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Molecular Mechanism of Antioxidant Synergism of Tocotrienols and Carotenoids in Palm Oil

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During repeated deep-fat frying of potato slices at 163 °C in yellow or red palm olein of comparable fatty acid profiles, the oxidative stability (peroxide value and anisidine value) of the palm oleins was similar, and in yellow palm olein, the rate of antioxidant depletion decreased in the order γ-T3 > α-T3 > δ-T3 (T3, tocotrienol). In red palm olein, which had a total tocopherol/tocotrienol content of 1260 vs 940 ppm in yellow palm olein and a corresponding longer induction period in the Rancimat stability test at 120 °C, only depletion of γ-T3 was significant among the phenols during frying and slower as compared to that in yellow palm olein. The carotenes in the red palm olein were depleted linearly with the number of fryings, apparently yielding an overall protection of the phenols. In antioxidant-depleted palm olein and in phospholipid liposomes with added increasing concentrations of phenols, γ-T3 was found to be a better antioxidant than α-T3. α-T3 and α-T (T, tocopherol) had a similar antioxidant effect in antioxidant-depleted palm olein in the Rancimat stability test, while in the liposomes the ordering as determined by induction period for the formation of conjugated dienes was γ-T3 > α-T3 > α-T. The addition of 100−1000 ppm β-carotene to antioxidant-depleted palm olein or liposomes (lycopene also tested) did not provide any protection against oxidation. In the liposomes, synergistic interactions were observed between β-carotene or lycopene and α-T, α-T3, or γ-T3 for carotene/phenol ratios of 1:10 and 1:2 but not for 1:1. In chloroform, carotenes were regenerated by tocopherols/tocotrienols from carotene radicals generated by laser flash photolysis as shown by transient absorption spectroscopy, suggesting that carotenes rather than phenols are the primary substrate for lipid-derived radicals in red palm olein, in effect depleting carotenes prior to phenols during frying. Regeneration of carotenes by the phenols also explains the synergism in liposomes. In the laser flash photolysis experiments, γ-T3 was also found to be faster in regenerating carotenes than α-T3 and α-T.

KEYWORDS: Palm oil; tocotrienols; carotenes; antioxidant synergism; frying conditions; radical kinetics

INTRODUCTION

Palm oil is the second largest vegetable oil in terms of world production, and today, about 50% of traded oils in the world is palm oil. Malaysia and Indonesia are the major producers of palm oil, and in importing markets such as India, the European Union, China, and Pakistan, palm oil is widely used as a frying and cooking oil in homes and in catering (*1*). Palm oil is oxidatively stable due to a fatty acid composition with low polyunsaturation and a high antioxidant content. While most vegetable oils provide mainly α- or γ-tocopherol, palm oil is unique in that it contains relatively large concentrations of homologue tocotrienols (α, γ, and δ), which together account for 72−80% of the total tocopherol and tocotrienol content of palm oil (600−1000 ppm). Moreover, palm oil is one of the richest sources of α- and β-carotene (β-C) (400−3500 ppm), and dependent on the refining process, a considerable amount can be retained as provitamin A in the refined palm oil of importance (*2*).

The antioxidant properties of tocotrienols in vegetable oils are sparsely documented (*3*−*6*), and only few studies have focused on the antioxidant properties of the tocotrienol homologues in model systems from which, moreover, conflicting ordering of the relative activity of the tocotrienols and the relative activity as compared to the tocopherols has been reported (*7*−*12*). Interactions between tocotrienols and carotenoids have also received only scarce attention, although antioxidants may act synergistically, in effect yielding a better overall protection when combined (*13*, *14*). In palm oil, tocotrienols and carotenes are present naturally in combination, making palm oil of relevance for a study of the interaction between tocotrienols and carotenes during lipid oxidation processes in the oil.

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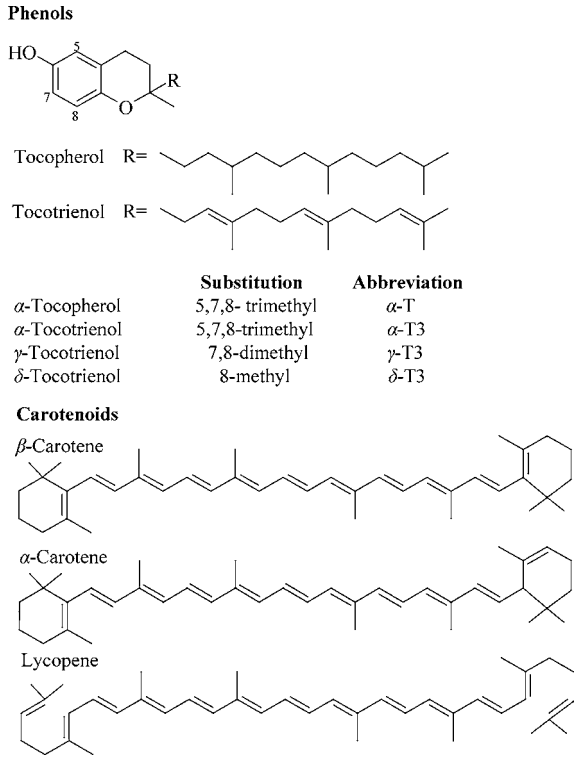

Figure 1. Chemical structure of α-T, the tocotrienols, and the carotenoids investigated.

The present study was designed to investigate the effect of α-tocopherol (α-T) and the homologues tocotrienols and carotene on lipid oxidation in palm olein during frying conditions and to rank the compounds relative to antioxidant activity in relation to their structure (**Figure 1**). These studies were based on practical frying experiments monitoring the consumption of the individual potential antioxidants and on the addition of the potential antioxidants alone in varying concentrations and in combination to antioxidant-depleted palm oil followed by determination of an induction period (IP) for oxidation. To obtain mechanistic insight in the antioxidant protection, the effect of similar combinations of antioxidants on the formation of conjugated dienes was studied in liposomes. Furthermore, laser flash photolysis with real time detection of transient phenoxyl radicals and carotene cation radicals formed during bleaching of carotenes in chloroform solutions was used to study reactions between carotenes and α-T and tocotrienols under oxidative stress. In the present investigation, α- and β-Cs were analyzed together in the red palm olein and not considered separately.

MATERIALS AND METHODS

Materials. Neutralized, bleached, and deodorized yellow [iodine value (IV) = 62.6 g I_2/100 g] and red (IV = 64.2 g I_2/100 g) double-fractionated palm olein were both obtained from standard productions at Unitata (Teluk Intan, Malaysia) and used fresh. α-T (purity ≥ 98.0%) and β-C (purity ≥ 97.0%) were purchased from Fluka Chemie GmbH (Steinheim, Germany), α-tocotrienol (α-T3) (purity = 99.7%) was from Eisai Co. Ltd. (Tokyo, Japan), and lycopene (lyco) (purity = 96.7%) was from Roche A/S (Hvidovre, Denmark). γ-Tocotrienol (γ-T3) (purity = 98.7%) was kindly provided by Carotech Bhd. (Ipoh, Malaysia). *p*-Anisidine and tocopherol standards were supplied by Merck (Darmstadt, Germany), butylated hydroxytoluene and L-α-phosphatidylcholine (PC) from soybean (~99%) were provided by Sigma-Aldrich Chemie GmbH (Steinheim, Germany), and 2,2′-azobis-(2-aminopropane)dihydrochloride (AAPH) was provided by Wako Chemicals Inc. (Richmond, VA). All chemicals were of analytical grade, and all solvents were of high-performance liquid chromatography (HPLC) grade.

