necessary, the reagent was purified by liquid–liquid extraction with carbon tetrachloride.

2.4. Preparation of the sample cartridges

The oxidation products of the reactions between ozone and β-carotene or β-ionone – mainly compounds containing one or two carbonilic groups in their structures – were derivatised, prior to analysis, directly in the sample cartridges, to their respective hydrazones. The derivatisation reaction was made in order to enhance the DAD detector's sensitivity, at the wavelength chosen for monitoring the compounds in the chromatograms (365 nm). The sample cartridges (Sep Pak Classic C18, 360 mg, Waters-Milford) were prepared immediately before use by impregnation with 2 mL of the DNPHi solution prepared as above. The cartridges were then dried in a gentle stream of nitrogen gas before use.

2.5. Ozonolysis of -carotene

The β-carotene solution (25 mL) was put in a glass impinger protected from light and ozone was then bubbled through the solution at a 1 L min^{-1} flow rate for seven hours. The experiments were carried out at four different ozone concentrations (0.8, 1.1, 1.5 and 2.5 ppm). Aliquots of the solution (1 mL) were sampled every hour from zero to seven hours in order to verify the β-carotene decay.

The oxidation products formed were collected and derivatised throughout the period of each ozonolysis experiment (7 h) in two DNPHi Sep Pak cartridges connected in series. Three cellulose filters impregnated with KI were mounted upstream from the cartridges in order to trap the ozone and thus prevent oxidation reactions of the carbonyl compounds (CC) sampled. After sampling, the hydrazones were directly eluted with ACN (2 mL) to an amber vial and analysed. A blank experiment was run with ACN and no β-carotene.

2.6. Ozonolysis of β-ionone

A model similar to that described above was used for β-ionone ozonolysis, in order to confirm the possibility that some of the secondary products formed from the oxidation of β-carotene were formed from this ketone. The β-ionone solution (15 μg mL^{-1} in ACN) was exposed to ozone for five hours, while the sampling conditions of the carbonyl compounds were the same as those described above.

2.7. β-carotene decay

The β-carotene decay was accomplished by the decrease in the peak area of this compound in the chromatogram of samples, taken each hour throughout the experiments. Chromatographic analysis were conducted in an LC column (Lichrospher-C18; 250 × 4.6 mm; 5 μm) using an isocratic mobile phase of ACN/ethyl acetate/methanol (60/20/20% v/v/v) at a flow rate of 1.5 mL min^{-1} and injection volumes of 20 μL. The β-carotene was monitored at 450 nm through a DAD.

2.8. Analysis of compounds

The oxidation compounds resulting from the ozonolysis of β-carotene and β-ionone were separated and analysed in an LC-DAD system (*Agilent 1100*, Agilent, Waldbronn, Germany) coupled with an ion-trap mass spectrometer (*Bruker Esquire 3000 plus*, Bruker Daltonics, Billerica, USA). The separation was performed on an XTerra MS C18 column (250 × 2.1 mm, 5 μm; Waters, Miford, USA), using a gradient of water (A) and ACN (B) as follows: 40% B to 99% B (30 min); 99% B (6 min); 99% B to 40% B (4 min); and 40% B (5 min), for a total run time of 45 min. The flow rate was kept at 0.25 mL min^{-1} and the injection volume was 10 μL. The conditions of the MS, operating with an ESI source in the negative mode, were as follows: nebulizer pressure – 22.0 psi; dry gas temperature – 300 °C; dry gas flow – 10 L min^{-1}; and capilar voltage – 4000 V. Prior to injection, samples were passed through a 0.22 μm Millipore membrane.

The compounds were tentatively identified by means of the $[M–H]^-$ ion of their mass spectra, along with the prediction of which probable structures could derive from the breaking down and reaction of the polyenic chain of β-carotene, at different positions. For those which standards were available – as in the case of glyoxal and β-ionone – the identity was confirmed by comparing their retention times to those of the standards in the DAD detector (λ = 365 nm).

3. Results and discussion

3.1. β-carotene decay

The decay study was conducted with ozone concentrations ranging from 0.80 to 2.54 ppm. These concentrations were based on previous studies which established their antimicrobial efficiency and influence on the food constituents (Akabas and Ozdemir, 2006; Zhao et al., 2005). In all evaluated ozone concentrations, there was a reduction in the initial quantities of β-carotene over the entire exposure period of seven hours. The percent decay of β-carotene after seven hours was 17.2%, 78.0%, 99.0% and 99.8%,

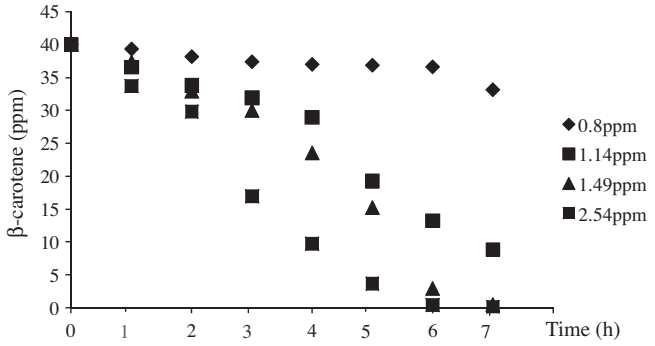

Fig. 1. Variation in the β-carotene concentration at different ozone concentrations as a function of time.

for initial ozone concentrations of 0.80, 1.14, 1.49 and 2.54 ppm, respectively.

