ELSEVIER

Food Chemistry 64 (1999) 315–321

Food Chemistry

Antioxidant activity of palm oil carotenes in organic solution: effects of structure and chemical reactivity

E. Olatunde Farombi*, George Britton

School of Biological Sciences, University of Liverpool, PO Box 147, Liverpool L69 3BX Liverpool, UK

Received 13 March 1998; accepted 28 April 1998

Abstract

The antioxidant effectiveness of palm oil α-carotene and comparison with β-carotene in organic solution containing egg-yolk phosphatidycholine (EYPC) in the presence of lipid soluble 2,2[1]-azobis (2,4-dimethyl valeronitrile) (AMVN)-generated peroxyl radicals was investigated by measuring the formation of phosphatidyl choline hydroperoxide (PCOOH) and thiobarbituricacid reacting substances (TBARS). Lutein and zeaxanthin (xanthophylls), similar to α and β carotenes, respectively, but differing in containing hydroxyl groups on the two rings (3,3[1]-diol), were also included in the investigation. The carotenes were more rapidly oxidised than the xanthophylls in the solution. The initial rates of oxidation of the carotenoid tested were 0.39 μM min^{-1} (α-carotene), 0.44 μM min^{-1} (β-carotene), 0.30 μM min^{-1} (lutein) and 0.33 μM min^{-1} (zeaxanthin). Incubation of EYPC with AMVN at 37°C induced the accumulation of PCOOH at the linear rate of 1.8 μM min^{-1}. Although, all the carotenoids tested at 1 mol % relative to EYPC retarded ($p < 0.05$) the chain propagation reaction of PCOOH formation α-carotene had the highest activity but this was less than α-tocopherol. α-Tocopherol, α-carotene, β-carotene, lutein and zeaxanthin reduced PCOOH accumulation by 78, 65, 40, 60 and 43%, respectively. AMVN incubated with EYPC for 2 h induced the formation of TBARS compared to the control ($p < 0.001$). α-Carotene significantly suppressed the TBARS formation by 68% whilst β-carotene, lutein and zeaxanthin elicited 50, 64 and 53% reductions, respectively. α-Tocopherol retarded the TBARS formation by 80%. These results suggest that α-carotene, a carotenoid abundantly present in human diets, especially red palm oil, may better attenuate peroxyl radical-dependent lipid peroxidation than β-carotene in organic solution.

1. Introduction

Accumulating evidence from several lines of investigation suggests that increased consumption of dietary antioxidants such as vitamin C, vitamin E and β-carotene, has chemopreventive activity (Ames et al., 1993). Epidemiological findings suggest that intake of foods containing carotenoids, including the vitamin A precursor β-carotene, protects against the development of human cancer, especially cancer of the lung (Nutrition Policy Board, Peto et al., 1981; Paganini-Hill et al., 1987). In experimental animal models, β-carotene has been shown to protect against chemically-induced cancer formation (Temple and Basu, 1987; Shklar and Schwartz, 1988). In cultured cells, β-carotene has been reported to inhibit physical- and chemical-mediated neoplastic transformation (Pung et al., 1988; Som et al., 1984).

Reports of various studies suggest that the anticarcinogenic properties of β-carotene may be related to its intrinsic activity as antioxidant and are independent of its provitamin A activity (Peto et al., 1981; Zhang et al., 1991). It has been demonstrated that β-carotene can prevent singlet oxygen-mediated lipid peroxidation of methyl linoleate (Terao et al., 1980) or formation of peroxyl radicals (Terao, 1989) and prevent free-radical-mediated oxidation in liposomal lipids (Krinsky and Deneke, 1982). Dietary β-carotene has also been reported to be a potent antioxidant at low oxygen tension (Stocker et al., 1987; Vile and Winterbourn, 1988; Burton, 1989). The effectiveness of other carotenoids, especially the xanthophylls such as lutein, zeaxanthin asthaxanthin, as antioxidants have been examined (Palozza et al., 1992; Terao, 1989; Woodall et al., 1995, 1996).

Palm oil contains a high amount carotenoids (Tan et al., 1986; Berger, 1983) and 80–90% of its carotenoids are β-carotene and α-carotene in the ratio 2:1 (Tan and Chu, 1991). These lipid-soluble pigments have been isolated and identified in palm oil (Tan et al., 1986; and Ng

* Corresponding author: Department of Biochemistry, University of Ibadan, Nigeria.