Fatty Acid Composition. The fatty acid composition was performed according to the AOCS Ce 1-62 Fatty Acid Composition by Gas Chromatography Method (*15*). The fatty acids of the palm oleins were converted into methyl esters and analyzed with gas liquid chromatography using a Hewlett 5890 Packard Series II Gas Chromatograph (Palo Alto, CA). The injection temperature was 240 °C, the carrier gas was purified helium (99.9995%), the flow rate was 0.8−1.0 mL/min, and the temperature program rose from 195 to 220 °C at 4.5 °C/min. The fatty acid methyl esters were separated by a 25 m × 0.32 mm × 0.5 μm capillary column with HP-FFAP as the stationary phase (J&W Scientific, Folsom, CA) and detected with a flame ionization detector set at 250 °C. Identification of the fatty acid methyl esters was done by comparison to standards of fatty acid methyl esters.

α-T and Tocotrienols. The content of α-T and tocotrienols was determined according to the AOCS Ce 8-89 Determination of Tocopherols and Tocotrienols Method (*16*) with some modifications. α-T and tocotrienols in the olein samples were quantified by HPLC using a photodiode array detector (Waters 996, Milford, MA). In addition to this, the system included a Waters 600 Controller, a Waters 600 Pump, and a 3.9 mm × 300 mm Nova-Pak Silica 6 μm Column. The mobile phase was a mixture of 99% hexane and 1% isopropyl alcohol with a flow rate of 0.7 mL/min. The detection wavelength was set to 292 nm. Olein samples exposed to frying were filtered through a fast Whatman no. 41 filter paper. Two grams of filtered olein sample was dissolved in hexane to give a total volume of 25 mL after which 20 μL of the dissolved olein sample was injected into the HPLC. Identification and calculation of concentrations of α-T and tocotrienols in the olein samples were done by comparison to a standardized red palm olein containing α-T as well as α-, γ-, and δ-T3s.

Carotenes. Carotenes were determined according to the PORIM test method (*17*). The olein samples exposed to frying were filtered through a fast Whatman no. 41 filter paper and dissolved in isooctane. Absorbance was measured at a Hitachi UV−vis spectrophotometer 150-20 (Tokyo, Japan) at a wavelength of 446 nm.

Peroxide Value (PV). The PV was determined according to the AOCS Cd 8-53 Peroxide Value Acetic Acid-Chloroform Method (*18*).

Anisidine Value (AV). The AV was determined according to the AOCS Cd 18-90 *p*-Anisidine Value Method (*19*). Olein samples exposed to frying were filtered through a fast Whatman no. 41 filter paper. Absorbance was measured at a Hitachi UV−vis spectrophotometer model 150-20 at a wavelength of 350 nm.

Deep-Fat Frying. A 2.5 L amount of yellow or red palm olein was heated to 161−165 °C in a kitchen fryer (Kenwood Ltd., Hants, United Kingdom). The heating time was approximately 8.5 min. A 100 g amount of sliced potatoes was fried in the olein for 3 min. The fried potatoes were removed, and 100 g of new potatoes was fried in the same olein for 3 min. This procedure was repeated 60 times. For every tenth frying (after 0, 10, 20, 30, 40, 50, and 60 fryings), 100 mL of olein sample was taken out for analyses. Stability parameters, PV, and AV as well as antioxidant concentrations (α-T, tocotrienols, and carotenes) were followed during the 60 fryings.

Purification of Palm Olein. Yellow palm olein was depleted of antioxidants by alumina column chromatography according to Fuster et al. (*20*) in order to obtain the oil without naturally occurring tocopherol, tocotrienols, peroxides, and trace metals. Quantification of α-T and γ-tocopherol was carried out by reverse-phase liquid chromatography (HPLC) with fluorescence detection by using external standard solutions of known concentrations of α-T and γ-tocopherol. Oil samples (25 mg) were dissolved in 3 mL of ethanol/1-butanol (75:25, v/v) and directly analyzed. An HPLC analytical column (4 mm × 125 mm) (Agilent Technologies, Karlsruhe, Germany) packed with C18 phase and containing a mean particle size of 5 μm was used. Methanol/water (94:6, v/v) was used as the mobile phase with a flow of 1 mL/min. The fluorescence detector was operated with the excitation wavelength set at 292 nm and the emission wavelength set at 330 nm. The antioxidant-depleted oil was found to contain undetectable amounts of tocopherol (<0.5 ppm).

Rancimat Stability Test. For the Rancimat stability test, a 679 Rancimat Control Unit with a 679 Rancimat Wet Section (Methrom,

Glostrup, Denmark) was applied. A 2.5 g amount of the olein samples was carefully weighed directly into the bottom of the reaction vessels and subjected to 120 °C and an air flow of 20 L/h in the vessels. Analyzed were the following palm olein samples: control (antioxidant depleted palm olein), yellow palm olein, red palm olein, and antioxidant-depleted palm olein with added antioxidant(s). The resistance to oxidation was expressed in terms of IP. Each sample was assessed in duplicate.