Fig. 1 presents the β-carotene decay curves as a function of the initial ozone concentration. A trend of sigmoid shapes is observed, except for the concentration of 0.80 ppm. This type of shape is typical for some kinetic models of carotenoid losses during storage and food processing (Limbo, Torri, & Piergiovanni, 2007; Goldman, Horev, & Saguy, 1983). The three distinct regions are known as the induction period; the main region, in which the reaction is fast; and, finally, a region of low decay rates.

In foods, degradation reactions of the different components usually follow zero order or first order kinetic models. For β-carotene in foods, most papers report first order kinetics.

On the other way, zero order kinetics were reported by several authors for β-carotene decay in organic solvents and in aqueous media, as, for instance, in the following: ozone and oxygen reactions of carotenoids in aqueous systems (Henry, Catignani & Schwartz, 1998); the reaction of β-carotene with oxygen in toluene (El-Tinay and Chichester, 1970); the oxidation of carotenoids in cyclohexane (Minguez-Mosquera and Jaren-Galan, 1995); the decomposition of β-carotene by UV radiation in dichloromethane solution (Gao, Deng, & Kispert, 2005); and the thermal degradation of carotenoids in aqueous media (Kanasawud and Crouzet, 1990).

In the present work, a zero order kinetic model was observed in the four cases, according to the following equation:

$$C = C_o - kt, \quad \text{(I)}$$

where: C = β-carotene concentration at time t; C_0 = initial β-carotene concentration; K = rate constant of reaction; and t = time (h).

The rate constants for the main region of the curves ranged between 0.8 and 6.3 ppm h^{-1}, for initial ozone concentrations of 0.80 and 2.54 ppm, respectively.

3.2. Identification of the oxidation products of β-carotene

All of the double bonds which are present in the chain of the carotenoid molecules are potential sites for the occurrence of the reactions with ozone, leading to a large variety of oxidation products. Although the carbonyl compounds and epoxides are the most cited in the literature as oxidation products of β-carotene, in the present study compounds from other classes, such as acids and hydroxy aldehydes, were also proposed. Table 1 presents the main oxidation products in the experiments of β-carotene ozonolysis in solution, tentatively identified through their $[M–H]^-$ fragment in their mass spectra.

It can be seen from Table 1 that, for 15-apo-β-carotenal, both non-derivatised and derivatised forms were identified in the samples. On the other hand, 3,7,11,11-tetramethyl-10,15-dioxo-hexadec-2,4,6,8-tetra-enal was identified as two different hydrazones:

Table 1
Main oxidation products tentatively identified in the β-carotene ozonolysis in solution.

Compound	MW[a]	[M–H][b]
15-Apo-β-carotenal	284	283
Pyruvic acid	88	267[c]
5,9,13,13-Tetramethyl-12,17-dioxo-octadec-2,4,6,8,10-pentenoic acid	358	537[c]
15-Apo-β-carotenal	284	463[c]
14́-Apo-β-carotenal	310	489[c]
3,7,11,11-Tetramethyl-10,15-dioxo-hexadec-2,4,6,8-tetra-enal	316	675[d]
2-Methyl-buten-2-dial	98	457[d]
Etanedial (glyoxal)	58	417[d]
2-Oxo-propanal (methyl glyoxal)	72	431[d]
3,7,11,11-Tetramethyl-10,15-dioxo-hexadec-2,4,6,8-tetra-enal	316	495[c]
7-Apo-β-caroten-7-al (β-cyclo-citral);	152	331[c]
6,6-Dimethyl-undec-3-en-2,5,10-trione	224	583[d]
4,9,13,17,17-Pentamethyl-16,21-dioxo-docos-2,4,6,8,10,12,14 -heptaenal	408	587[c]
12́-Apo-β-carotenal	350	529[c]
5,6-Epoxy-12́apo-β-carotenal;	366	365
5,6 epoxy-10́-apo-β-carotenal)	392	391

Observation:
[a] molecular weight of the oxidation product formed.
[b] [M–H] ion, identified by ESI-MS.
[c] hydrazone formed through the reaction of one carbonyl group.
[d] hydrazone formed through the reaction of two carbonyl groups.

one of them resulting from the reaction between DNPHi and one carbonyl group and the other from the reaction between DNPHi and two carbonyl groups of the CC. This fact shall be related to differences in the reaction rates between DNPHi and each different CC structure.

The factors which can interfere with these rates include the electrophilic character of the carbonylic carbons, the steric conditions in the molecules (for example, for aldehydes the carbonyl group is located in the molecule's extremity, making easier the approximation of a reagent than in ketones), the pH of the media and the DNPHi concentration (Bicking and Cooke, 1998). The time elapsed between the sampling and the elution of the cartridge for analysis is also a critical factor, depending on the type of compound reacting with the derivatisation agent (Van Leeuwen, Hendriksen, & Karst, 2004). Some authors (Zwiener, Glauner, & Frimmel, 2002) have demonstrated that a minimum of 1 h of contact is necessary for the compounds which have high reaction rates with DNPHi, such as monocarbonyl compounds. However, for the dicarbonyl and hydroxicarbonyl compounds, a minimum of 12 h may be necessary to carry out the reaction in the experimental conditions evaluated. In this study, as stated in section *2.5*, the total elapsed time between the sampling and the elution of the cartridges was seven hours.

