Effects of Lutein, Lycopene, Annatto, and γ-Tocopherol on Autoxidation of Triglycerides

Katri M. Haila,* Satu M. Lievonen, and Marina I. Heinonen

Department of Applied Chemistry and Microbiology, Food Chemistry Division, P.O. Box 27, FIN-00014 University of Helsinki, Finland

The effects of lycopene, lutein, annatto, and γ-tocopherol were examined on autoxidized triglycerides. Oxidation was followed by measuring hydroperoxide formation as peroxide value. The loss of the orange color of carotenoids was followed spectrophotometrically. Lutein and lycopene were prooxidants, whereas the natural food color annatto and γ-tocopherol effectively inhibited hydroperoxide formation. By adding γ-tocopherol, the prooxidant effect of carotenoids was inhibited and loss of yellow carotenoid color was retarded. Moreover, a combination of lutein and γ-tocopherol was more efficient than γ-tocopherol in inhibiting the hydroperoxide formation of triglycerides. The benefit of a combination of a carotenoid and tocopherol as an antioxidant may be due to the effect of γ-tocopherol to retard the formation of degradation products of the carotenoid. The results suggest that potential prooxidant effects of carotenoids should be considered when carotenoids are proposed for color in lipid-containing foods.

Keywords: *Lutein; lycopene; annatto; γ-tocopherol; autoxidation; color; antioxidant*

INTRODUCTION

Carotenoids are commonly found in orange, yellow, and green fruits and vegetables. In foods, naturally occurring carotenoid pigments are added to enhance the color quality. The following carotenoids are permitted by the European Union as food colors: β-carotene, mixed carotenes, annatto, bixin, norbixin, paprika extract, capsanthin, capsorubin, lycopene, lutein, canthaxanthin, β-apo-8′-carotenal and ethyl ester of β-apo-8′-carotenic acid (European Parliament and Council Directive, 1994). The increasing use of carotenoids is due to the growing interest in natural food additives. Annatto, containing bixin or norbixin as a carotenoid, is a natural orange-yellow color widely added to foods (Lauro, 1991; Collins, 1992). However, care must be taken in applications of carotenoids such as β-carotene as food color, since the highly unsaturated structure of β-carotene is responsible not only for the color but also for the unstability of the molecule (Johnson, 1995). Moreover, carotenoid colors could be more universally used if their light stability can be improved (Marcus, 1994).

Carotenoids such as β-carotene, lutein, and lycopene act as antioxidants in lipid phases by trapping free radicals or physically quenching singlet oxygen (Sies et al., 1992). Antioxidant activity of carotenoids in organic solutions is related to oxygen concentration, the chemical structure of carotenoids, and the presence of other antioxidants (Krinsky, 1993). Carotenoids have been shown to maintain the oxidative stability of oils (Kiritsakis and Dugan, 1985; Fakourelis et al., 1987; Lee and Min, 1988, 1990; Min and Lee, 1989; Jung and Min, 1991). However, there are some contradictory results obtained in studies of carotenoids in oxidation of oils. β-Carotene has been shown to act as a prooxidant during lipid oxidation in the light (Terao et al., 1980; Faria and Mukai, 1983; Haila and Heinonen, 1994) and in the dark (Warner and Frankel, 1987; Suzuki et al., 1989).

* Author to whom correspondence should be addressed (e-mail katri.haila@helsinki.fi).

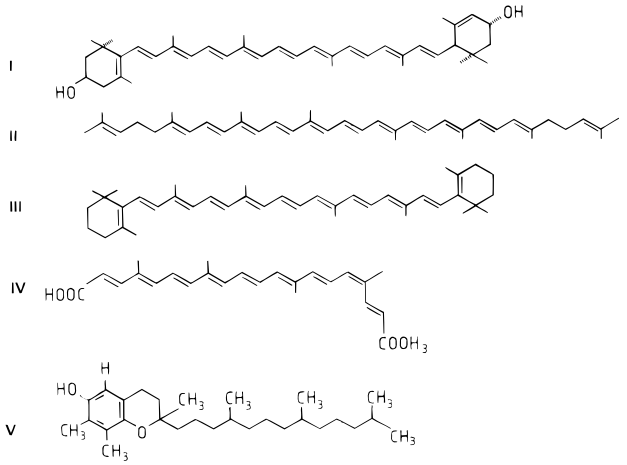

Figure 1. Structures of lutein (I), lycopene (II), β-carotene (III), bixin (IV), and γ-tocopherol (V).

