β-Apo-8′-carotenoic Acid and Its Esters in Sunflower Oil Oxidation[1]

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ABSTRACT: The oxidation kinetics of sunflower oil (SO) and pure triacylglycerols of sunflower oil (TGSO) in the presence of different concentrations (0.0008–0.02%, 1.9–32.7 x 10^{-5} M) of β-apo-8′-carotenoic acid (CA), ethyl β-apo-8′-carotenoate (EC), and β-apo-8′-carotenoylglycerol (CG) were studied. The process was performed at high (kinetic regime) and low (diffusion regime) oxygen concentrations at room temperature and at 100°C and in the dark and in daylight. CA, EC, and CG were not antioxidants in TGSO systems. However, the carotenoid derivatives, especially CA, increased the stability of tocopherol-containing SO at room temperature and in daylight. The stabilization effect was more evident in a kinetic regime of oxidation. The synergism between the carotenoids and tocopherols was characterized by the increase of the stabilization factor *F* and activity *A*. *F* and *A* were highest for CA (*F* = 1.2–5.5, *A* = 2.4–78.6), followed by EC (*F* = 1.2–3.5, *A* = 1.7–14.6) and CG (*F* = 1.1–2.1, *A* = 1.6–5.5) in the kinetic regime for SO exposed to daylight at room temperature.

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KEY WORDS: β-Apo-8′-carotenoic acid, β-apo-8′-carotenoylglycerol, ethyl β-apo-8′-carotenoate, oxidation, sunflower oil.

Carotenoids are common food ingredients and are used as natural colorants (1), as a component in dietary fats (2), and as antioxidants (2,3). However, carotenoids have also been reported to act as prooxidants (4,5).

Numerous investigations demonstrate that the behavior of carotenoids in the oxidation process depends strongly on their concentration (6), the environment (7), and illumination conditions (8). The anti- or prooxidative activity of carotenoids is also closely related to both oxygen concentration (9) and the presence of other antioxidants (10). Numerous reviews discuss the ability of carotenoids to act as chain-breaking antioxidants and as singlet oxygen quenchers (11,12).

β-Apo-8′-carotenoic acid (CA) is a naturally occurring carotenoid (13) that is commercially available as the ethyl ester, ethyl β-apo-8′-carotenoate (EC). The glyceryl ester of β-apo-8′-carotenoic acid, β-apo-8′-carotenoylglycerol (CG), is an intermediate substrate for the synthesis of biologically active glycerides (14). Limited data are available regarding the role of these pigments in lipid oxidation.

The objective of this study was to investigate the role of CA, EC, and CG on lipid oxidation of sunflower oil (SO) and pure triacylglycerols of sunflower oil (TGSO).

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EXPERIMENTAL PROCEDURES

Materials. EC was a gift from BASF AG (Ludwigshafen, Germany). CA was obtained by stirring EC (350 mg), *Candida antarctica* B (CAB)-lipase (2 g), and decahydronaphthalene/water (50:10) for 15 d and purifying using chromatography (14). CG was obtained by transesterification of EC (73 mg) with glycerol (83 mg) in the presence of CAB-lipase (350 mg) and decahydronaphthalene (10 mL) at 35°C at reduced pressure (10 Torr) for 4 d. CAB-lipase was a gift from Novo Nordisk (Bagsvaerd, Denmark). Decahydronaphthalene and glycerol were purchased from Fluka (Buchs, Switzerland). β-Carotene was purchased from E. Merck (Darmstadt, Germany)

A commercially available sample of SO was used without purification. The TGSO were obtained by removing pro- and antioxidants and trace metals by adsorption chromatography (15). SO (50 g) was dissolved in hexane (500 mL), passed through a column (i.d. 2 cm) filled with activated alumina (35 g, type 507C, neutral, Fluka AG) and collected under nitrogen in the dark. The solvent was removed by rotary evaporator at 30°C in the dark. The product obtained was stored in an inert atmosphere at –20°C and used within 10 d.