Although the formation of some of the compounds highlighted in this work (e.g., 5,6-epoxy-12́-apo-β-carotenal, 7-apo-β-caroten-7-al (β-cyclocitral), 12́-apo-β-carotenal, amongst others) as oxidation products of β-carotene is well documented, there is not a well-defined mechanism in the literature for their formation yet (Rodriguez and Rodriguez-Amaya, 2007, Waché, Bosser-Deratuld, LY, & Belin, 2002). In fact, it is inadequate to propose just one mechanism for all of the products obtained, considering that the precursor molecule is highly unsaturated and offers several possibilities for the initial ozone attack. In addition, the products that are initially formed may also react with ozone themselves, giving rise to new products as described previously. When a double bond is broken, several different compounds may be formed; for example, when the double bond localised inside the ring is broken, epoxyde and secocarotenoids are formed.

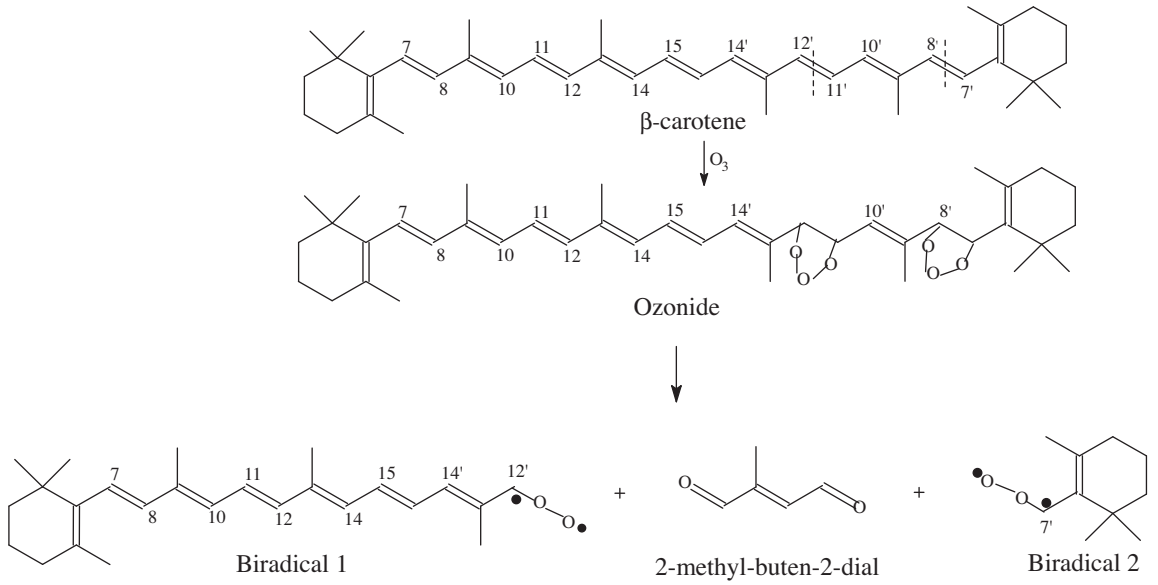

Fig. 2. Proposed mechanism of the formation of 2-methyl-buten-2-dial from the ozonolysis of β-carotene.