The present work aimed to study the effects of lutein, lycopene, and annatto, and interactions of carotenoids and γ-tocopherol, on hydroperoxide formation of autoxidized triglycerides and to follow the loss of the orange carotenoid color.

MATERIALS AND METHODS

Materials. Lycopene, lutein, and β-carotene were gifts from Roche Oy (Espoo, Finland). Annatto containing 3.8% bixin was kindly provided by Chr. Hansen's Lab. (Hørsholm, Denmark). γ-Tocopherol was purchased from E. Merck (Darmstadt, Germany). The structures of carotenoids and γ-tocopherol are presented in Figure 1. The control was a triglyceride sample without added carotenoid or γ-tocopherol. Organic solvents of HPLC grade were purchased from Rathburn Chemicals Limited (UK).

Preparation of Triglycerides. The triglycerides were purified from low-erucic acid rapeseed oil (received from Van Den Bergh Foods, Helsinki, Finland) by the chromatographic method described by Lampi et al. (1992). The polyunsaturated acid content of the triglycerides was 0.1% 14:0, 3.7% 16:0, 0.2% 17:1, 1.4% 18:0, 53.8% 18:1$n-9$, 2.4% 18:1$n-7$, 22.1% 18:2$n-6$, 10.9% 18:3$n-3$, 0.4% 20:0, 1.3% 20:1$n-9$, 0.2%

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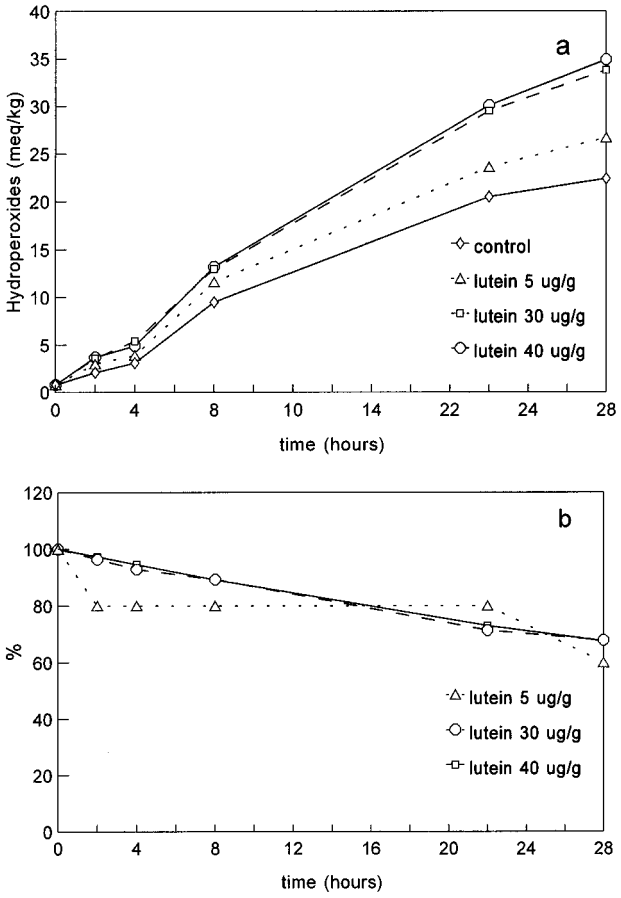

Figure 2. (a) Effect of lutein on hydroperoxide formation (mequiv/kg) of triglycerides autoxidized under dark at 40 °C. The γ-tocopherol content of triglycerides was <1 μg/g. The concentrations of lutein were ◊ = control, △ = 5 μg/g, □ = 30 μg/g, and ○ = 40 μg/g. (b) Concentration (%) of lutein during autoxidation.

22:0, and 0.7% 22:1n − 9 (Hyvönen et al., 1993). The characterization for the remaining tocopherols was performed by HPLC as described in a previous study (Haila and Heinonen, 1994). The determination limit of tocopherols was 1 μg/g. Peroxide value (PV) was determined by the ferric thiocyanate method (FID-IDF, 1974; Ueda et al., 1986).