0308-8146/98/$—see front matter
PII: S0308-8146(98)00108-3

and Tan, 1988). α-Carotene is similar to β-carotene in structure but a slight difference occurs in the double bond located on the β-ionone ring. It also has provitamin A activity like β-carotene. However, little is known about the antioxidant activity of α-carotene in homogeneous/heterogeneous organic solutions, though its inhibitory effects on the proliferation of the human neuroblastoma cell line and protection against chemically-induced neoplastic transformation have been reported (Murakoshi et al., 1989; Bertram et al., 1991). Also, the antioxidant activities of α-and related carotenes have been reported in Fe^{3+}-ADP/NADPH and in paraquat/NADPH systems (Kim, 1990).

The purpose of this study was to examine the antioxidant effectiveness of α-carotene and to compare it with β-carotene in organic solution containing phosphatidyl choline in the presence of chemically generated peroxyl radicals. Also lutein and zeaxanthin, similar to α- and β-carotenes, respectively, but differing in possessing hydroxyl groups on the rings, were included in the investigation to elucidate the chemical reactivity and structural determinants of their antioxidant activity.

2. Materials and methods

2.1. Chemicals

Palm oil (*Elaeis guineensis*) was imported from Nigeria. β-carotene, lutein and zeaxanthin were donated by F. Hoffman-La Roche and Co. Ltd, Basle, Switzerland. The carotenoids were shown to be pure by HPLC analysis. α-Tocopherol, 2,2[1]-azobis (2,4 dimethyl valeronitrile) (AMVN), thiobarbituric acid (TBA), trichloroacetic acid, egg-yolk-phosphatidyl choline (EYPC) and ethylenediamine tetraacetic acid (EDTA) were purchased from Sigma, Chemical Co., Poole, Dorset. Magnesium oxide and Kieselgur G were purchased from E.Merck, Darmstadt. Solvents were redistilled before use. All other reagent and solvents were of analytical grade.

2.2. Extraction and isolation of carotenes from palm oil

Two grams of palm, oil were dissolved in about 20 ml of diethyl ether. To this was added 100 ml of 30% KOH in methanol (w/v). The mixture was transferred into a separating funnel and kept in the dark for 5 h. The saponified mixture was exhaustively washed with distilled water to remove the lower soap solution until the pH of the solution was neutral. The extract was concentrated using a rotary evaporator with the water bath temperature maintained at 30°C. The concentrated extract was transferred into a sample vial after adding a small volume of petroleum ether and flushed with nitrogen. The sample was stored in the freezer at −20°C.

2.3. Column chromatography

Ten grams of alumina were deactivated to Brokman III by the addition of 0.6 ml of water. The slurry was added into the column already plugged with wool. Petroleum ether was gently added and allowed to run through for a few minutes. Two millilitres of the extract was introduced to the top of the column. Petroleum ether was used for the elution. The hydrocarbon fractions containing the carotenes were collected into sample vials and dried under nitrogen. Other fractions containing the xanthophylls were also collected using appropriate solvent systems. The fractions were taken up in different solvents (ether, petrol and chloroform) and subjected to UV/visible absorption spectrophotometry. Carotenoids were tentatively identified by comparison of their wavelengths and spectra with literature reports.

2.4. Thin-layer chromatography (TLC)

α- and β-Carotenes were separated on a Mgo/Kieselgur G plates using cyclohexane as the mobile phase. Two bands that emerged after the chromatography were scraped off and eluted with diethyl ether and evaporated under nitrogen. The two fractions were further purified on silica gel TLC plates to ensure purity of the samples. The two fractions were tentatively identified by UV/visible spectrophotometry.

2.5. Carotenoid pigment analysis by HPLC

The purity of carotenoid samples used for antioxidant activity was confirmed by reverse-phase HPLC. A Waters 600E solvent delivery system was used with a Waters 990 on-line multichannel photo diode array detector (Waters chromatography Watford, Herts.) scanning from 250–550 nm. The stationary phase used was a spherisorb ODD 2 reversed-phase column, 250× 4.6 mm diameter, 5 μm particle size (Phase separation Ltd.). The mobile phase consisted of a linear gradient of 10–100% ethylacetate in acetonitrile–water (9:1,v/v) over 25 min at a flow rate of 1 ml min^{-1}.

2.6. Quantitative determination of carotenoids

The concentrations of carotenoids used for antioxidant activity were determined spectrophotometrically (Cecil Ce 599 automatic scaning spectrophotometer) in an appropriate solvent according to Britton (1985).