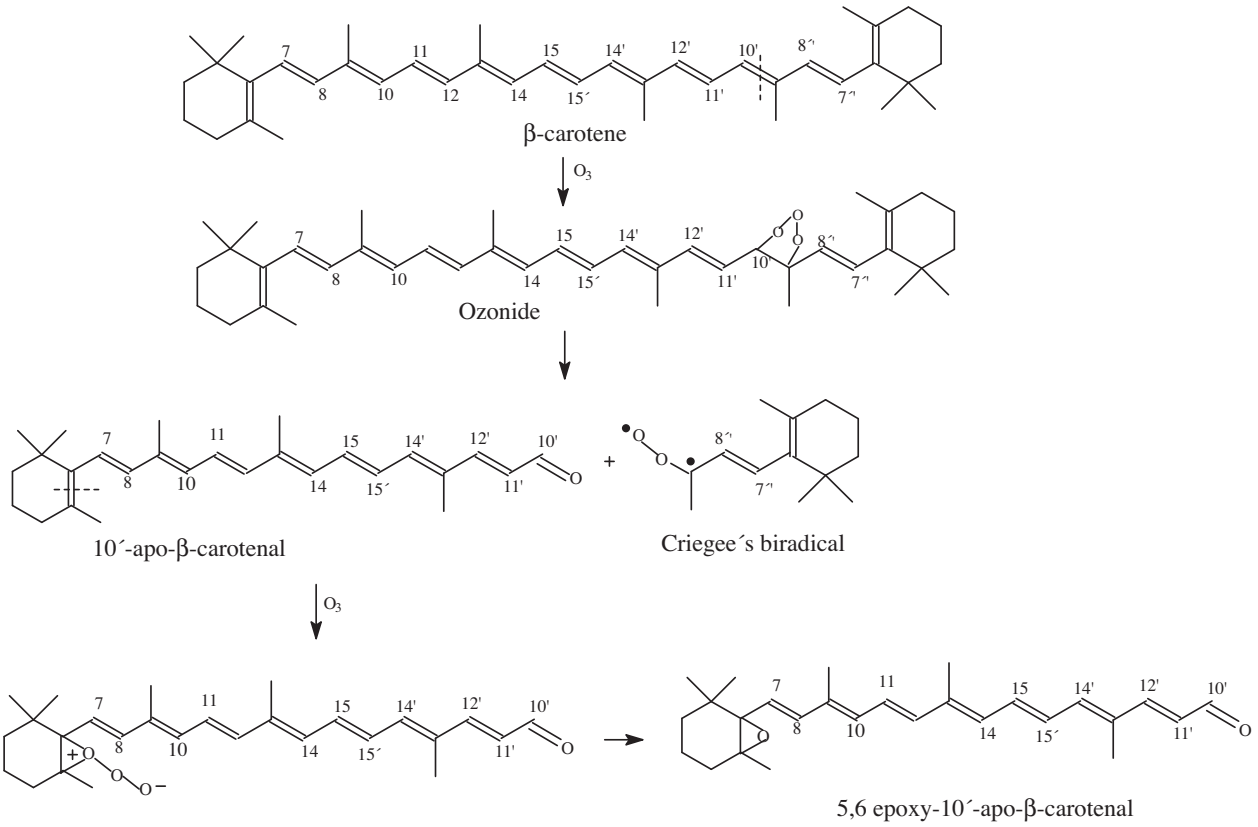

Fig. 3. Proposed mechanism of the formation of epoxycarotenoids (e. g. 5,6-epoxy-1ó-apo-β-carotenal) from the ozonolysis of β-carotene.

Fig. 2 shows the mechanism proposed for the formation of 2-methyl-buten-2-dial, beginning with the ozone attack on the double bonds between C_{12}–C_{11} and C_8–C_7. A highly unstable ozonide is then formed, followed by the dienal and two Criegee's biradicals.

Fig. 3 shows, on the other way, the proposed mechanism for the formation of an epoxycarotenoid, which is originally based on that suggested by other authors for the ozonolysis of alkenes (Bailey, Mann, & Maittlis, 1975). The β-carotene initially reacts with ozone, on the double bond between C_{10}–C_9 and resulting in an ozonide, followed by a Criegee's biradical and the 1ó-apo-β-carotenal, which is then oxidised by the ozone in the ring's double bond, giving rise to the 5,6-epoxy-1ó-apo-β-carotenal.

The formation of epoxides as a product of the terpene ozonolysis has a few mentions in the literature. However, it is fairly common during the oxidation of carotenoids. The attack on the molecule tends to be in positions 5,6 or 5′,6́ in carotenoids which