Antioxidant Activity in Liposomes. The preparation of liposomes, the peroxidation of phospholipids in liposomes, and the measurement of conjugated dienes were performed as described by Roberts and Gordon (*21*) with only a few modifications. Liposome suspensions with incorporated antioxidant(s) were prepared by adding 2 mL of 0.75 mM soybean PC solution and 1 mL of lipid-soluble antioxidant(s) solution to a 25 mL pear-shaped flask. The flask was covered with aluminum foil throughout the experiment to avoid light-induced oxidation of the lipid. The solvent was removed under reduced pressure on a rotary evaporator (Rotavapor R-144, Büchi, Flawil, Switzerland) with a vacuum pump (Julabo F25, Seelbach, Germany) in a waterbath (Waterbath B-840, Büchi) set at 30 °C. Thereafter, nitrogen was introduced to reestablish atmospheric pressure. The lipid residue was rehydrated with 10 mL of thermostated (37 °C) phosphate buffer, flushed with nitrogen, and quickly sealed with a cap before it was vortex-mixed for 10 min producing a homogeneous white suspension of multilamellar liposomes. The multilamellar liposome suspension was then shaken while being sonicated for 30 s in an ultrasonic bath. Large unilamellar liposomes were obtained by transferring the liposome suspension to an Avestin Lipsofast Basic small volume extrusion device (Avestin, Mannheim, Germany). The suspension was passed 21 times through a double layer of polycarbonate membranes with 100 nm pore diameter. Three milliliters of the unilamellar liposome suspensions with antioxidant(s) were pipetted into quartz cuvettes and incubated for 10 min at 37 °C within the temperature-regulated cell holder of a Shimadzu UV−vis scanning spectrophotometer model 2101 (Kyoto, Japan). Lipid peroxidation was initiated by the addition of 30 μL of 0.75 mM AAPH solution. The following concentrations of α-T, α-T3, γ-T3, β-C, and lyco were analyzed individually: 100, 500, and 1000 ppm. Furthermore, combinations of β-C (100, 500, and 1000 ppm) and α-T, α-T3, or γ-T3 (1000 ppm) were analyzed in a full factor experiment as well as combinations of 100 ppm lyco and 1000 ppm α-T, α-T3, or γ-T3. The control consisted of 3 mL of liposome suspension, with 30 μL of AAPH solution but without antioxidant added. In addition to this, a blank consisting of 3 mL of sodium phosphate buffer and 30 μL of AAPH solution was incubated to measure the AAPH absorbance, which was subtracted from the measured absorbance of all samples and controls. The resistance to oxidation was expressed in terms of the IP for increase in absorbance at 234 nm as an indication of the formation of conjugated dienes.

Radical Kinetics by Laser Flash Photolysis. Laser flash photolysis experiments were carried out following the procedure proposed by Mortensen and Skibsted (*22*). The samples investigated contained solutions of 1.0×10^{-5} M β-C alone or in combination with 1.0×10^{-3} M α-T, α-T3, or γ-T3 in chloroform. An LKS.50 spectrometer from Applied Photophysics Ltd. (Leatherhead, United Kingdom) was used to perform the experiments. The third harmonic at 355 nm of a pulsed Q-switched Nd:YAG laser Spectron Laser System (Rugby, United Kingdom) was used for excitation. A 1P28 photomultiplier tube from Hamamatsu (Hamamatsu, Japan) was used to detect transient absorption at wavelengths below 550 nm. Near-infrared detection was conducted with an S1336-44BK silicon photodiode from Hamamatsu. Appropriate UV cutoff filters were used to minimize the sample degradation in the blue-green spectral region, whereas red band-pass filters were used for near-infrared measurements. The samples were excited in 1 cm × 1 cm fluorescence cells from Hellma (Mulheim, Germany). All samples were prepared using fresh solutions thermostated at 25 ± 0.5 °C. Because of degradation of the carotenoids by the laser pulse, each sample was subjected to no more than five laser pulses. Each sample was assessed in duplicate.

Statistics. The statistical analyses were carried out in SAS 8.3 (Statistical Analysis System Inc., Cary, NC). Analyses of variance were used to test the effects of the experimental factors for each response variable in the deep-fat frying experiment. To test whether significant increases/decreases of the response variables from 0 to 60 fryings were different between yellow and red palm olein, pairwise comparisons of the changes in the two oleins were carried out by *t*-tests. To investigate whether the response variable was a linear function of the number of fryings, the linear model was tested against the analysis of variance in an *F*-test. Analyses of variance were carried out to investigate the effects of the experimental factors of the response variables as well as for synergy in the Rancimat and liposome experiments.

Table 1. Characteristics of the Studied Palm Oleins[a]

parameter	yellow palm olein	red palm olein
C14:0	0.78 ± 0.02 a	0.92 ± 0.00 a
C16:0	35.28 ± 0.03 a	34.99 ± 0.08 b
C18:0	3.81 ± 0.01 a	3.95 ± 0.02 a
C18:1	47.22 ± 0.12 a	45.88 ± 0.08 b
C18:2	12.63 ± 0.09 a	13.88 ± 0.02 b
C18:3	0.27 ± 0.01 a	0.38 ± 0.01 a
PV	0.5 ± 0.1 a	0.1 ± 0.1 b
AV	3.5 ± 0.2 a	4.3 ± 0.3 b
α-T	193 ± 8 a	288 ± 9 b
α-T3	245 ± 20 a	336 ± 18 b
γ-T3	393 ± 3 a	471 ± 22 b
δ-T3	111 ± 4 a	166 ± 3 a
total α-T + T3	944 ± 15 a	1260 ± 48 b
carotenes		534 ± 8

[a] Data are means ± SD ($n = 4$; except for fatty acid composition, $n = 2$). The content of a fatty acid is expressed in percent of total fatty acid composition. The PV is expressed as milliequivalents of peroxides per kg of oil (meq/kg). α-T, tocotrienol (T3), and carotene concentrations are expressed in ppm. Different letters within rows represent significant differences ($p < 0.05$).

RESULTS

Palm Olein Characterization. Characterization of yellow and red palm olein used in the present study may be seen in **Table 1**. Red palm olein contained a significantly higher amount of linoleic acid as compared to yellow palm olein. On the other hand, red palm olein seemed to be better protected against lipid oxidation by antioxidants since it had a significantly higher total content of α-T and tocotrienols as compared to yellow palm olein. Furthermore, red palm olein contained a considerable amount of carotenes, which were completely absent in yellow palm olein. Both yellow and red palm olein are expected to be rather stable toward lipid oxidation due to a low content of polyunsaturated fatty acids and a high content of antioxidant. The low values for both PV as an indicator of primary oxidation products and AV including secondary oxidation products indicate that both the yellow and red palm oleins used were of superior quality.

Deep-Fat Frying. The fresh yellow and red palm oleins as characterized in **Table 1** were used for frying of potato slices, and oil quality deterioration was followed during 60 subsequent fryings.

α-Tocopherol and Tocotrienols. From **Figure 2** and the statistical analysis of the data used in **Figure 2**, it is evident that the total content of α-T and tocotrienols decreased from 0 to 60 fryings in both yellow and red palm oleins ($p < 0.0001$). The decrease in total content of α-T and tocotrienols from 0 to 60 fryings was found to be significantly larger for yellow palm olein with a decrease of 24.5% as compared to red palm olein with a decrease of 10.3% ($p = 0.0321$). For both oleins, the concentration of total α-T and tocotrienols could be described as a linear function of the number of fryings ($p > 0.05$), indicating that the decrease in antioxidant concentration per frying was the same during the 60 fryings for each of the two oleins. During the 60 successive fryings in yellow palm olein

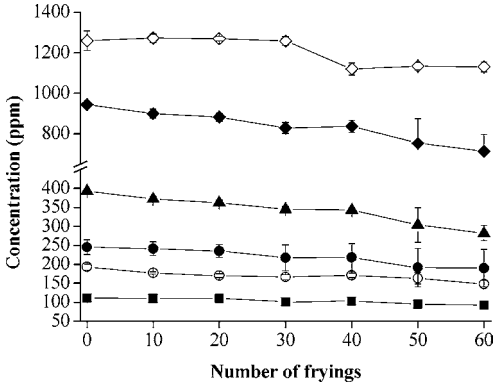

Figure 2. Concentrations of total α-T and tocotrienols in yellow palm olein (◆) and red palm olein (◇) and of α-T and tocotrienol homologues in yellow palm olein during deep-fat frying of potato slices: α-T (○), α-T3 (●), γ-T3 (▲), and δ-tocotrienol (■) with standard deviations ($n = 2$).