2.7. Reactions of carotenoids with AMVN

Carotenoids (α, β carotenes, lutein and zeaxanthin) at 1 μM concentration were dissolved in hexane and pre-incubated at 37°C for 5 min in a 3 cm^3 quartz

cuvette. The reaction was initiated by the addition of freshly-prepared AMVN (1 mM, final concentration). Loss of absorbance at the wavelength maximum for each carotenoid was monitored by UV-Visible spectrophotometry for 30 min.

2.8. Assay of phospholipid hydroperoxide formation and residual carotenoid

The methods of Stocker et al. (1987) and Terao (1989) as modified by Woodall (1994) were used. Egg-yolk phosphatidycholine (0.5 μM) of (100 mg ml^{-1} in chloroform) was diluted with 5 ml of chloroform and added to the dried antioxidant. The carotenoid/phospholipid mixtures were redissolved by vigorous vortex mixing for at least 1 min.

The reaction was initiated by the addition of freshly-prepared AMVN (10 mM) to give the following final concentrations: AMVN (5 mM) carotenoids/α-tocopherol (0.05 mM) and phosphatidylcholine (5 mM). Incubation was done at 37°C in the dark and in a thermostatted water bath. Aliquots of samples were removed for the measurement of phosphatidylcholine hydroperoxide (PCOOH) formation by HPLC or of residual carotenoid concentrations by UV-visible spectrophotometry.

2.9. HPLC analysis of PCOOH

PCOOH were determined according to the modification of the methods of Stocker et al. (1987) and Lim et al. (1992). A Sperisorb 25 cm×4.6 mm silica (S5N) column, particle size 5 μm, as the stationary phase, was used. An isocratic mobile phase consisting of methanol/water (85:15, v/v) was used with a flow rate of 1.5 ml min^{-1} at ambient temperature. The hydroperoxides were detected by ultraviolet absorption at 235 nm. Samples were loaded with 25 ml syringes (Hamilton co. Ltd Saffron Walden U.K.) to fill a 20 ml sample loop, fitted to a rheodyne injector. Phospholipid hydroperoxides eluted around 3.5 min as a relatively broad peak. Standard PCOOH was prepared according to Terao et al. (1985).

2.10. Induction of lipid peroxidation and assay of thiobarbituric acid-reactive substances (TBARS)

For the assay of TBARS, the samples were prepared as described above but incubation was done at 37°C for 2 h. TBARS were determined according to the modification of Jackson et al. (1983), as described by Woodall (1994). After incubation, the mixture was added to an equivalent colume of TBA reagent (0.61 M, TCA, 55.5 mM TBA and 1 mM EDTA). The mixture was heated at 100°C for 12 min and cooled on ice. The chromogen was extracted into 3 ml butan-l-ol. The formation of TBARS was measured at 532 nm by UV-visible spectrometry.

2.11. Statistics

The data were analyzed by a two-tailed students' t-test, p-values less than 0.05 were considered statistically significant.

3. Results

The oxidation of four carotenoids (Fig. 1) tested in this study by azo-initiated peroxyl radicals is shown in Fig. 2. For all the carotenoids, the relative time courses show a linear rate of decrease in concentration. β-carotene and α-carotene are the most rapidly oxidised in the system whilst zeaxanthin and lutein exhibit the lowest rates of oxidation. The initial rates of oxidation of the carotenoids were α-carotene, 0.39 μM min^{-1}, β-carotene, 0.44 μM min^{-1}, lutein 0.30 μM min^{-1}) and zeaxanthin, 0.33 μM min^{-1}, respectively. The hydrocarbon carotenes are more reactive in AMVN-generated peroxyl radicals than the xanthophylls.

The results of the effects of the four carotenoids at 0.5 μmol (1 mol %) relative to egg-yolk phosphatidycholine hydroperoxides appear in Fig. 3. Incubation of egg-yolk phosphatidylcholine with AMVN at 37°C