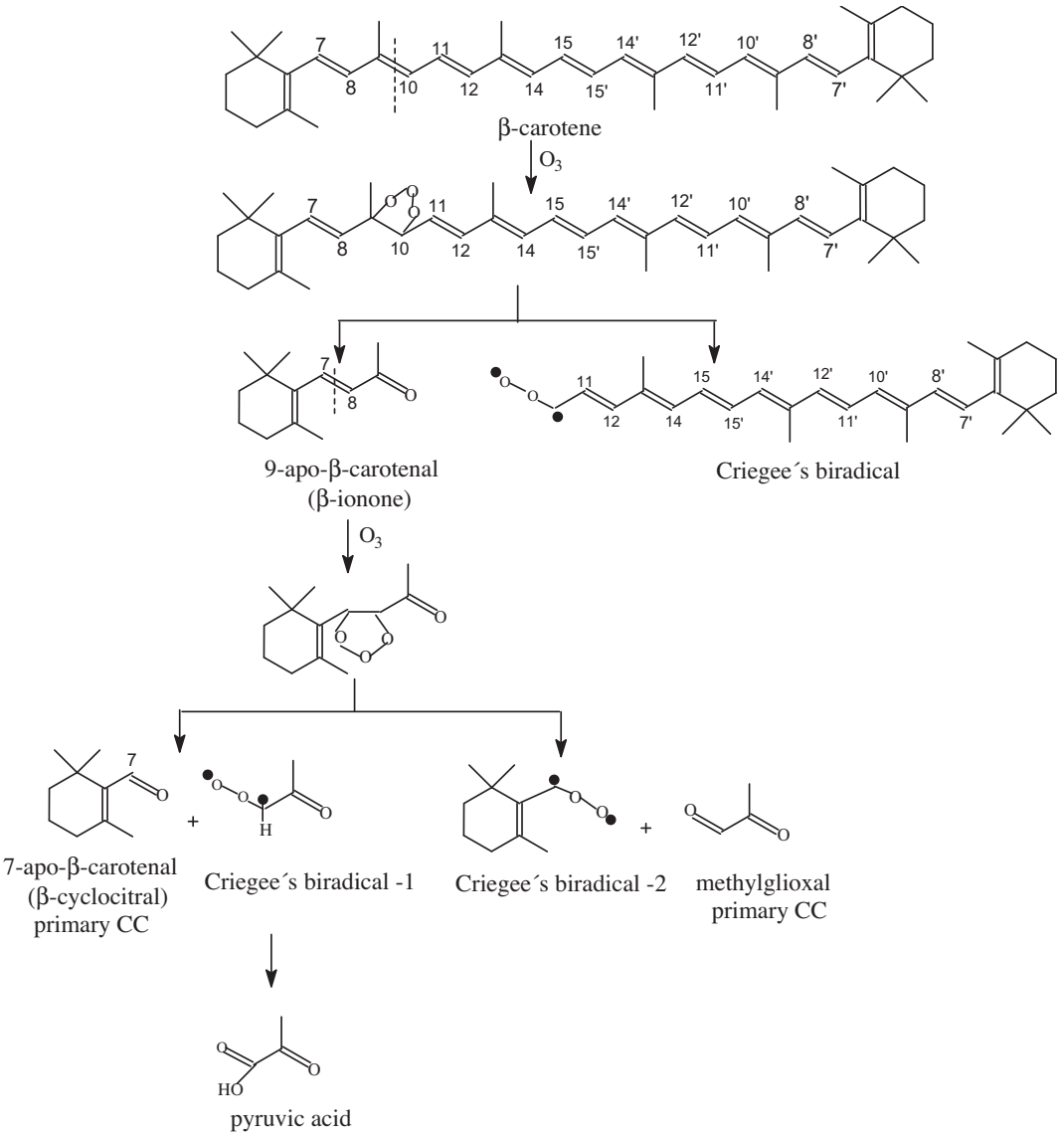

Fig. 4. Proposed scheme of the formation of pyruvic acid from the ozonolysis of β-ionone, an intermediary product formed in the ozonolysis of β-carotene.

have a β-ionone ring. This preference is due to the fact that terminal double bonds have high electronic densities and, consequently, favour the attack of the electrophilic species (Chichester and McFeeters, 1971). To the best of our knowledge, this work is the first to examine compounds originating from the oxidation of carotenoids that have an epoxy function in the β-ionone ring and a carbonyl function in the main chain.β-Apo-acid-carotenoids may also be formed during β-carotene oxidation. In this study, the compounds 5,9,13,13-tetramethyl-12,17-dioxo-octadec-2,4,6, 8,10-pentenoic and pyruvic acid were tentatively identified, with the latter compound being the most abundant product.

Pyruvic acid, which is considered to be a secondary product in the ozonolysis of β-carotene, was found in the chromatogram as two peaks that were eluted during distinct retention times (8.0 and 9.4 min). The *sin* and *anti* isomers, which are eluted at 8.0 and 9.4 min, respectively, were likely responsible for these two peaks. Both peaks were tentatively identified using mass spectrometry, through the 267 ion which is characteristic of the $[M–H]^-$ fragment from the corresponding hydrazone.

Pyruvic acid could have been formed either by the direct ozonolysis of β-carotene or from its primary oxidation products. One of the possible pathways for the formation of pyruvic acid is shown in Fig. 4. Initially, the ozone reacts with the $C_9–C_{10}$ double bond of β-carotene, resulting in an ozonide which then gives rise to β-ionone and a Crieege's biradical. A new reaction then occurs with ozone in the $C_7–C_8$ double bond of β-ionone, which forms the mono and dicarbonyls β-cyclocitral and methyl glyoxal, respectively, and the corresponding Crieege's biradicals 1 and 2. In this pathway, pyruvic acid is then formed from a rearrangement of Crieege's biradical 1. The present study suggests that other intermediary products, such as 15-apo-β-carotenal and 3,7,11,11-tetramethyl-10,15-dioxo-hexadec-2,4,6,8-tetraenal, both of which are highly reactive species, are prone to subsequent oxidation. New products, such as pyruvic acid, may be then formed during this further oxidation.

Fig. 5 shows possible mechanisms for the formation of the following compounds: 15-apo-β-carotenal; 14́-apo-β-carotenal; 12́-apo-β-carotenal; 5,6-epoxy-12́apo-β-carotenal; 5,6-epoxy-10́-apo-β-carotenal; 5,9,13,13-tetramethyl-12,17-dioxo-octadec-2,4, 6,8,10-pentenoic acid; 3,7,11,11-tetramethyl-10,15-dioxo-hexadec-2,4,6,8-tetraenal; and 4,9,13,17,17-pentamethyl-16,21-dioxo-docos-2,4,6,8,10,12,14-heptaenal. All of these compounds were