Sample preparation. Lipid samples containing 0.0008–0.02% (1.86×10^{-5}–3.70×10^{-4} M) of the carotenoids CA, EC, or CG were prepared by adding aliquots of an acetone solution of the carotenoids to SO or TGSO. The acetone was removed by flushing the samples with nitrogen.

Analytical methods. The fatty acid composition of SO was determined by gas chromatography of the methyl esters using a Pye Unicam (Philips, United Kingdom) instrument, model 304, equipped with a dual flame-ionization detector and a glass capillary column (30 m × 0.2 mm i.d.) coated with SILAR 10C (Supelco Inc., Bellefonte, PA). The carrier gas was nitrogen at a flow rate of 14 mL/min. The temperature was maintained at 165°C for 5 min and then increased to 220°C at 2°C/min.

The tocopherol content of the sunflower oil was determined by normal-phase high-performance liquid chromatography using a Merck Hitachi apparatus equipped with an L-6000 pump and a fluorescence detector (Merck, Hitachi F-1050) (λ_{ex} = 295 nm, λ_{em} = 330 nm). A Nucleosil SI 50-5, 250 × 4 mm column (Macherey Nagel, Düren, Germany) and an elution system of hexane/dioxane (96:4) at a rate of 1 mL/min were used during the separation.

Carotenoid analysis of SO was performed after saponification (16) and solvent extraction, followed by nonaqueous reversed-phase liquid chromatography (17).

Oxidation. Oxidation at room temperature, 22°C (±2°C), in the dark and in daylight (southern window) was carried out

in oxygen-sufficient conditions (kinetic regime), and oxygen-insufficient conditions (diffusion regime). The kinetic regime was realized by storage of the samples in 1-mm layers in petri dishes having a diameter of 52 mm. The diffusion-controlled oxidation was performed in 10-mm layers in glass jars. Oil samples were thoroughly stirred before sampling.

Oxidation at 100°C (±0.2°C) was carried out by blowing air through the samples (2 g) in the dark at a rate of 100 mL/min. The oxidation process was monitored by withdrawing samples at various time intervals and determining the peroxide value (PV) (18).

Evaluation of the antioxidative action. The influence of the carotenoid derivatives on lipid oxidation was estimated on the basis of the induction periods (IP) determined by the method of the tangents to the two parts of the kinetic curves (19). The oxidation rate of the control sample (W_o) and of the samples containing CA, EC, and CG (W_{CA}, W_{EC}, and W_{CG}) were found from the tangents to the initial phase of the kinetic curves of peroxide accumulation and expressed as M s^{-1}. Recalculation of the rate from meq kg^{-1} h^{-1} into M s^{-1} was performed according to the following formula:

$$1 \text{ meq kg}^{-1} \text{ h}^{-1} = 1.4 \times 10^{-7} \text{ M s}^{-1} \quad [1]$$

The following kinetic parameter characterized the lipid oxidation during its initial stage: stabilization factor *F*, oxidation rate ratio ORR, and activity *A* (20).

F is a measure of the effectiveness,

$$F = \text{IP}_{\text{Car}}/\text{IP}_0 \quad [2]$$

where IP_{Car} is the induction period in the presence of the carotenoid, and IP_0 is the induction period of SO without additive.

ORR is an inverse measure of the strength:

$$\text{ORR} = W_{\text{Car}}/W_0 \quad [3]$$

where W_{Car} is the oxidation rate in the presence of the carotenoid, and W_0 is the initial oxidation rate of the control sample.