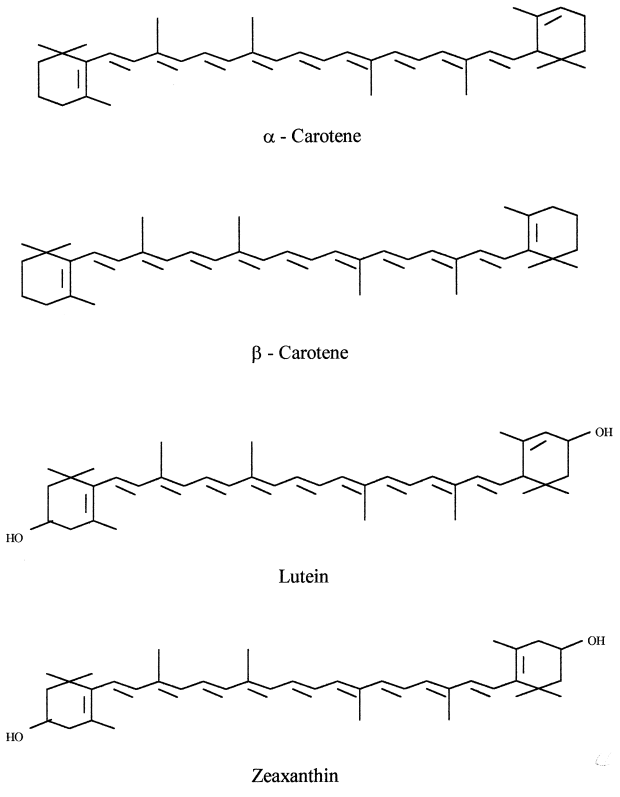

Fig. 1. Structures of carotenoids.

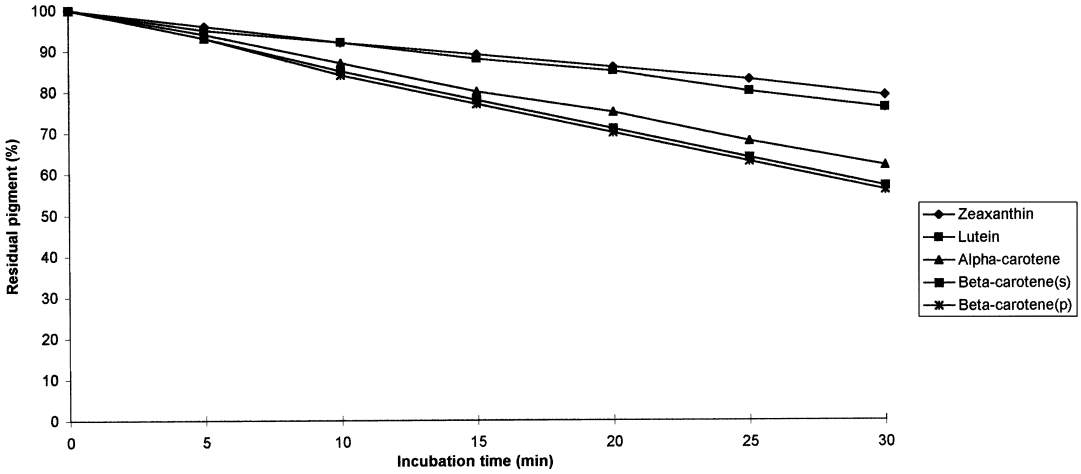

Fig. 2. AMVN-induced oxidation of carotenoids. Each point represents the mean of seven experiments β-Carotene(s) = synthetic β-carotene; β-carotene (p) = β-carotene from red palm oil.

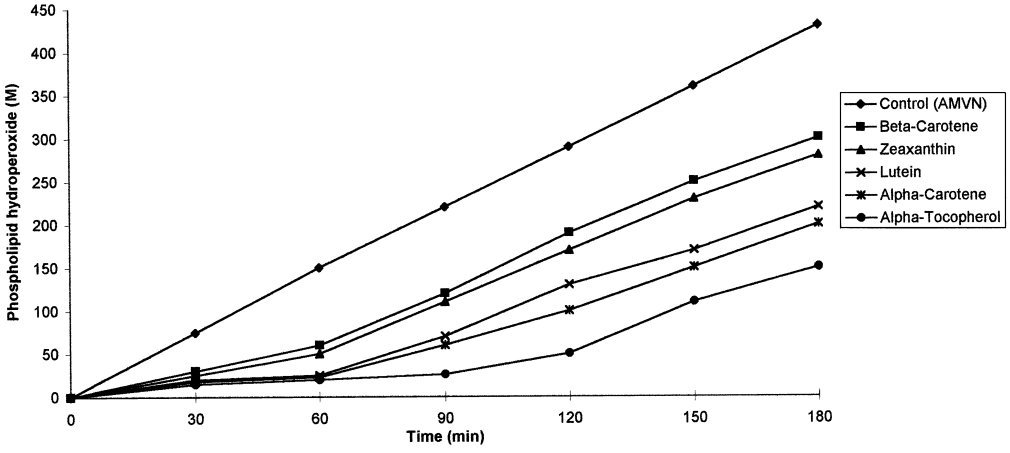

Fig. 3. Effect of carotenoids and alpha-tocopherol on AMVN-induced phopspholipid hydroperoxide formation. The points represent the means of four experiments.