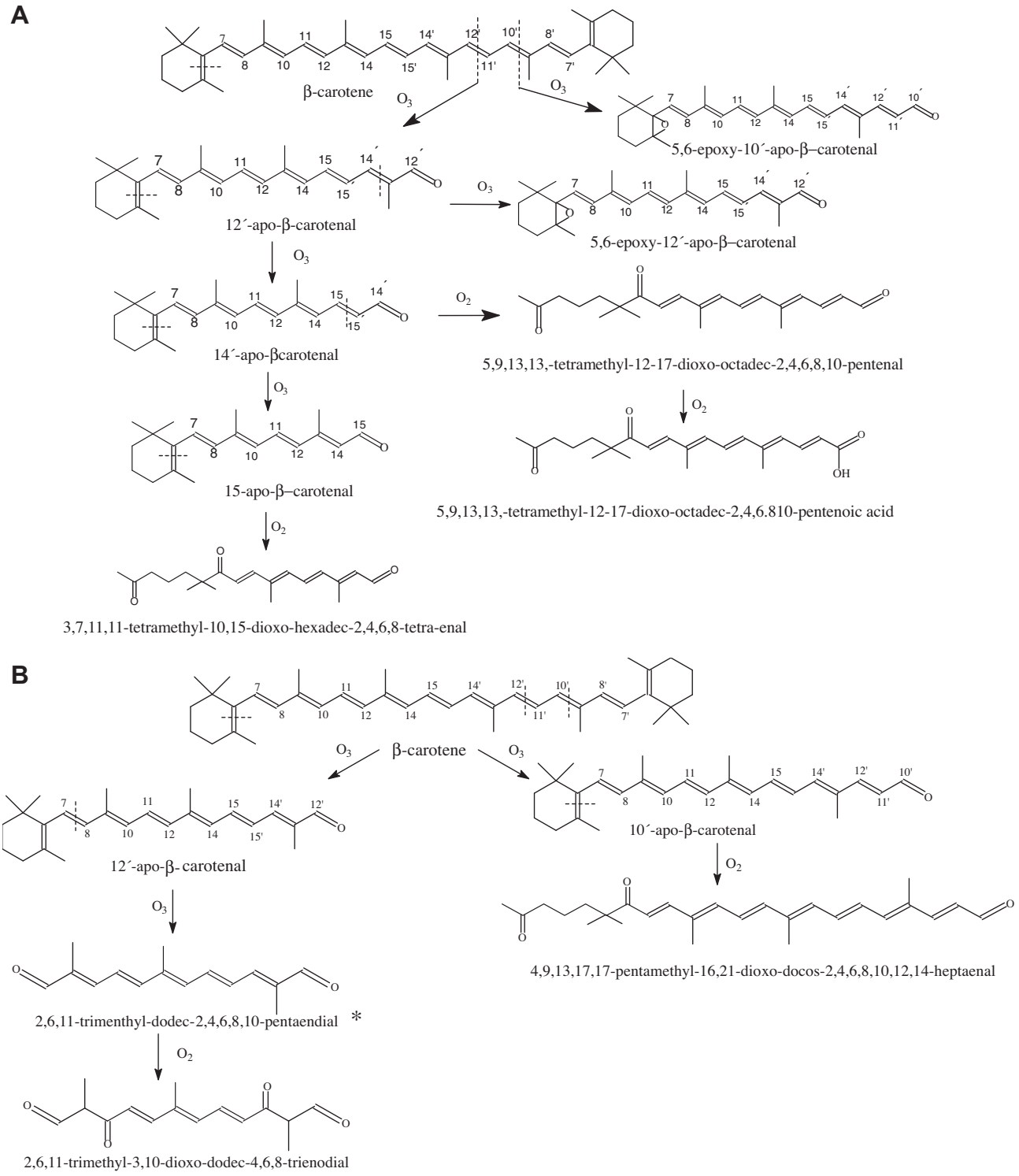

Fig. 5. Proposed schemes of the formation of the following compounds from the ozonolysis of β-carotene: 5A: 15-apo-β-carotenal; 14́-apo-β-carotenal; 12́-apo-β-carotenal; 5,6-epoxy-12́apo-β-carotenal; 5,6-epoxy-10́-apo-β-carotenal; 5,9,13,13-tetramethyl-12,17-dioxo-octadec-2,4,6,8,10-pentenoic acid; and 3,7,11,11-tetramethyl-10,15-dioxo-hexadec-2,4,6,8 -tetraenal. 5B: 12́-apo-β-carotenal; 10́-apo-β-carotenal; 4,9,13,17,17-pentamethyl-16,21-dioxo-docos-2,4,6,8,10,12,14-heptaenal; and 2,6,11-trimethyl-3,10-dioxo-dodec-4,6,8-trienedial.

proposed as oxidation products from the β-carotene ozonolysis in solution during the present study, based on their tentative identification through LC-MS.

Secocarotenoids, such as 4,9,13,17,17-pentamethyl-16,21-dioxo-docos-2,4,6,8,10,12,14-heptaenal and 3,7,11,11-tetramethyl-10,15-dioxo-hexadec-2,4,6,8-tetra-enal, have not been assessed in the literature to date, since oxidation products originating from the breakdown of the ring's double bond, producing a keto function, are not very common (Britton, 1995). The 5,6-seco-β-carotene-5,6-dione is a possible exception, although it has been

identified as product of β-carotene oxidation in permanganate solutions (Chou and Labuza, 1984), thus in a different condition of this work.

Other compounds observed, including β-cyclocitral, 15-apo-β-carotenal, 14́-apo-β-carotenal, 12́-apo-β-carotenal, 5,6-epoxy-12́-apo-β-carotenal and 5,6-epoxy-10́-apo-β-carotenal, had been identified previously by other researchers, although using different model systems, as, for instance, exposure to UV light (Chou & Labuza, 1984), in combination with photo-sensitizers (Ojima, Sakamoto, Ishiguro & Terao, 1993 and Stratton, Schaefer & Liebler, 1993), through autooxidation at 20 and 80 °C (Ojima, Sakamoto, Ishiguro &Terao, 1993) and in the presence of permanganate (Rodriguez et al., 2007), amongst other methods. It is generally accepted that the initial compounds formed, during the oxidation of β-carotene, are epoxides and apocarotenals. β-cyclocitral is frequently mentioned as a product of the reaction of the double bond between the C_7–C_8 carbons of β-carotene (Glória, Grulke & Gray, 1993; Sommerburg, Langhans & Arnhold, 2003), since this bond has a high mobility index which favours its break-down and results in the formation of this carbonyl compound.

