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/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
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%,