Autoxidation Studies. After adding lutein (5, 20, 30, 40 μg/g), lycopene (20 μg/g), annatto (20, 30, 60 μg/g as bixin), β-carotene (20 μg/g), γ-tocopherol (10, 15 μg/g), lutein + γ-tocopherol (20 + 15 μg/g, 1:1 in molar ratio) or lutein + γ-tocopherol (20 + 10 μg/g, 1,5:1 in molar ratio) in hexane to triglycerides, hexane was evaporated by N_2. The samples (3 g each) were oxidized under air in sealed clear glass bottles (18 mm i.d.) of pharmacopeia quality. The oxidation was performed in a light cabinet (10 000 lux) (Sanyo MLR-350T, Sanyo Electrics Co., Ltd., Osaka, Japan) with white fluorescent lamps (Sanyo FL40SSW/37, Sanyo Electrics Co., Ltd., Osaka, Japan) at 25 °C or under dark at 40 °C. The methods for the determinations of light power and light intensity were described in a previous study (Haila and Heinonen, 1994). The effects of lutein, lycopene, annatto, and γ-tocopherol on the hydroperoxide formation of autoxidized triglycerides was measured as PV. The loss of lutein and lycopene was followed spectrophotometrically (PE Lambda 11/Bio UV/VIS Spectrometer, Perkin-Elmer Ltd., Überlingen, Germany) at 447 and 452 nm, respectively, and the loss of γ-tocopherol by HPLC was described in a previous study (Haila and Heinonen, 1994). All analyses were done in duplicate, and the results were calculated by one-way analysis of variance (Wagner, 1992).

RESULTS

The results show that lutein at 5, 20, 30, and 40 μg/g concentration was a prooxidant ($p < 0.05$) (Figures 2a and 3a). The higher the concentration of lutein was, the more hydroperoxides were formed of autoxidized triglycerides under dark (Figure 2a). The loss of yellow color is shown in Figure 2b as the consumption of lutein. Moreover, lycopene at 20 μg/g was a prooxidant ($p < 0.05$) (Figure 5a).

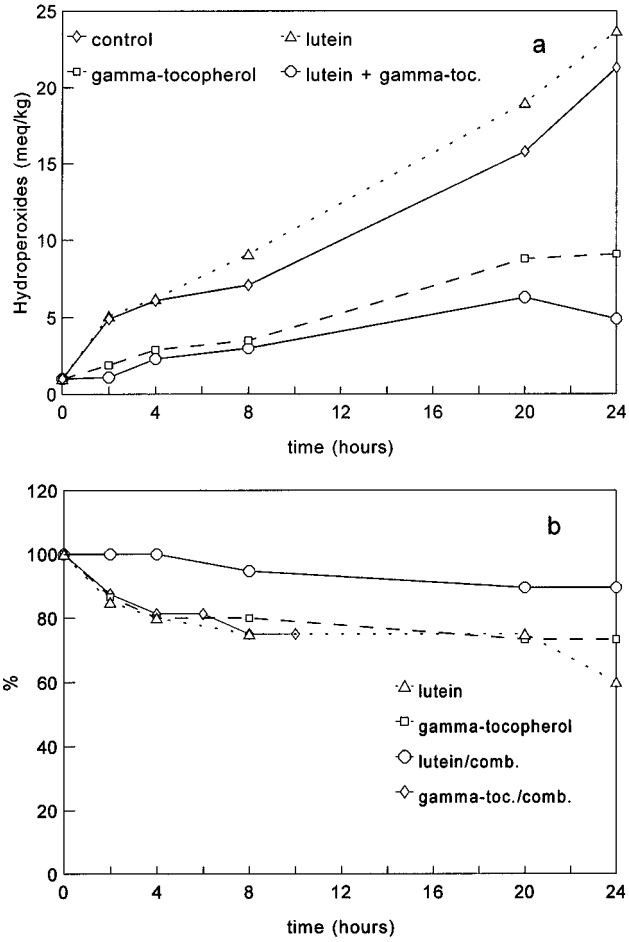

Figure 3. (a) Effect of lutein on hydroperoxide formation (mequiv/kg) of triglycerides autoxidized under light at 25 °C. The γ-tocopherol content of triglycerides was <1 μg/g. The samples were ◊ = control, △ = 20 μg/g lutein, □ = 15 μg/g γ-tocopherol, and ○ = combination sample of 20 μg/g lutein + 15 μg/g γ-tocopherol (molar ratio 1:1). (b) Concentrations (%) of △ = lutein, □ = γ-tocopherol, ○ = lutein in the combination sample of γ-tocopherol and lutein, and ◊ = γ-tocopherol in the combination sample of lutein and γ-tocopherol during autoxidation.