3.3. Identification of the oxidation products of β-ionone

β-Ionone (9-apo-β-carotenone) has been mentioned in several studies (Glória, Grulke & Gray, 1993 and Waché, Bosser-Deratuld, Ly & Belin, 2002) as an oxidation product of β-carotene. However, this compound was not detected in our experiments. Since β-ionone still has double bonds in its structure which can react with ozone, this study proposes that β-ionone could have been completely oxidised during the experiments, giving rise to secondary oxidation products. As predicted, in our experiments the ozonolysis of β-ionone gave rise to three carbonilic compounds which had been also tentatively identified as products of β-carotene ozonolysis, namely methyglyoxal, β-cyclocitral and 6,6-dimethyl-undec-3-en-2,5,10-trione. It is worth to mention that methylglyoxal and β-cyclocitral had also been found previously in the gas-phase reactions between β-ionone and ozone in Teflon chambers (Forester, Ham & Wells, 2007).

4. Conclusions

The oxidation of β-carotene, under different ozone concentrations, was found to follow a zero order kinetic model relative to β-carotene in the main region of the curves. During the experiments, the percentage of β-carotene decay varied from 17.2% to 99.8% for ozone concentrations ranging, respectively, from 0.80 to 2.54 μg mL^{-1}.

Although the model used in this work doesn't simulate real food matrices, once they constitute, in general, complex systems, it represents an attempt to identify the formed products which can also be possible products in foods.

The β-carotene ozonolysis with the model system in solution made it possible to propose, through tentative identification, fourteen oxidation products: 15-apo-β-carotenal; pyruvic acid; 5,9,13,13-tetramethyl-12,17-dioxo-octadec-2,4,6,8,10-pentenoic acid; 14́-apo-β-carotenal; 3,7,11,11-tetramethyl-10,15-dioxo-hexadec-2,4,6,8-tetra-enal; 2-methyl-buten-2-dial; glyoxal; methylglyoxal; β-cyclocitral; 6,6-dimethyl-undec-3-en-2,5,10-trione; 4,9,13,17,17-pentamethyl-16,21-dioxo-docos-2,4,6,8,10,12,14-heptaenal; 12́-apo-β-carotenal; 5,6-epoxy-12́apo-β-carotenal; and 5,6 epoxy-10́-apo-β-carotenal.

Of these products, eight (pyruvic acid; 5,9,13,13-tetramethyl-12,17-dioxo-octadec-2,4,6,8,10-pentenoic acid; 3,7,11,11-tetramethyl-10,15-dioxo-hexadec-2,4,6,8-tetraenal; 2-methyl-but-2-enodial; glyoxal; methylglyoxal; 6,6-dimethyl-undec-3-en-2,5,10-trione and 4,9,13,17,17-pentamethyl-16,21-dioxo-docos-2,4,6,8,10,12,14-heptaenal) had not previously been cited in the literature as oxidation products of β-carotene. Their occurrence was probably due to the high oxidant power of ozone. On the other hand, compounds that are normally present in β-carotene oxidation, such as β-ionone, have not been identified. This suggests that these compounds reacted completely during exposure to ozone and were thus converted to secondary products observed during these experiments. The experiment conducted with β-ionone alone supports this hypothesis, since methylglyoxal, β-cyclocitral and 6,6-dimethyl-undec-3-en-2,5,10-trione were formed and all of these compounds were also tentatively identified during the ozonolysis of β-carotene.

Acknowledgements

The authors wish to thank the National Research Council (CNPq), the State of Bahia Foundation for Support to Research (FAPESB), PRONEX, FINEP, CAPES and UNEB (DCV 1). We would also like to thank M.Sc. Eliane Teixeira Sousa for her valuable help in the LC-MS analysis.

References

Akabas, M. Y., & Ozdemir, M. (2006). Effectiveness of ozone for inactivation of *Escherichia coli* and *Bacillus cereus* in pistachios. *International Journal Food Science and Technology, 41*, 513–519.

Aschmann, S. M., Arey, J., & Atkinson, R. (2002). OH radical formation from the gas-phase reactions of ozone with a series of terpenes. *Atmospheric Environment, 36*, 4347–4355.

Bailey, P. M., Mann, B. E., & Maittlis, P. M. (1975). Ozonolysis of cis- and trans-1, 2-difluoroethylene. Stereoselectivity in epoxide formation. *Journal of the American Chemical Society, 97*, 1276–1278.

Bicking, M. K. L., & Cooke, W. M. (1998). Effect of pH on the reaction of 2,4-dinitrophenylhydrazine with formaldehyde and acetaldehyde. *Journal of Chromatography, 455*, 310–315.

Britton, G. (1995). Structure and Properties of carotenoids in relation to function. *Federation of American Societies for Experimental Biology, 9*, 1551–1558.

Chichester, C. O., & McFeeters, R. (1971). *Pigment degeneration during processing and storage. The biochemistry of fruits and their products*. London: Academic Press.