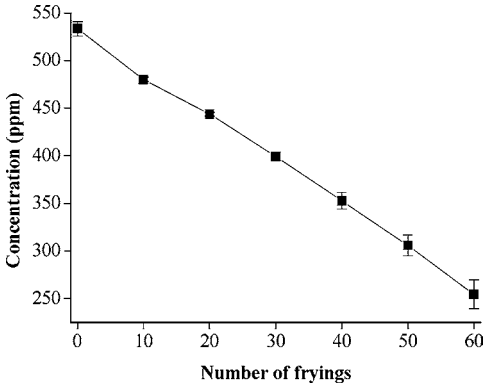

Figure 3. Concentration of carotenes in red palm olein during deep-fat frying of potato slices with standard deviation ($n = 2$).

(**Figure 2**) and in red palm olein (results not shown), neither α-T nor any of the tocotrienols were totally depleted. In yellow palm olein, the concentration of γ-T3 decreased by 28.2% from 0 to 60 fryings ($p < 0.0001$), and α-T and α-T3 decreased from 0 to 60 fryings with 23.3 and 22.5%, respectively ($p = 0.0181$ and $p = 0.0039$, respectively). In contrast, the decrease in concentration of δ-T from 0 to 60 fryings was not significant ($p = 0.3129$). From statistical analysis of the data presented in **Figure 2**, it is concluded that the antioxidant efficiency of α-T and tocotrienols in yellow palm olein during deep-fat frying under the circumstances of the present study decreases in the following order: γ-T3 > α-T3 ≈ α-T > δ-T3. In red palm olein, the decrease of γ-T3 after 60 fryings was 18.1% ($p < 0.0001$), while α-T3 and δ-T3 showed a nonsignificant decrease ($p = 0.3323$ and $p = 0.4102$, respectively). Thus, γ-T3 in red palm olein was also found to be the antioxidant consumed most rapidly and, accordingly, a more effective antioxidant as compared to α-T and the other tocotrienol homologues. Notably, it was found that for both yellow and red palm oleins, the concentration of γ-T3 decreased as a linear function of the number of fryings ($p > 0.05$). On the basis of this observation, it was concluded that the reduction in antioxidative capacity of the most active antioxidant, i.e., γ-T3, per frying was the same during each 60 fryings.

Carotenes. From **Figure 3** and the statistical analysis of the data, it is apparent that the carotene concentration in red palm olein decreased linearly with the number of fryings for the 60 fryings investigated ($p > 0.05$). The linear relationship corresponded to a decrease in carotene concentration of approximately 5 ppm per frying or a decrease of 52.3% after 60 fryings.

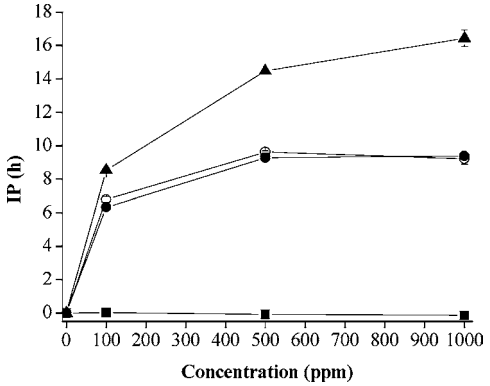

Figure 4. IP (h) for Rancimat stability test at 120 °C for antioxidant-depleted yellow palm olein with added potential antioxidants: α-T (○), α-T3 (●), γ-T3 (▲), and β-C (■). IP is subtracted IP for control and given with the standard deviation ($n = 2$).

PV. The PV increased significantly for every tenth frying during the 60 deep-fat fryings for both yellow and red palm oleins ($p < 0.0001$) (results not shown). In yellow palm olein, the PV increased from 0.5 mequiv/kg for the fresh oil to 8.1 mequiv/kg after 60 fryings. In red palm olein, the PV increased from 0.1 to 7.7 mequiv/kg following 60 fryings. The increases in the PV from 0 to 60 fryings in yellow and red palm olein were not found to be significantly different ($p = 0.7499$). Both in yellow and in red palm oleins during the 60 fryings, the PV value did not advance to the stage where the concentration of hydroperoxides had reached a maximum value and had started to decrease due to faster formation of secondary oxidation products than of hydroperoxides.

AV. The AV increased significantly for every tenth frying during the frying experiment for both yellow and red palm oleins ($p < 0.0001$) (results not shown). In yellow palm olein, the AV increased from 3.5 for the fresh oil to 19.2 following 60 fryings. In red palm olein, the AV increased from 4.3 to 20.9 from 0 to 60 fryings. The increase in the AV was found to be parallel (no two-way interaction) for the two oleins during the 60 fryings with the level of the AV significantly higher for red palm olein ($p < 0.0001$).

Rancimat Stability Test of Oleins. In the Rancimat stability test, yellow palm olein (IP = 12.30 ± 0.42 h) and red palm olein (IP = 14.05 ± 0.07 h) were found to be more stable as compared to the control (IP = 1.33 ± 0.11 h), since both oleins had a significantly longer IP than the control ($p < 0.0001$), which consisted of yellow palm olein from which tocopherols and tocotrienols were removed. Notably, red palm olein appeared as more stable as compared to yellow palm olein when exposed to 120 °C during the test in the Rancimat ($p = 0.0064$). The increase in IP for oxidation of palm olein as a function of concentration of antioxidant added to antioxidant depleted (yellow) palm olein at 120 °C in the Rancimat test is presented in **Figure 4**. Any concentration of α-T3, γ-T3, and α-T tested increased the stability of palm olein significantly as compared to the control ($p < 0.0001$) indicating that these compounds each had an antioxidative effect in the antioxidant depleted palm olein. On the other hand, β-C did not show any antioxidative effect as compared to the control when added alone to the antioxidant-depleted palm olein ($p > 0.05$). γ-T3 proved to be the most effective of the antioxidants tested in prolonging the IP of the antioxidant-depleted palm olein and in a dose-dependent way, as a significant increase in olein stability was observed for a concentration increase in the range between 100 and 1000 ppm ($p < 0.0001$). The addition of α-T3 and α-T was found to have a similar effect on the IP of the antioxidant-