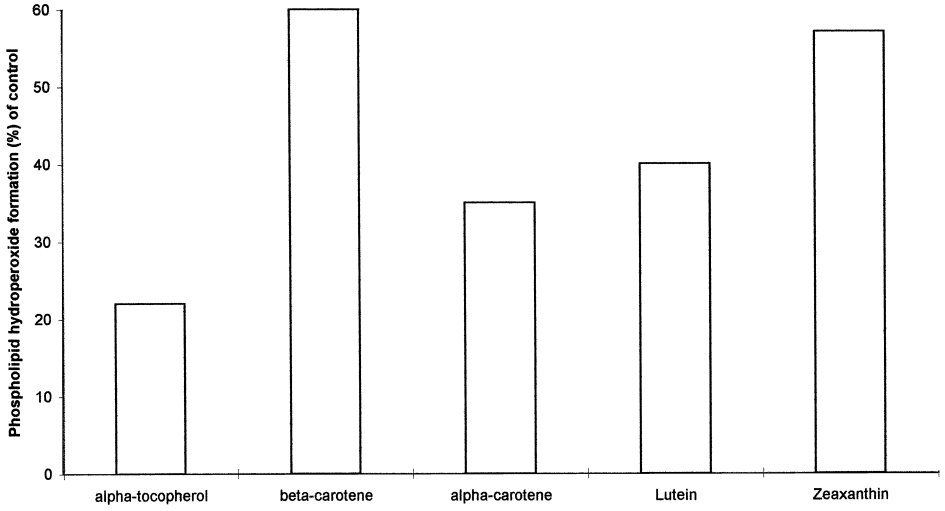

Fig. 4. Effect of 1 mol % carotenoids and alpha-tocopherol on the formation of phospholipid hydroperoxide induced by AMVN. The bar lines indicate the mean of four experiments.

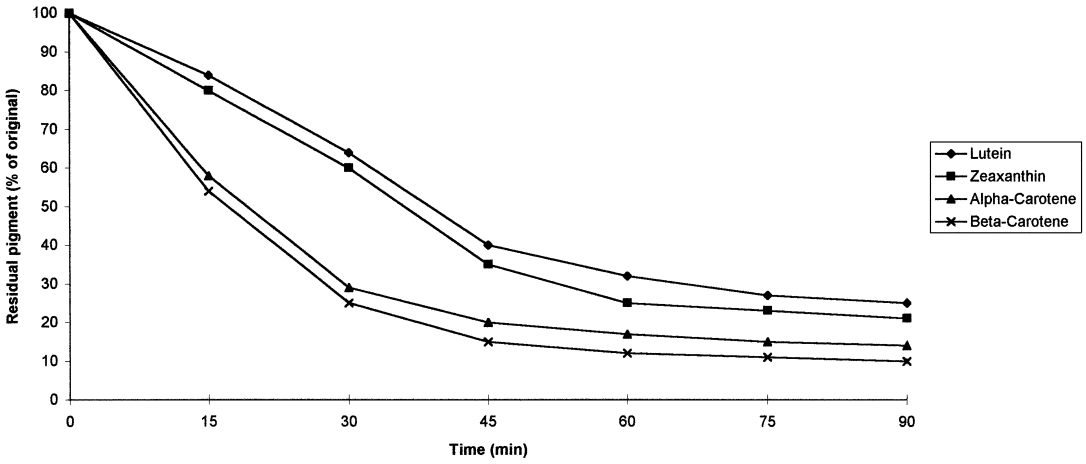

Fig. 5. Loss of carotenoids and alpha-tocopherol during AMVN-mediated oxidation of phosphatidyl choline in solution. Each point represents the mean of four experiments.