The general parameter activity *A* unifies the effectiveness and the strength of the additive in the oxidation process:

$$A = F/\text{ORR} \quad [4]$$

Statistical analysis. All kinetic curves of peroxide accumulation were the main result of three independent experiments. The standard deviation (SD) of PV determination (in meq kg^{-1}) for different mean values of PV was as follows: PV = 11.7, SD = 1.1; PV = 33.2, SD = 1.5; PV = 70.7, SD = 5.0; PV = 155.3, SD = 14.0; PV = 405.3, SD = 15.3 (21). The SD for different mean values of IP determined according to Doerffel (22) was (in days) as follows: IP = 1.5, SD = 0.2; IP = 3.6, SD = 0.3; IP = 7.5, SD = 0.4; IP = 10.5, SD = 0.6; IP = 14.3, SD = 0.8; IP = 20.8, SD = 1.1; IP = 42.5, SD = 2.4; IP = 105, SD = 5.0. The SD for different mean values of IP, obtained at 100°C, was as follows (in hours): IP = 1.6, SD = 0.1; IP = 3.9, SD = 0.4; IP = 7.7, SD = 0.5; IP = 14.3, SD = 1.0; IP = 30.3, SD = 2.5; IP = 60.9, SD = 3.8. The initial oxidation rate of the control samples W_0 and the oxidation rate in the presence of the carotenoids W_{Car} were quite constant, varying by less than 3%.

RESULTS AND DISCUSSION

Control oxidation experiments with TGSO in the presence of 0.01 and 0.02% citric acid demonstrated that the chelating agent had no effect on oxidation kinetics. The initial PV of the TGSO at the start of each experiment was zero. The fatty acids of TGSO were palmitate 5%, stearate 4%, oleate 23%, and linoleate 68%. The SO contained 670 ppm tocopherols (Toc): α-Toc 88.7%, β-Toc 3.8%, γ-Toc 5.0%, δ-Toc 1.4%, and α-tocotrienol (α-Toc-3) 1.1%. The initial PV of the commercial SO was 7.2 meq kg^{-1}. Carotenoids were not detected in SO, which is in accordance with the statement that carotenoids are destroyed during the refining process.

The kinetic results obtained with SO showed that CA, EC, and CG at room temperature and in the dark had no influence on the oxidation stability of SO in a kinetic (IP = 35 d, data not shown) or a diffusion regime (IP 105 d, data not shown). The same ineffectiveness was observed when SO was oxidized at 100°C in the dark (IP 8.5 h, data not shown). A similar inability for carotenoids to act as antioxidants was observed during the thermal oxidation of safflower seed oil at 75°C (23).

Some of the kinetic curves of oxidation obtained are presented in Figures 1 and 2. Figure 1 illustrates the peroxide accumulation in SO in the presence of EC during oxidation at room temperature under daylight and in a kinetic regime. EC increases the induction period IP and decreases the rate of the process *W* in the initial stage. Figure 2 presents peroxide accumulation in SO in the presence of CG during oxidation at room temperature under daylight and in a diffusion regime. CG does not change the initial oxidation rate during the induction period *W*. CG increases the IP to a lower degree in comparison with EC (data presented in Fig. 2).

The kinetic parameters *F*, ORR, and *A*, obtained after processing the kinetic curves for all investigated concentrations of CA, EC, and CG, are given in Tables 1, 2, and 3. The presented data illustrate that CA, EC, and CG retard the oxidation of SO under light to a greater degree in the kinetic regime than in the diffusion regime. The same effect was observed when SO was oxidized in the presence of β-carotene (24).

No effect of different concentrations of CA, EC, and CG on TGSO oxidation at room temperature under daylight, in the dark, in a kinetic, or in a diffusion regime, was observed. The carotenoids did not change the kinetics of the peroxide accumulation in TGSO at these conditions (data not shown). CA, EC, and CG did not change the kinetics of TGSO autoxidation at 100°C in the dark (IP = 0.5 h).