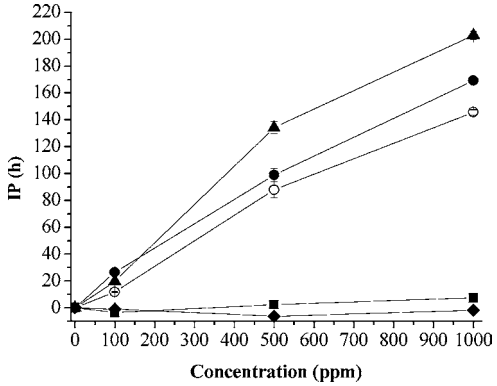

Figure 5. IP (min) for formation of conjugated dienes in phospholipid liposomes with added potential antioxidants: α-T (○), α-T3 (●), γ-T3 (▲), β-C (■), and lyco (◆). IP is subtracted IP for control and given with the standard deviation ($n = 2$).

depleted palm olein. Upon increasing the concentration of α-T or α-T3 from 100 to 500 ppm in the palm olein, the stability was found to increase significantly ($p < 0.0001$), but the effect leveled off when the antioxidant concentration was increased from 500 to 1000 ppm ($p = 0.6197$ and $p = 0.0629$ for α-T3 and α-T, respectively). The antioxidative properties of the individual tocotrienols and α-T in palm olein in the Rancimat at 120 °C may accordingly be concluded to decrease in the following order: γ-T3 > α-T3 = α-T. Combined addition of 100 ppm β-C with 1000 ppm α-T3, γ-T3, or α-T in antioxidant-depleted palm olein did not show any synergistic antioxidative effect (results not shown).

Liposome Experiments. The IP for the formation of conjugated dienes as a function of antioxidant concentration in PC liposomes is presented in **Figure 5**. All concentrations of α-T3, γ-T3, and α-T tested increased the IP prior to lipid oxidation in the PC liposomes significantly ($p < 0.0001$) as compared to the control (IP = 4.0 ± 3.2 min) indicating that the antioxidants protected the phospholipids in the liposomes against oxidation. In contrast, the addition of β-C and lyco to the liposomes did not increase the IP indicating the lack of any antioxidative effect as compared to the control ($p > 0.05$). The tocotrienols and α-T were all found to react in a dose-dependent manner resulting in significant increases in stability of PC liposomes when the antioxidant concentrations were increased from 100 to 500 ppm and from 500 to 1000 ppm ($p < 0.0001$). The highest antioxidative protection was seen by γ-T3 when compared to the same concentrations (500 or 1000 ppm) of α-T3 and α-T ($p < 0.0001$). Moreover, α-T3 was a more efficient antioxidant as compared to α-T at all concentrations tested ($p < 0.0001$). The antioxidant activity measured for the PC liposomes as the IP for formation of conjugated dienes decreased in the following order: γ-T3 > α-T3 > α-T.

Interactions of Carotenes with Tocopherol/Tocotrienol. α-T and the tocotrienols were each combined with β-C and lyco and for all combinations (**Figure 6**); the liposomes analyzed for conjugated dienes showed a significantly increased oxidative stability as compared to the control ($p < 0.0001$). Furthermore, the addition of 100 ppm β-C combined with 1000 ppm α-T, γ-T, or α-T clearly prolonged the IP found for the PC liposomes as compared to the IP calculated from the IP found in individual experiments with 100 ppm β-C and 1000 ppm α-T or tocotrienol assuming an additive model. It was accordingly concluded that the inhibition of lipid oxidation by the mixture of two potential antioxidants was greater than the sum of the inhibition obtained individually by the same two compounds. A similar pattern was observed for combinations of 100 ppm lyco with 1000 ppm

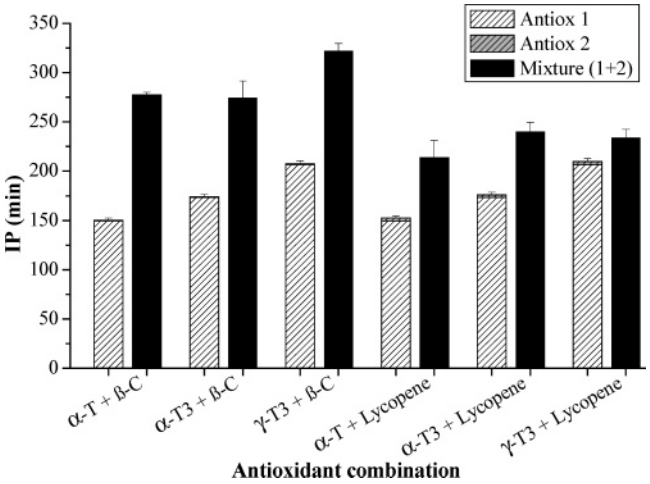

Figure 6. Induction time (IP in min with standard deviation $n = 2$) for formation of conjugated dienes in phospholipid liposomes with combinations of potential antioxidants added as compared to IP calculated from experiments with each potential antioxidant added separately assuming additivity. Antioxidant 1 is 1000 ppm α-T or tocotrienols, and antioxidant 2 is 100 ppm β-C or lyco.

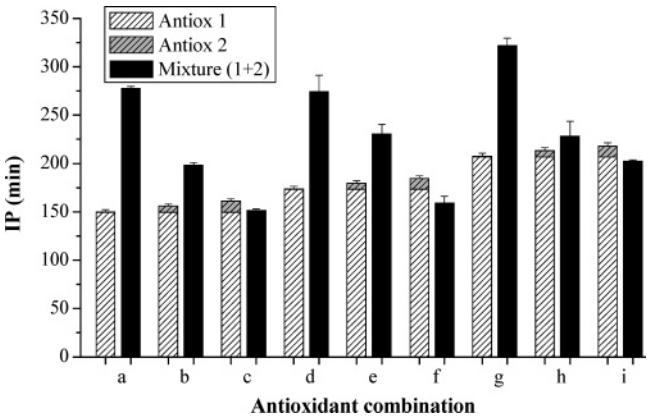

Figure 7. Induction time (IP in min with standard deviation $n = 2$) for formation of conjugated dienes in phospholipid liposomes with combinations of potential antioxidants added as compared to IP calculated from experiments with each potential antioxidant added separately assuming additivity. Antioxidant 1 is α-T or a tocotrienol (α- or γ-T3), and antioxidant 2 is β-C: a = 1000 ppm α-T + 100 ppm β-C; b = 1000 ppm α-T + 500 ppm β-C; c = 1000 ppm α-T + 1000 ppm β-C; d = 1000 ppm α-T3 + 100 ppm β-C; e = 1000 ppm α-T3 + 500 ppm β-C; f = 1000 ppm α-T3 + 1000 ppm β-C; g = 1000 ppm γ-T3 + 100 ppm β-C; h = 1000 ppm γ-T3 + 500 ppm β-C; and i = 1000 ppm γ-T3 + 1000 ppm β-C.