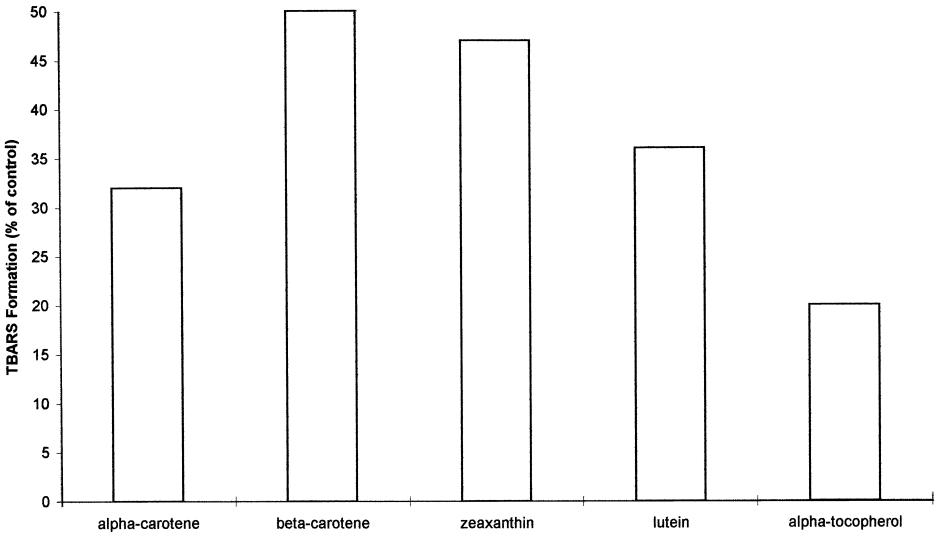

Fig. 6. Effect of 1 mol % carotenoids and alpha-tocopherol on TBARS formation in phosphatidyl choline stressed with AMVN in solution. The bar lines indicate the mean of four experiments.

induced the accumulation of PCOOH at a reasonably linear rate of about 1.8 μM min^{-1}. In the presence of the carotenoids (α,β carotenes, lutein and zeaxanthin), there was a significant reduction in the rate of AMVN-induced PCOOH formation compared to controls ($p < 0.001$). α-Tocopherol and the carotenoids produced a lag in PCOOH formation to varying extents during the first hour of the reaction (Fig. 3). Comparison of the percentage PCOOH formed relative to that of control (Fig. 4) indicates that α-tocopherol, α-carotene and lutein are better antioxidant as revealed by their ability to attenuate PCOOH formation. The order of antioxidant potency of the carotenoids and α-tocopherol is α-tocopherol > α-carotene > lutein > zeaxanthin = β-carotene. Even after the first hour, α-tocopherol, α-carotene and lutein retarded PCOOH formation more than β-carotene and zeaxanthin (Fig. 3). It can be seen from Fig. 5 that the xanthophylls were consumed at a slower rate than α-carotene or β-carotene.

Incubation of egg-yolk phosphatidylcholine in the presence of 5 mM AMVN for 2 h induced the formation of TBARS compared to control ($p < 0.001$) (Fig. 6). α-Tocopherol, α-carotene, β-carotene, lutein and zeaxanthin suppressed the initiator to different degrees. Again, α-carotene was the most potent antioxidant of the carotenoids tested in the TBA test.

4. Discussion

β-Carotene has received considerable attention in recent times as a putative chain-breaking biological antioxidant and its ability to interact with free radicals such as peroxyl radicals and to scavenge and quench

singlet oxygen is well documented (Palozza et al., 1992; Lim et al., 1992).

Other potential dietary antioxidants which exist in human plasma such as lycopene, lutein, zeaxanthin and β-cryptoxanthin have been examined, but α-carotene, which is abundantly present in palm oil and exists in the same concentration as β-carotene in human plasma (0.1–0.2 μml^{-1}) (Terao, 1989; Miller et al., 1984), has not been fully examined. To date, the antioxidant efficacy of α-carotene in homogeneous solution has not been reported.

We have used azo-initiated peroxyl radicals to induce the peroxidation of egg-yolk phosphatidylcholine in organic solution to assess the antioxidant potency of α-carotene and related carotenoids. Our results indicate that AMVN induced peroxidation of phosphatidylcholine, resulting in the formation of PCOOH and TBARS (Figs. 3 and 6). This is in consonance with previous findings (Lim et al., 1992; Terao, 1989). All the carotenoids exhibited reactivity towards peroxyl radicals in solution. The hydrocarbon carotenes (α- and β-carotenes) are more reactive in this system than the xanthophylls (lutein and zeaxanthin). Lutein and zeaxanthin are more polar than α and β-carotenes and may possibly not react in a non-polar solvent like hexane due to the presence of hydroxyl groups. In studies on the reactivity of lutein with free radicals in organic solution, it was observed that the reactivity of this xanthophyll decreased with decreasing polarity of the solvent (Chopra et al., 1993).