Figure 3a shows that γ-tocopherol at 15 μg/g and a combination of lutein and γ-tocopherol at 20 μg/g + 15 μg/g, respectively, inhibited hydroperoxide formation of autoxidized triglycerides under light. Figures 3a and 4a show that the antioxidant effect of a combination of lutein and γ-tocopherol was stronger ($p < 0.05$) than of γ-tocopherol alone. Figures 3b and 4b show that lutein was consumed faster without added γ-tocopherol, whereas the consumption of γ-tocopherol was not affected by added lutein. In comparison, the concentration of lutein was 89.5% in the presence of added γ-tocopherol and 60.0% without added γ-tocopherol after 24 h storage (Figure 3b). Figure 4a demonstrates that, in the presence of lutein and remaining 3 μg/g of γ-tocopherol in triglycerides, hydroperoxide formation was inhibited. γ-Tocopherol at 10 μg/g and a combination of lycopene and γ-tocopherol at 10 μg/g + 20 μg/g, respectively, inhibited hydroperoxide formation of autoxidized triglycerides under light (Figure 5a). In comparison, the concentration of lycopene was 82.6%

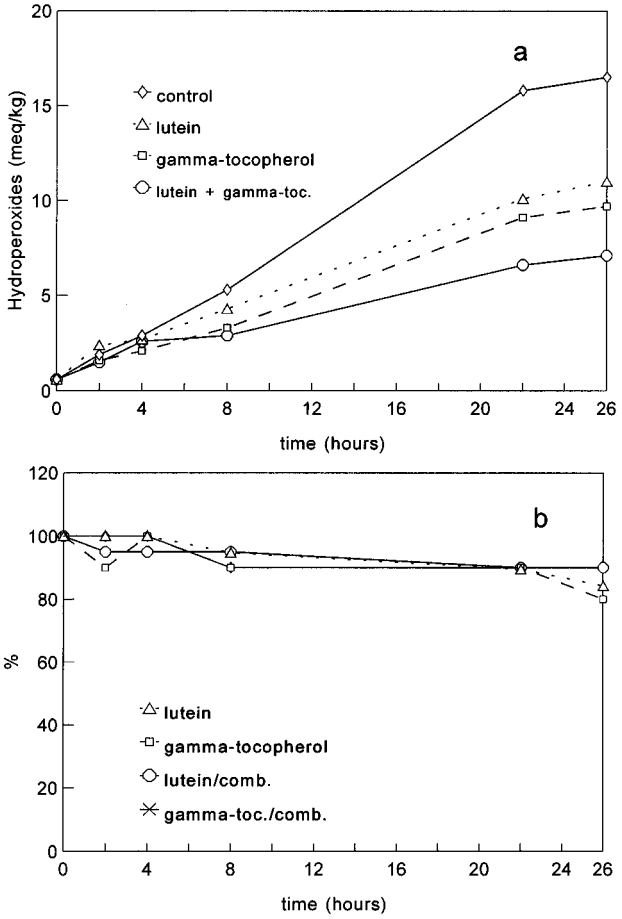

Figure 4. (a) Effect of lutein on hydroperoxide formation (mequiv/kg) of triglycerides autoxidized under light at 25 °C. The γ-tocopherol content of triglycerides was 3 μg/g. The samples were ◊ = control, △ = 20 μg/g lutein, □ = 10 μg/g γ-tocopherol, and ○ = combination sample of 20 μg/g lutein + 10 μg/g γ-tocopherol (molar ratio 1.5:1). (b) Concentrations (%) of △ = lutein, □ = γ-tocopherol, ○ = lutein in the combination sample of γ-tocopherol and lutein, and ∗ = γ-tocopherol in the combination sample of lutein and γ-tocopherol during autoxidation.