α-T3, γ-T3, or α-T. However, the synergy was weaker than observed for the β-C combinations. In an additional series of experiments, β-C was added in increasing concentration to 1000 ppm α-T3, γ-T3, or α-T in order to investigate the influence of the relative concentrations of the potential antioxidants on lipid oxidation in PC liposomes. The results are presented in **Figure 7**. The IP was for all combinations of β-C and α-T or tocotrienols investigated and was found to be significantly longer, indicating an increased oxidative stability as compared to the control ($p < 0.0001$). However, for an increasing β-C concentration relative to 100 ppm in the combinations, the length of the IP decreased for all combinations ($p < 0.0001$) while in a mixture of 100 ppm β-C and 1000 ppm α-T3, γ-T3, or α-T the two potential antioxidants were observed to interact synergistically; this synergy decreased for all combinations when β-C

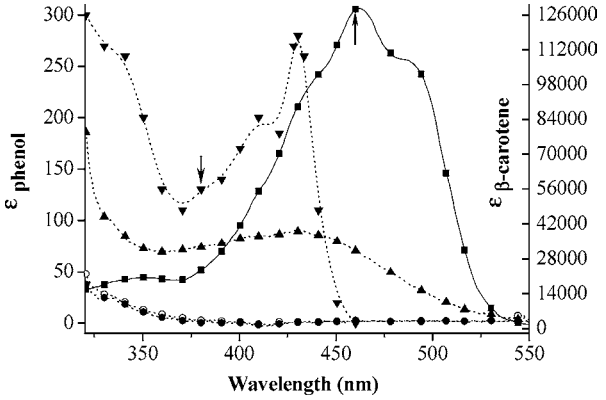

Figure 8. Absorption spectra of 1.0×10^{-5} M β-C (■), 1.0×10^{-3} M α-T (○), 1.0×10^{-3} M α-T3 (●), and 1.0×10^{-3} M γ-T3 (▲) in chloroform. Absorption spectra reported by Bisby and Parker (*25*) for α-tocopheryl radicals following 308 nm laser flash photolysis of an aqueous micellar solutions of 1.0×10^{-3} M α-T (▼). Arrows indicate the wavelength chosen for the determination of phenoxyl radicals (380 nm) and bleaching of parent β-C (460 nm); ϵ = extinction coefficient.

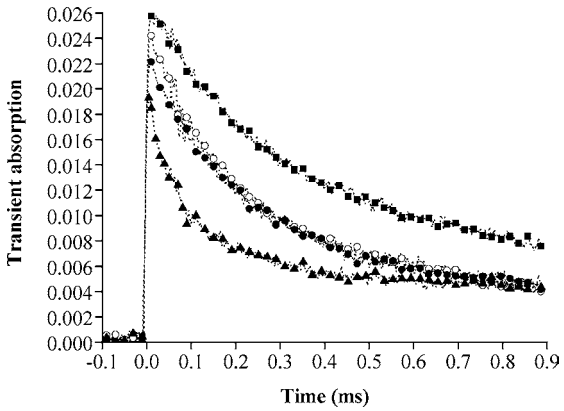

Figure 9. Time trace of absorption at 920 nm of β-C radicals following laser flash photolysis at 355 nm of 1.0×10^{-5} M β-C and 1.0×10^{-3} M α-T (○), 1.0×10^{-3} M α-T3 (●), 1.0×10^{-3} M γ-T3 (▲), or without phenolic compound (■) in chloroform.

was increased to 500 ppm, and when 1000 ppm β-C was added, even a weak antagonistic effect was observed.

Free Radical Kinetics. Laser flash photolysis of β-C in chloroform leads to photooxidation of the carotenoid as evidenced by β-C bleaching. Photooxidation in chloroform initiated by electron transfer to the solvent was previously shown to proceed via carotenoid cation radical species (*23*). Kinetic studies of interactions of carotenoid radicals with tocopherols became possible using transient absorption spectroscopy with fast detection and in the present study extended to the tocotrienols (*22*, *24*). Carotenoid concentration could be monitored in the visible absorption region ($\lambda_{max} = 460$ nm), radical adducts between chloroform and β-C ($\lambda = 920$ nm) together with carotenoid radical cations ($\lambda = 1000$ nm) in the near-infrared spectral region and tocopherols ($\lambda_{max} = 290$ nm) and tocopheroxyl radicals ($\lambda_{max} = 430$ nm) in the UV absorption region. To follow formation and disappearance of various transients, similar spectral regions were selected in the present study: 920 nm for the formation of β-C radicals, 460 nm for the bleaching of β-C, and 380 nm (rather than 430 nm to minimize spectral overlap with β-C) for the formation of phenoxyl radicals (**Figure 8**). The lifetime of the β-C radicals was found to be shorter in the presence of α-T, α-T3, or γ-T3 as monitored at 920 nm, and γ-T3 was found to react faster with β-C radicals than the

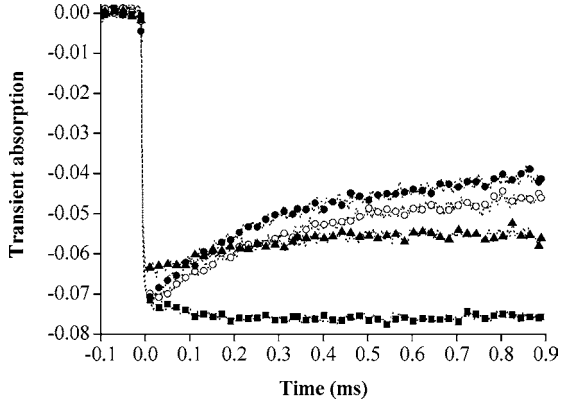

Figure 10. Time trace of absorption at 460 nm of bleaching of β-C following laser flash photolysis at 355 nm of 1.0×10^{-5} M β-C and 1.0×10^{-3} M α-T (○), 1.0×10^{-3} M α-T3 (●), 1.0×10^{-3} M γ-T3 (▲), or without phenolic compound (■) in chloroform.

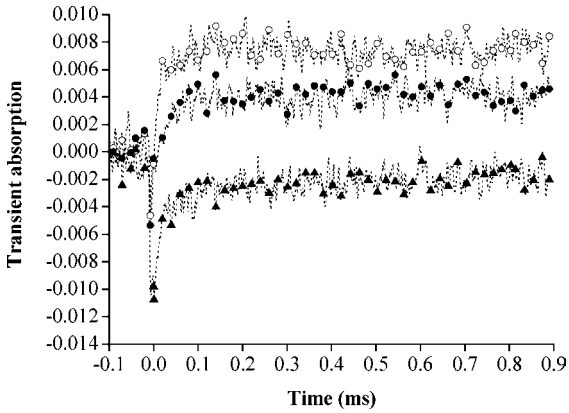

Figure 11. Time trace of absorption at 380 nm of bleaching of β-C and formation of phenoxyl radicals following laser flash photolysis at 355 nm of 1.0×10^{-5} M β-C and 1.0×10^{-3} M α-T (○), 1.0×10^{-3} M α-T3 (●), and 1.0×10^{-3} M γ-T3 (▲) in chloroform.