At concentrations of 1 mol % relative to phosphatidylcholine, all the carotenoids reduced the levels of PCOOH and TBARS formed compared to control samples but none was as effective an antioxidant as α-tocopherol. This is in agreement with other studies where the antioxidant activity of carotenoids has been compared with that of a α-tocopherol to protect various substrates from peroxyl radical mediated oxidation in organic solution (Burton and Ingold, 1984; Stocker et al., 1987; Terao, 1989). These results could be interpreted to mean that in the presence of carotenoids, phosphatidylcholine in organic solution is protected from peroxidation, the carotenoids preferentially reacting with peroxyl radicals and being consumed in the process, thus acting as sacrificial radical-trapping antioxidants.

In the solvent system, lutein was as effective an antioxidant as α-carotene and zeaxanthin as β-carotene but α-carotene and lutein were better antioxidants than β-carotene and zeaxanthin, respectively. This suggests that the presence of hydroxyl groups on the two rings of lutein and zeaxanthin did not influence their ability to protect phosphatidyl choline from free-radical oxidation. This has been confirmed in studies of the reactivity of zeaxanthin with peroxyl radicals in solution. (Terao, 1989; Woodall, 1994).

Cis or trans configuration on β-ionone rings of α and β-carotenes did alter the antioxidant activity of the two carotenes. In α-carotene, the double bond of the second ring (cis configuration) is not part of the chromophore, leaving 9 conjugated double bonds with the β-ring. This arrangement, therefore presumably enhances the ability of α-carotene to better trap radical species than β-carotene. Moreover, the results of our autoxidation reaction (Fig. 2) indicate that α-carotene is more resistant to this chain reaction than β-carotene.

Thus a cis configuration on the β-ionone ring reduces the reactivity of the α-carotene radical intermediate with oxygen in the system, thus reducing the extent of autoxidation and increasing the amount of α-carotene to act as antioxidant compared with β-carotene. α-Carotene has been shown to be a better antioxidant than β-carotene in Fe^{3+}-ADP/NADPH and in paraquat/NADPH systems (Kim, 1990).

The present investigation has demonstrated that α-carotene could possibly be a better antioxidant than β-carotene in peroxyl radical-dependent lipid peroxidation. Further studies are necessary to elucidate the effectiveness of α-carotene as an antioxidant, especially in membranes which are highly ordered and anisotropic in nature. This ordered environment may possibly affect free-radical-trapping antioxidants and differ from the present system where the carotenoids exist in free solution in an isotropic monomeric state.

Acknowledgements

This work was supported by a Staff development research fellowship, through National Universities (Nig) commission under the World Bank Project Scheme, to E.O.F., while in Liverpool University, U.K.

References

Ames, B. N., Shingenaga, M. K. and Hagen, T. M. (1993) Oxidants, antioxidants and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences U.S.A.* **90**, 7915–7922.

Berger, K. G. (1983) In *Handbook of Tropical Foods*, ed. H. T. Chan, pp. 443–468. Marcel Dekker, New York.

Bertram, J. S., Pung, A., Churley, M., Kappock, T. J., Wilkins, L. R. and Cooney, R. V. (1991) Diverse carotenoids protect against chemically-induced neoplastic transformation. *Carcinogenesis* **12**, 671–678.

Britton, G. (1985) General carotenoid methods. *Methods in Enzymology* **111**, 113–149.

Burton, G. W. and Ingold, K. U. (1984) β-carotene: an unusual type of lipid antioxidant. *Science* **224**, 569–573.

Burton, G. W. (1989) Antioxidant action of carotenoids. *Journal of Nutrition* **119**, 109–111.

Chopra, M., Wilson, R. L. and Thurnham, D. I. (1993) Free radical scavenging of lutein in vitro. *Annals New York Academy of Science* **1**, 246–249.

Jackson, M. J., Jones, D. A. and Edwards, R. H. T. (1983) Lipid peroxidation of skeletal muscle: an *in vitro* study. *Bioscience Reports* **3**, 609–619.

Kim, H. (1990) Comparison of antioxidant activity of α-carotene, lutein and lycopene by high pressure liquid chromatography. *Korea Journal of Nutrition* **23**, 434–442.

Krinsky, N. I. and Deneke, S. M. (1982) Interaction of oxygen and oxy-radicals with carotenoids. *Journal of the National Cancer Institute* **69**, 205–210.

Lim, B. P., Nagao, A., Terao, L., Tanaka, K., Suzuki, T. and Takama, K. (1992) Antioxidant activity of xanthophylls on peroxy radical-mediated phospholipid peroxidation. *Biochimica et Biophysica Acta*. **1126**, 178–184.

Miller, K. W., Lorr, N. A. and Yang, C. S. (1984) . *Anal. Biochem.* **138**, 340–345.

