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Spectroscopic studies of neutral and chemically oxidized species of β-carotene, lycopene and norbixin in CH_2Cl_2: Fluorescence from intermediate compounds

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ABSTRACT

Radical cations, dications and oxidized intermediate species of three carotenoids, namely, β-carotene, lycopene and norbixin, were generated in CH_2Cl_2 solutions *via* chemical oxidation using anhydrous $FeCl_3$. UV–vis, fluorescence and fluorescence-excitation spectroscopic studies were performed to understand and compare the nature of intermediate species generated during the chemical oxidation process and subsequent degradation. The intense emission observed at 550 nm can be assigned to the $S_2 \rightarrow S_0$ ($1^1B_u \rightarrow 1^1A_g$) transition of the carotenoid molecules. The 350 nm excitation during the oxidation process for β-carotene, lycopene and norbixin exhibit intense fluorescence peaks at 492 nm, 493 nm and 500 nm, respectively. These peaks are assigned to intermediate peroxy/epoxy compounds of the three molecules that are formed with molecular oxygen prior to the formation of oxidized short-chain stable compounds.

1. Introduction

Carotenoids (Car) are linear C_{40} tetraterpenoid hydrocarbons composed of eight C_5 isoprene units. The extended conjugated polyene chain with a delocalized π electrons system serves as the light absorbing chromophore [1–4], which imparts brilliant colors to these molecules. Carotenoids are multifunctional naturally occurring pigments found in many plants, animals, algae, among others, [5,6] and they participate in redox reactions as electron donors, generating Car radical cations ($Car^{\bullet+}$) [7,8]. These reactive $Car^{\bullet+}$ species are involved in many reactions, one of which is the reaction with O_2 to produce the epoxide form of $Car^{\bullet+}$, which subsequently undergoes cleavage to form apo-carotenals, apo-carotenones and other various oxidized products [9–13]. The potential health risks associated with these molecules were recently reported in an article [12] that indicated that the oxygenated products of β-carotene that were generated *in vitro* increased the oxidative stress of isolated rat liver. The ability of Car to quench singlet oxygen ($^1O_2$), especially under photosensitized oxidation conditions to form $Car^{\bullet+}$ oxygen adducts, were also reported by many workers [11–13]. However, the role of oxygenated Car in biological systems has not been fully explained.

Further, the electronic absorption properties of many Car species and their radical species have been extensively studied [14–16]. However, their luminescence properties have not been addressed adequately to our knowledge. In this study, both electronic absorption and fluorescence spectroscopic techniques were used to gain a better understanding of the chemical oxidation process of Car. It is essential to elucidate the role of epoxy Car, especially in the area of biological research because oxidation products may have a beneficial or a harmful effect on human health. Three Car species, namely, β-carotene, lycopene and norbixin (Fig. 1), were investigated in this work, and anhydrous ferric chloride ($FeCl_3$) in CH_2Cl_2 was used as the oxidizing agent.

2. Experimental

2.1. Sample preparation and HPLC characterization

β-carotene (I) of a purity ≥93% was purchased from Sigma chemicals (PVT) Ltd. Lycopene (II) was extracted from well-matured fruits of watermelon "Dark Belly" species according to the method described in Rodriguez-Amaya [2,3]. Norbixin (III) was extracted [17] from the outer coating of the Annatto seeds of the *Bixa orellana* tree by washing with hexane, followed by solvent removal by rotary evaporation. Aqueous alkali was added to the

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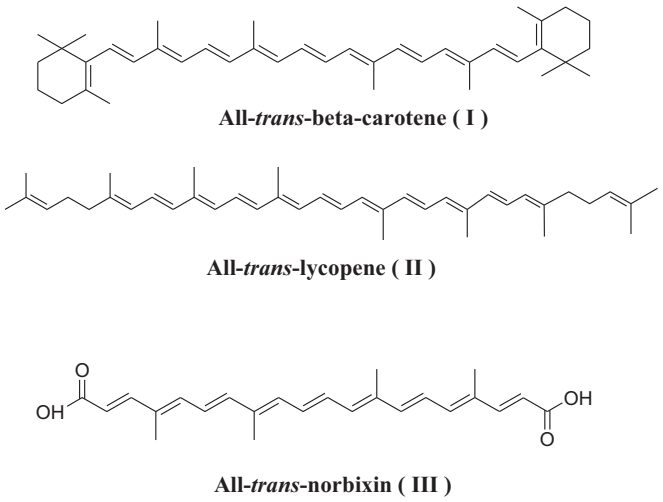

Fig. 1. Molecular structures of the all-*trans* carotenoids (I) β-carotene, (II) lycopene and (III) norbixin.

resultant powder and then heated for hydrolysis, followed by cooling. An aqueous solution was filtered and acidified with 5 M HCl to precipitate norbixin, washed with cold hexane, and then dried to obtain solid norbixin. All of the samples were N_2 purged and stored at −20 °C. Compounds were characterized using UV–vis spectrophotometry and HPLC techniques. A HPLC series 1200 (Agilent, Waldbronn Germany) apparatus equipped with a multi-wavelength/photodiode array detectors was used for chromatographic analysis. The mobile phase consisting acetonitrile, methanol and ethyl acetate with 0.05% (v/v) triethylamine was used at a flow rate 0.5 ml/min. Anhydrous ferric chloride ($FeCl_3$) and HPLC grade dichloromethane (CH_2Cl_2) were purchased from Hemsons International and Sigma Chemicals (PVT) Ltd., respectively.