other phenolic compounds (**Figure 9**). In the presence of α-T3, slightly less β-C radicals were formed as compared to α-T but the decay rate of β-C radicals was the same. The degree of initial bleaching of β-C was not reduced markedly by addition of the phenols; however, the bleaching became partially reversible by the presence of the phenols (**Figure 10**). γ-T3 was the phenol that reduced the initial β-C bleaching most, whereas the partial recovery of absorption at 460 nm was not so pronounced as for α-T and α-T3. The slower increase in β-C absorption in the presence of γ-T3 could, however, be explained by the decay of the γ-tocotrienyl radical, which absorbs in the same spectral region as β-C. At 380 nm, an instantaneous bleaching of β-C was overlayed with the (positive) transient absorption of phenol radicals, the transient absorption of the bleaching of γ-T3, and β-C decomposition products formed in slower processes, as seen in **Figure 11**. It is further seen from **Figure 11** that γ-T3 reacts faster with the β-C radical cation in agreement with the results obtained at 920 nm (**Figure 9**). The initial negative transient absorption seen for γ-T3 is due to the higher absorption of γ-T3 at 380 nm and the more significant bleaching of γ-T3 due to higher relative absorption at the wavelength of excitation (355 nm), which results in the formation of a higher initial concentration of tocopheroxyl radicals. In conclusion, the reduction of the lifetime of the β-C radical, the partial recovery of β-C, and the increasing concentration of phenoxyl radicals following reaction with initially formed β-C radicals suggests that α-T, α-T3, and γ-T3 each are capable of reducing the β-C radicals,

in effect regenerating β-C from the one-electron oxidized form, the β-C cation radical. γ-T3 was the most effective as it reacted faster, while α-T3 and α-T reacted almost with identical rates.

DISCUSSION

Relative Antioxidant Activity of α-T3 and α-T. α-T3 and α-T were found to have similar effect as antioxidants in antioxidant-depleted palm olein when evaluated by the Rancimat test at 120 °C and to be depleted with the same rate in yellow palm olein when used for deep-fat frying of potato slices. α-T3 and α-T have also in other studies of palm olein and coconut oil using the Rancimat stability test at 100 and 160 °C, respectively, been found to have the same antioxidant activity (*4*, *6*). In addition to this, all available studies on homogeneous model systems showed α-T3 and α-T to possess equal antioxidant activities (*7*, *10*−*12*), suggesting that unsaturation in the side chain in the chromanols has little effect on the chemical reactivity, phenolic hydrogen bond energy, or resonance stabilization of the phenoxyl radical. However, in a simulated frying experiment at 185 °C with palm olein with the natural antioxidant content of the oil, α-T3 has been reported to act as a more effective antioxidant as compared to α-T (*5*).

The antioxidative activity of chain-breaking antioxidants in heterogeneous systems is influenced not only by the chemical reactivity toward radicals but also by other factors including localization and mobility in the microenvironment and conflicting reports on the relative antioxidant activity of α-T3 and α-T in membranous systems have appeared. In the present study, α-T3 was found to exert a higher antioxidant activity than α-T in PC liposomes with the antioxidants incorporated. This result is in agreement with previous findings by Serbinova et al. (*9*) and Suzuki et al. (*11*) on PC liposomes and rat liver microsomes. The higher antioxidant activity of α-T3 as compared to α-T in liposomes as also observed in the present study has previously been explained by Serbinova et al. (*9*) by the combined effect of three properties: (i) α-T3 has a higher recycling efficiency from the chromanoxyl radical, (ii) α-T3 is less associated in clusters and more uniformly distributed in membrane bilayers, and (iii) α-T3 has a strong disordering effect on membrane lipids, which makes interaction of the chromanols with lipid radicals more efficient. The higher mobility of α-T3 in liposomes as compared to α-T finds a parallel in the higher molecular mobility of polyenoic lipids in membranes as compared to saturated lipids (*26*). α-T3 may also be located closer to the membrane surface as compared to α-T promoting reaction with free radicals formed at the water−lipid interface as suggested by Suzuki et al. (*11*).

Relative Antioxidant Activity of α-, *γ*-, and *δ*-T3. γ-T3 was found to have the highest antioxidant activity in yellow palm olein during deep-fat frying followed by α-T3 and with γ-T3 as the least active based on their rate of depletion in agreement with the findings of Simonne and Eitenmiller (*5*) for antioxidant depletion after 6 h of simulated deep-fat frying at 185 °C in palm olein: γ-T3 > α-T3 > δ-T3. In red palm olein, γ-T3 also disappeared at the fastest rate; however, the relative antioxidant activity of α-T3 and δ-T3 could not be distinguished as evidenced by a similar rate of depletion. In the Rancimat stability test as performed in the present study, antioxidant-depleted palm olein with 100−1000 ppm tocotrienol homologues added also showed γ-T3 to be more effective than α-T3 in prolonging the stability of the antioxidant-depleted palm olein. γ-T3 has in agreement with the present finding been reported to possess twice or almost three times the activity of α-T3 in antioxidant-depleted palm olein added tocotrienols (*3*, *4*). Likewise, coconut oil has been shown to be better protected by γ-T3 as compared to α-T3 (*6*). In PC liposomes, γ-T3 was in the present study also found to be more effective in prolonging IP as measured by conjugated dienes when compared to α-T3, while for homogeneous model systems (methyl myristate, methyl linoleate, or ethanol) the rate of disappearance of antioxidants showed that α-T3 was depleted faster than γ-T3 during lipid oxidation accelerated by either azo-initiators or a stream of oxygen at 70 °C (*7*, *10*, *12*). α-T3 has been found to be a faster hydrogen atom donor than γ-T3 and δ-T3 by direct measurement of radical formation by ESR spectroscopy. The rate of antioxidant depletion seems to correlate with the reactivity of the phenol as a hydrogen atom donor while the length of IP may rather be considered as a measure of antioxidant capacity as suggested by Goh et al. (27), but antioxidant capacity and activity do not always follow the same order, since other factors such as dimerization of the antioxidant may be important.

Antioxidant Activity of Carotenes. The antioxidant activity of carotenes was tested in antioxidant-depleted palm olein and liposomes, and no activity was observed in agreement with the weak radical scavenging effect often assigned to carotenoids (*28*). Carotenoids have been found to show both prooxidative and antioxidative effects depending on the actual oxidation substrate and the specific conditions (*29*−*31*). Localization of carotenes in a heterogeneous system is important for a possible antioxidative effect and in the liposomes β-C and lyco are concentrated in the hydrophobic core of the lipid bilayer and susceptible to attack by lipid peroxyl radicals but are less accessible to radicals generated in the aqueous phase as those generated by AAPH in the present study (*29*, *32*). Lyco has been shown prooxidative in AAPH-initiated lipid peroxidation in liposomes but to inhibit the formation of phospholipid hydroperoxides by the lipophile AMVN radical initiator (*29*). Oxygen partial pressure may also be important, although Chen and Djuric (*31*) found no antioxidant effect of β-C and lyco in liposomes against AAPH-initiated lipid oxidation at varying concentrations and oxygen tensions.