Chou, H., & Labuza, T. P. (1984). Antioxidant effectiveness in intermediate moisture content model systems. *Journal of Food Science, 39*, 479–485.

El-Tinay, A. H., & Chichester, C. O. (1970). Oxidation of β-carotene. Site of initial attack. *The Journal of Organic Chemistry, 35*, 2290–2293.

Forester, C. D., Ham, J. E., & Wells, J. R. (2007). β-ionone reactions with ozone and OH radical: Rate constants and gas-phase products. *Atmospheric Environment, 41*, 8758–8771.

Gao, G., Deng, Y., & Kispert, L. D. (2005). Photoactivated ferric chloride oxidation of carotenoids by near-UV to visible light. *The Journal of Physical Chemistry B, 101*, 7844–7849.

Glória, M. B. A., Grulke, E. A., & Gray, J. I. (1993). Effect of type of oxidation on β-carotene loss and volatiles products formation in model systems. *Food Chemistry, 46*, 401–406.

Goldman, M., Horev, B., & Saguy, I. (1983). Decolorization of β-carotene in model systems simulating dehydrated food. Mechanism and kinetic principles. *Journal of Food Science, 48*, 751–754.

Greene, A. K., Few, B. K., & Serafini, J. C. A. (1993). Comparrison of ozonation and chlorination for a disinfection of stainless steel sufaces. *Journal Dairy Science, 76*, 3617–3620.

Henry, L. K., Catignani, G. L., & Schwartz, S. J. (1998). The influence of carotenoids and tocopherols on the stability of safflower seed oil during heat-catalyzed oxidation. *Journal of the American Oil Chemists'Society, 75*, 1399–1402.

Kanasawud, P., & Crouzet, J. C. (1990). Mechanism of formation of volatile compounds by thermal degradation of carotenoids in aqueous medium. β-Carotene degradation. *Journal Agricultural Food Chemistry, 38*, 237–243.

Limbo, S., Torri, L., & Piergiovanni, L. (2007). Light-induced changes in an aqueous β-carotene system stored under halogen and fluorescent lamps, affected by two oxygen partial pressures. *Journal Agricultural Food Chemistry, 55*, 5238–5245.

Minguez-Mosquera, M. I., & Jaren-Galan, M. (1995). Kinetics of the decolouring of carotenoid pigments. *Journal of the Science of Food and Agriculture, 69*, 153–161.

Nunes, F. M. N., Veloso, M. C. C., Pereira, P. A., de, P., & de Andrade, J. B. (2005). Gas-phase ozonolysis of the monoterpenoids (S)-(+)-carvone, (R)-(−)-carvone, (−)-carveol, geraniol and citral. *Atmospheric Environment, 39*, 7715–7730.

Ojima, F., Sakamoto, H., Ishiguro, Y., & Terao, J. (1993). Consumption of carotenoids in photosensitized oxidation of human plasma and plasma low-density lipoprotein. *Free Radical Biology and Medicine, 15*, 377–384.

Rodriguez, E. B., & Rodriguez-Amaya, D. B. (2007). Formation of apocarotenoids and epoxycarotenoids from β-carotene by reactions and by autoxidation in model systems and processed foods. *Food Chemistry, 101*, 563–572.

Siems, E., Sommeburg, O., Schild, L., Augustin, W., Langhans, C. D., & Wiswedel, I. (2002). β-Carotene cleavage products induce oxidative stress in vitro by impairing mitochondrial respiration. *Federation of American Societies for Experimental Biology, 16*, 1289–1291.

Siems, E., WiswedeL, I., Salerno, C., Crifo, C., Augustin, W., Schild, L., et al. (2005). β-Carotene breakdown products may impair mitochondrial functions–potential site effects of hight-dose β-Carotene supplementation. *Journal Nutritional Biochemistry, 16*, 385–397.

Sommerburg, O., Langhans, C., & Arnhold, J. (2003). β-Carotene cleavage products after oxidation mediated by hypochlorous acid-a model for neutrophil-derived degradation. *Free Radical Biology and Medicine, 35*, 1480–1490.

Stratton, S. P., Schaefer, W. H., & Liebler, D. C. (1993). Isolation and identification of singlet oxygen oxidation products of β-carotene. *Chemical Research in Toxicology, 6*, 542–547.

Van Leeuwen, S. M., Hendriksen, L., & Karst, U. (2004). Determination of aldehydes and ketones using derivatization with 2,4-dinitrophenylhydrazine and liquid hromatography-atmospheric pressure photoionization-mass spectrometry. *Journal of Chromatography A, 1058*, 107–112.

Waché, Y., Bosser-Deratuld, A., LY, H. M., & Belin, J. (2002). Co-oxidation of β-carotene in biphasic media. *Journal of Molecular Catalysis B: Enzymatic, 19–20*, 197–201.

Zhao, L., Chen, F., Zhao, G., Wang, Z., Liao, X., & Hu, X. (2005). Isomeration of trans-astaxanthin induced by copper (II) ion in ethanol. *Journal of Agricultural and Food Chemistry, 53*, 9620–9623.

Zwiener, C., Glauner, T., & Frimmel, F. H. (2002). Method optimization for the determination of carbonyl compounds in disinfected water by DNPH derivatization and LC-ESI-MS-MS. *Analytical and Bioanalytical Chemistry, 372*, 615–621.