The results obtained showed that CA, EC, and CG did not modify the oxidation kinetics of Toc-depleted TG of SO. The carotenoids did not work as radical trapping (chain-breaking) antioxidants under the oxidation conditions of our experiments. The investigated carotenoids increase the oxidation stability when added to the natural Toc-containing SO under

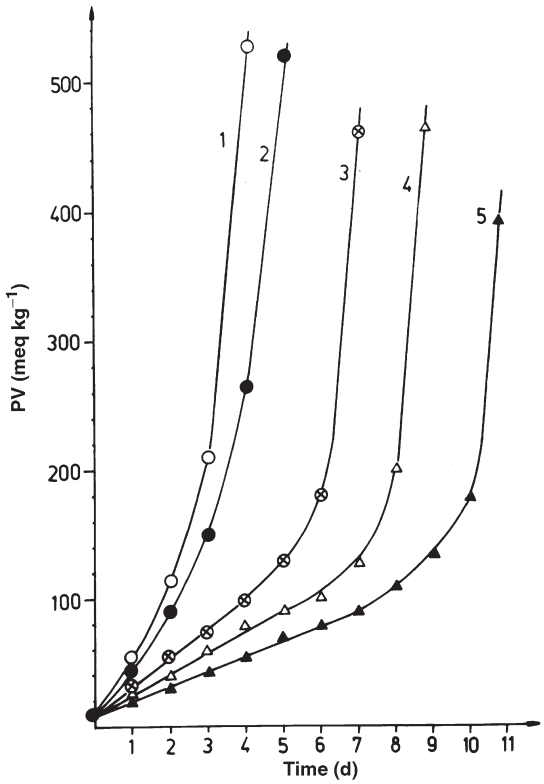

FIG. 1. Kinetic curves of peroxide accumulation during oxidation of sunflower oil at room temperature under light in a kinetic regime in the presence of different concentrations of ethyl β-apo-8′-carotenoate: (1) 0, (2) 1.86×10^{-5} M, (3) 9.32×10^{-5} M, (4) 1.86×10^{-4} M, and (5) 3.72×10^{-4} M.

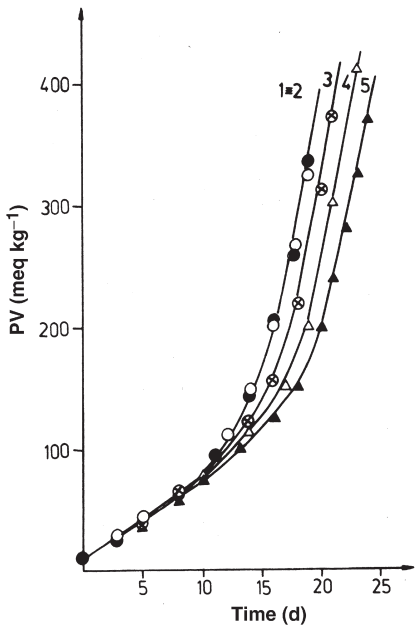

FIG. 2. Kinetic curves of peroxide accumulation during oxidation of sunflower oil at room temperature under light in a diffusion regime in the presence of different concentrations of β-apo-8′-carotenoylglycerol: (1) 0, (2) 1.86×10^{-5} M, (3) 9.32×10^{-5} M, (4) 1.86×10^{-4} M, and (5) 3.72×10^{-4} M.

daylight conditions. The natural sensitizers in SO, the chlorophylls, may generate singlet oxygen during oil oxidation at light exposure (25). The stabilizing effect of the investigated carotenoids could therefore originate from their ability to quench singlet oxygen (26), which may explain their cooperative action with Toc.

The presented study demonstrates that CA is a more active substance in retarding SO oxidation at room temperature under daylight than β-carotene (24) and EC (compare the data in Tables 1 and 2). The higher activity of CA in comparison to EC could be explained by the presence of an abstractable hydrogen in CA (27), which may promote the antioxidant synergism with tocopherols. The activity of CG was lower than EC (*cf.* Tables 2 and 3). The weaker antioxidant effect of CG may be related to the presence of the two hydroxy groups in the molecule. This structural feature can cause an acceleration of lipid oxidation (28,29).