2.2. UV–vis and fluorescence spectroscopy

The electronic absorption spectra were recorded using a Labomed, Inc., Spectro UV–vis double beam spectrophotometer Model UVD 2960. Fluorescence experiments were performed at 25 °C using a Thermo Scientific Lumina fluorescence spectrometer. The excitation wavelength used throughout this work was 350 nm. The probing wavelengths of the fluorescence excitation scans were recorded at 550 nm. The cuvette holder with the Peltier heater was used to obtain spectra at different temperatures in the range of 10–40 °C. Fluorescence quantum yields of I–III were measured using quinine sulfate as the standard [18]. Anhydrous $FeCl_3$ in CH_2Cl_2 and all of the solvents used throughout this work were scanned to confirm that they are free from fluorescing impurities.

3. Results and discussion

3.1. HPLC analysis of carotenoids

Fig. 2(A)–(C) shows the HPLC chromatograms of (I)–(III), respectively. HPLC chromatograms obtained for extracted lycopene and norbixin samples indicate that they have purities of approximately ≥96% and ≥90%, respectively. The purity of the samples was confirmed by using photodiode array spectra [2,3,19]. Sharp peaks without any shoulders indicate that molecules (I)–(III) are predominantly in all *trans* configuration.

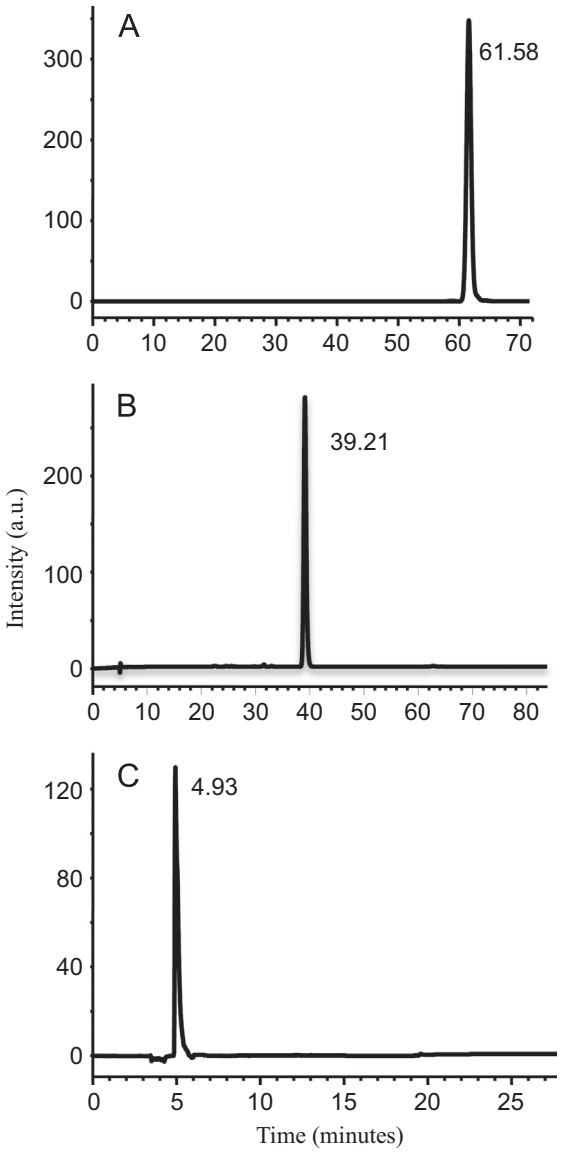

Fig. 2. HPLC chromatogram of (A) standard β-carotene, (B) lycopene extracted from watermelon and (C) norbixin extracted from Annatto seeds.

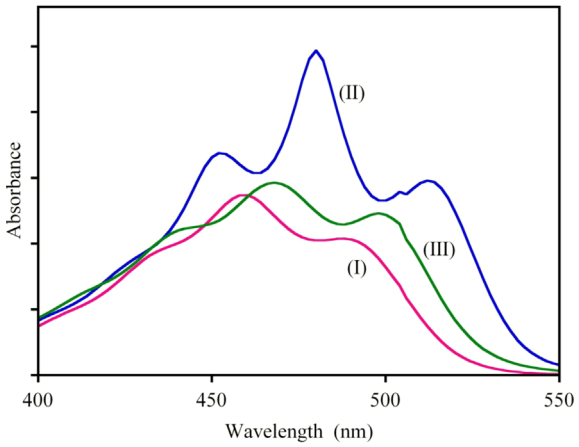

Fig. 3. UV–vis/NIR spectra of the carotenoids (I) β-carotene, (II) lycopene and (III) norbixin in CH_2Cl_2.