Murakoshi, M., Takayasu, J., Kimura, O., Koshimura, E., Nishino, H., Iwashima, A., Okuzumi, J., Sakai, T., Sugimoto, T., Imanishi, J. and Iwasaki, R. (1989) Inhibitory effects of α-carotene on proliferation of the human neuroblastoma cell line GoTO. *Journal of the National Cancer Institute* **81**, 1649–1652.

Ng, J. H. and Tan, B. (1988) Analysis of palm oil carotenoids by HPLC with diode array detection. *Journal of Chromatographic Science* **26**, 463–469.

Paganini-Hill, Chao, A., Ross, R. K. and Henderson, B. E. (1987) *Journal of the National Cancer Institute* **79**, 443–448.

Palozza, P., Moualla, S. and Krinsky, N. I. (1992) Effect of β-carotene and α-tocopherol on radical-initiated peroxidation of microsomes. *Free Radicals in Biology and Medicine* **13**, 127–136.

Peto, R., Doll, Bukley, J. D. and Sporn, M. B. (1981) Can β-carotene materially reduce human cancer rates? *Nature* **290**, 201–208.

Pung, A., Rundhaug, J. E., Yoshizawa, C. N. and Bertram, J. S. (1988) β-carotene and canthaxanthin inhibit chemically- and physically-induced neoplastic transformation in 10 T1/2 cells. *Carcinogenesis* **9**, 1533–1539.

Shklar, G. and Schwartz, J. (1988) Tumor necrosis factor in experimental cancer regression with alpha-tocopherol, beta-carotene, canthaxanthin and algae extract. *European Journal of Cancer and Clinical Oncology* **24**, 839–845.

Som, S., Chatterjee, M. and Baverjee, M. R. (1984) β-carotene inhibition of 7,12 dimethylbenzanthracene-induced transformation of murine mammary cell *in vitro*. *Carcinogenesis* **5**(7), 937–940.

Stocker, R., Yamamoto, Y., McDonagh, A. F., Glazer, A. N. and Ames, B. N. (1987) Bilirubin is an antioxidant of possible physiological importance. *Science* **235**, 1043–1046.

Tan, B. and Chu, F. L. (1991) Effects of palm carotenoids in rat hepatic cytochrome P450-mediated benzo(a) pyrene metabolism. *American Journal of Clinical Nutrition* **53**, 1071–1075.

Tan, B., Grady, C. M. and Gawienowski, A. M. (1986) Hydrocarbon carotenoid profiles of palm oil processed fractions. *Journal of the American Chemical Society* **63**, 1175–1179.

Temple, N. J. and Basu, T. K. (1987) Protective effect of β-carotene against colon tumors in mice. *Journal of the National Cancer Institute* **78**, 1211–1214.

Terao, J. (1989) Antioxidant activity of β-carotene-related carotenoids in solution. *Lipids* **24**, 659–661.

Terao, J., Yamauchi, R., Murakami, H. and Matsushita, S. (1980) Inhibitory effects of tocopherols and β-carotene on singlet oxygen-initiated photoxidation of methyl linoleate and soybean oil. *Journal of Food Process Preserv* **4**, 79–83.

Terao, J., Asano, I. and Matsushita, S. (1985) Preparation of hydroxy and hydroxy derivatives of rat liver phosphatidylcholine and phosphatidylethanolamine. *Lipids* **20**,312–317.

Vile, G. T. and Winterbourn, C. C. (1988) Inhibition of adriamycin-promoted microsomal lipid peroxidation by β-carotene, α-tocopherol and retinal at high and low oxygen partial pressures. *FEBS Letters* **238**, 353–356.

Woodall, A. A. (1994) Carotenoids and the protection of membrane against oxidative damage. Ph.D thesis. University of Liverpool, Liverpool, U.K.

Woodall, A. A., Britton, G. and Jackson, M. J. (1995) Antioxidant activity of catotenoids in phosphatidylcholine vesicles: chemical and structural considerations. *Biochemical Society Transactions* **23**, 133s.

Woodall, A. A., Britton, G. and Jackson, M. J. (1996) Dietary supplementation with carotenoids: effects on α-tocopherol levels and susceptibility of tissues to oxidative stess. *British Journal of Nutrition* **76**, 307–317.

Zhang, L., Conney, R. V. and Bertram, J. S. (1991) Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10T1/2 cells: relationship to their cancer chemopreventive action. *Carcinogenesis* **12**, 2109–2114.