A comparison of the activity *A* of EC (with 9 conjugated double bonds) and β-carotene (with 11 conjugated double

TABLE 1
Kinetic Parameters Characterizing the Oxidation of Sunflower Oil at Room Temperature Under Light in the Presence of Different Concentrations of β-Apo-8′-carotenoic Acid (CA), PV_o = 7.2 meq kg^{-1}

Concentration[a]				
[CA], %	[CA] × 10^5, M	F^b	ORR[c]	A^d
Kinetic regime: IP_0 = 3.0 d, $W_0 = 2.46 \times 10^{-7}$ M s^{-1}				
0.0008	1.86	1.2	0.50	2.4
0.004	9.32	2.5	0.25	10.0
0.008	18.60	4.4	0.14	31.4
0.016	37.20	5.5	0.07	78.6
Diffusion regime: IP_0 = 13 d, $W_0 = 3.9 \times 10^{-8}$ M s^{-1}				
0.0008	1.86	1.1	0.77	1.4
0.004	9.32	1.3	0.68	1.9
0.008	18.60	1.4	0.68	2.1
0.016	37.20	1.6	0.68	2.4

[a]PV_0 = initial peroxide value.
[b]*F*, stabilization factor (Eq. 2).
[c]ORR, oxidation rate ratio (Eq. 3).
[d]*A*, activity (Eq. 4). IP_0 induction period without additive; W_0, initial oxidation rate of control sample.

TABLE 2
Kinetic Parameters[a] Characterizing the Oxidation of Sunflower Oil at Room Temperature Under Light in the Presence of Different Concentrations of Ethyl β-Apo-8′-carotenate (EC), PV_o = 7.2 meq kg^{-1}

Concentration				
[EC], %	[EC] × 10^5, M	*F*	ORR	*A*
Kinetic regime: IP_0 = 3.0 d, $W_0 = 2.46 \times 10^{-7}$ M s^{-1}				
0.00086	1.86	1.2	0.69	1.7
0.0043	9.32	2.1	0.49	4.3
0.0086	18.60	2.8	0.39	7.2
0.0172	37.20	3.5	0.24	14.6
Diffusion regime: IP_0 = 13 d, $W_0 = 3.9 \times 10^{-8}$ M s^{-1}				
0.00086	1.86	1.2	0.86	1.4
0.0043	9.32	1.4	0.76	1.8
0.0086	18.60	1.5	0.69	2.2
0.0172	37.20	1.7	0.56	3.0

[a]For abbreviations see Table 1.

TABLE 3
Kinetic Parameters[a] Characterizing the Oxidation of Sunflower Oil at Room Temperature Under Light in the Presence of Different Concentrations of β-Apo-8′-carotenoylglycerol (CG), PV_0 = 7.2 meq kg^{-1}

Concentration				
[CG], %	[CG] × 10^5, M	*F*	ORR	*A*
Kinetic regime: IP_0 = 3.0 d, $W_0 = 2.46 \times 10^{-7}$ M s^{-1}				
0.00094	1.86	1.1	0.67	1.6
0.0047	9.32	1.5	0.59	2.5
0.0094	18.60	1.6	0.48	3.3
0.019	37.20	2.1	0.38	5.5
Diffusion regime: IP_0 = 13 d, $W_0 = 3.9 \times 10^{-8}$ M s^{-1}				
0.00094	1.86	1.0	1.0	1.0
0.0047	9.32	1.1	1.0	1.1
0.0094	18.60	1.3	1.0	1.3
0.019	37.20	1.4	1.0	1.4

[a]For abbreviations see Table 1.

bonds) (24) indicates that β-carotene is more active in retarding SO oxidation under light. This may be explained by the fact that in structurally related carotenoids, increasing the number of the conjugated double bonds results in increased singlet oxygen ability of the carotenoids (30).

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