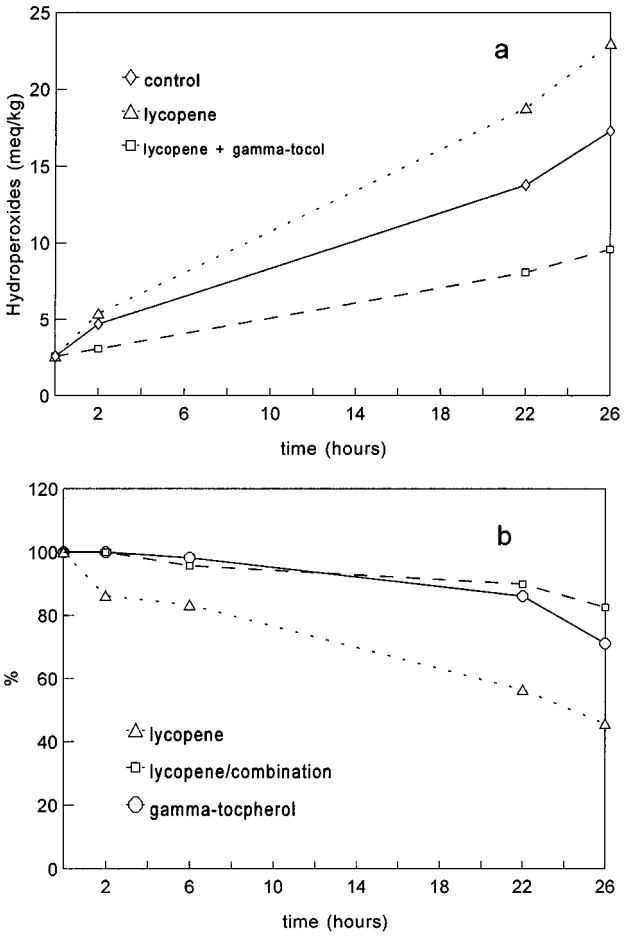

Figure 5. (a) Effect of lycopene on hydroperoxide formation (PV mequiv/kg) of triglycerides autoxidized under light at 25 °C. The γ-tocopherol content of triglycerides was <1 μg/g. The samples were ◊ = control, △ = 20 μg/g lycopene, and □ = combination sample of 20 μg/g lycopene + 10 μg/g γ-tocopherol. (b) Concentrations (%) of △ = lycopene, ○ = γ-tocopherol, and □ = lycopene in the combination sample of γ-tocopherol and lycopene during autoxidation.

in the presence of added γ-tocopherol and 45.7% without added γ-tocopherol after 26 h storage (Figure 5b).

Figure 6 shows that the antioxidant effect of natural food color annatto was significant ($p < 0.05$) at concentration of 30 and 60 μg/g of bixin, but not at 20 μg/g of bixin. In comparison, β-carotene at 20 μg/g was a prooxidant.

DISCUSSION

The results show that lutein, lycopene, and β-carotene increased the hydroperoxide formation of autoxidized triglycerides (Figures 2−6). Lutein was a prooxidant during oxidation of triglycerides both in the dark and in the light (Figures 2a and 3a). The prooxidant effects of lutein and lycopene support the earlier findings that β-carotene may promote the oxidation of vegetable oils under light (Terao et al., 1980; Haila and Heinonen, 1994) and under dark (Warner and Frankel, 1987; Suzuki et al., 1989). However, carotenoids such as lutein, lycopene, and β-carotene have been reported to inhibit the photosensitized oxidation of purified oil (Kiritsakis and Dugan, 1985; Fakourelis et al., 1987; Lee and Min, 1988, 1990; Min and Lee, 1989; Jung and Min, 1991). The contradictory results could be due to differences in experimental conditions, including the oil involved, the content of remaining other antioxidants such as tocopherols in triglycerides, and the photosensitizer for singlet oxygen production and solvent used in other food oil models. In oxidation model studies other than those of triglycerides, it has been observed that oxygen-containing carotenoids are better antioxidants than β-carotene, which was not observed in the present food lipid model. For example, Miki (1991) observed that lutein was a better antioxidant than β-carotene against malondialdehyde formation in a linoleic acid system.

Prooxidant effect of a carotenoid should be evaluated if carotenoid is proposed as a food color. Our results show that even minor concentrations of γ-tocopherol may inhibit the prooxidant effect of lutein and lycopene (Figures 3−5). A combination of lutein and γ-tocopherol was more efficient than γ-tocopherol in inhibiting the hydroperoxide formation of triglycerides (Figures 3 and 4). This carotenoid−tocopherol interaction has been previously demonstrated in food lipids (Terao et al., 1980; Haila and Heinonen, 1994) and in a membrane model system (Palozza and Krinsky, 1992). The benefit of a combination of a carotenoid and tocopherol as antioxidant may be due to the effect of γ-tocopherol in inhibiting the degradation of lutein (Figures 3b and 4b) and lycopene (Figure 5b). Instead, both the hydroperoxide formation of triglycerides and the consumption of

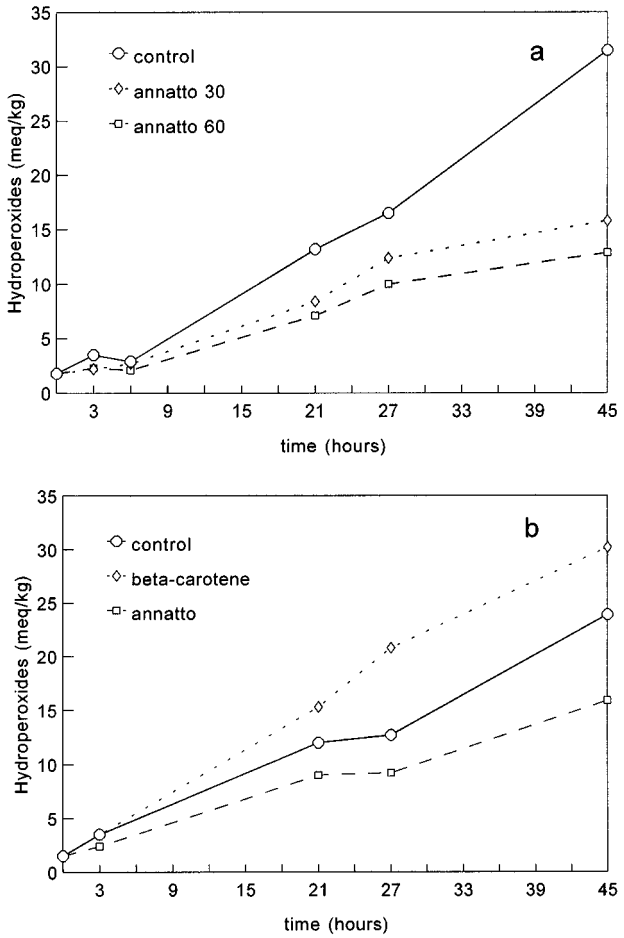

Figure 6. (a) Effect of annatto on hydroperoxide formation (PV mequiv/kg) of triglycerides autoxidized under light at 25 °C. The γ-tocopherol content of triglycerides was <1 μg/g. A. The samples were ○ = control, ◊ = annatto as 30 μg/g bixin, and □ = annatto as 60 μg/g bixin. (b) Samples were ○ = control, ◊ = 20 μg/g β-carotene, and □ = annatto as 20 μg/g bixin.

lutein was increased without added γ-tocopherol, which indicates the prooxidative role of oxidation products of these carotenoids (Figures 3 and 4). Carotenoids are suggested to inhibit lipid oxidation primarily by trapping peroxyl radicals by an addition mechanism (Burton and Ingold, 1984; Kennedy and Liebler, 1992; Liebler, 1993; Jørgensen and Skibsted, 1993) and most efficiently at low oxygen partial pressure (Burton and Ingold, 1984; Vile and Winterbourn, 1988; Palozza and Krinsky, 1991; Kennedy and Liebler, 1992; Jørgensen and Skibsted, 1993). The autoxidation products of β-carotene have recently been identified to be a complex mixture of products with epoxy, hydroxy, and carbonyl groups (Kennedy and Liebler, 1991; 1992; Liebler and Kennedy, 1992; Liebler, 1993; Handelman et al., 1991; Mordi et al., 1991; Yamauchi et al., 1993). The present results suggest that γ-tocopherol may retard the formation of carotenoid radical adduct and its further degradation to autoxidation products. Moreover, Marcus (1994) proposed a combination of antioxidants such as rosemary extract to stabilize natural colors such as carotenoids.

In contrast to the prooxidant effects of lutein and lycopene, annatto, containing bixin as a coloring component, was an antioxidant (Figures 6a and 6b). The results indicate that both antioxidant and color effect may be achieved by adding natural annatto to foods. However, the annatto product contained an unidentified fluorescent component coeluting in the HPLC analysis of tocopherols. In this food colorant bixin may not be the only component responsible for the antioxidant effect of annatto.

In conclusion, to maintain good color quality the bleaching of carotenoids should be avoided. The present observations suggest that the loss of the orange color as well as the prooxidant effect of carotenoids such as lutein and lycopene may be retarded by a very low level of γ-tocopherol.

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