Antioxidant Interactions. During the 60 repeated fryings, no differences in the oxidation parameters, PV and AV, were noted between yellow and red palm oleins despite the fact that red palm olein was slightly more polyunsaturated as compared to yellow palm olein. Red palm olein was even found more stable against oxidation as compared to yellow palm olein in the Rancimat test at 120 °C. The increased oxidative stability of red palm olein could, however, be assigned to both the carotene content in red palm olein and to the higher total tocotrienol/tocopherol content. Notably, carotenes were found to protect α-T and the tocotrienols during deep-fat frying as the depletion of carotene in red palm olein was paralleled by a slower decrease in the total content of α-T and tocotrienols when compared to yellow palm olein. Carotene depletion in red palm olein was moreover faster than the total depletion of α-T and the tocotrienols, an observation that could be explained by the carotenoids being the primary substrate for lipid radicals or by a mechanism where the carotenes are regenerating the oxidized phenols. In nonpolar solvents, Edge et al. (*33*) found using pulse radiolysis that 7,7-dihydro-β-C was able to regenerate α-T from the tocopheroxyl radical cation, while Tan and Saleh (*13*) reported that natural tocotrienol mixtures were able to reduce oxidized palm carotenes. A regeneration of α-T or the tocotrienols by carotenes as could be indicated by a slower rate of total tocotrienol/tocopherol depletion in red palm oil as com-

pared to yellow palm oil in the deep-fat frying experiment will appear as antioxidant synergism since a weaker antioxidant regenerates the more effective chain-breaking antioxidant. Notably, such a mechanism has been demonstrated for mixtures of quercetin and α-T (*34*). However, the carotenoid cation radicals generated during laser flash photolysis were regenerated by α-T, α-T3, and γ-T3, rather than α-T and the tocotrienols being regenerated by the carotenes. The carotenes being depleted prior to the phenols during the frying seem accordingly rather to indicate that carotenes are the primary substrate for lipid-derived radicals rather than the phenols. The carotenes are, however, not chain-breaking antioxidants, capable of stopping the oxidation. In contrast, the one-electron oxidized carotenes are subsequently partly regenerated by the phenols, as evidenced by the laser flash photolysis experiments, in competition with further degradation processes. γ-T3 seems to react faster than the other phenols as seen both from the laser flash photolysis and from the faster depletion of γ-T3 as compared to α-T and α-T3. The carotenoid may accordingly not be classified as a chain-breaking antioxidant but rather as a protector of the tocopherols and the tocotrienols through sacrificial oxidation in effect resulting in antioxidant synergism. A final proof of such a mechanism will depend on the design of experiments where the free radical reactions can be monitored in a lipid system like the palm olein rather than in a strongly electron-accepting solvent like chloroform.

The liposome experiment supports the suggestion of a synergistic interaction of carotenes with α-T and the tocotrienols. The synergistic effect in the liposomes may also be explained by a sacrificial oxidation of β-C or lyco through reaction with lipid-derived radicals in effect protecting α-T and the tocotrienols. Mixtures of β-C and α-T have in another study been found not to act synergistic in liposomes, with a β-C and α-T ratio of 1:1 and 1:2 (*21*). Synergism seems to require that carotenoids are present in concentrations considerably lower than the tocopherol concentration (*35*−*37*), as also observed in the present study in which the effect was found to vanish when the ratio approached unity in the liposomes. High concentrations of carotenes have been found to act prooxidatively also in other systems (*32*).

Two major results seem to appear from the present study. First, for conditions of high temperatures in homogeneous lipid systems exemplified by palm olein and in liposomes at ambient temperatures, γ-T3 affords higher protection against lipid oxidation as compared to α-T3 and α-T, while α-T3 and α-T provide similar protection in palm olein. For the heterogeneous system, the higher molecular mobility may be of importance for the better protection observed for α-T3 with the unsaturated phytyl tail as compared to α-T. Second, during deep-fat frying in red palm olein, carotenes protect α-T and tocotrienols against oxidation in a process which may be termed sacrificial oxidation. In liposomes, this protection of the phenol by the carotenes was manifested as antioxidant synergism between β-C or lyco and α-T, α-T3, or γ-T3, when the carotene concentration was considerably lower than the phenol concentration (1:10 or 1:2). During the sacrificial oxidation of carotene, the tocotrienol/tocopherol may partly regenerate carotenes from the carotenoids radicals leading to some phenol degradation when carotenes are still present. Notably, this hypothesis of regeneration of carotenes by α-T and tocotrienols in palm olein during deep-fat frying and in liposomes was supported by real-time detection of decay of β-C radicals and simultaneous formation of phenoxyl radicals in a chloroform model system following laser flash photolysis.

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NOTE ADDED AFTER ASAP PUBLICATION

The original posting of April 4, 2006 has been corrected. A percentage in the first paragraph of the Materials and Methods section was corrected in the revised ASAP posting of April 5, 2006.

LITERATURE CITED

(1) Mielke, T. *Oil World Annual 2004. Global Analysis. All Major Oilseeds, Oils & Oilmeals. Supply, Demand and Price Outlook*; ISTA Mielke GmbH: Hamburg, Germany, 2004.

(2) Ooi, C. K. Minor components of palm oil. *PORIM Bull.* **1999**, *38*, 29−32.

(3) Gapor, A. B.; Ong, A. S. H.; Kato, A.; Watanabe, H.; Kawada, T. Antioxidant activities of palm vitamin E with special reference to tocotrienols. *JOPR* **1989**, *1* (1), 63−67.

(4) Feng, H. P. Preparative techniques for isolation of vitamin E homologues and evaluation of their antioxidant activities. *Diss. Abstr. Int.* **1995**, *56* (10), 207.

(5) Simonne, A. H.; Eitenmiller, R. R. Retention of vitamin E and added retinyl palmitate in selected vegetable oils during deep-fat frying and in fried breaded products. *J. Agric. Food Chem.* **1998**, *46*, 5273−5277.

(6) Wagner, K. H.; Wotruba, F.; Elmadfa, I. Antioxidative potential of tocotrienols and tocopherols in coconut fat at different oxidation temperatures. *Eur. J. Lipid Sci. Technol.* **2001**, *103* (11), 746−751.

(7) Lehmann, J.; Slover, H. T. Relative antioxidative and photolytic stabilities of tocols and tocotrienols. *Lipids* **1976**, *11* (12), 853−857.

(8) Yamaoka, M.; Carrillo, M. J. H.; Nakahara, T.; Komiyama, K. Antioxidative activities of tocotrienols on phospholipid liposomes. *JAOCS* **1991**, *68* (2), 114−118.

(9) Serbinova, E.; Kagan, V.; Han, D.; Packer, L. Free radical recycling and intermembrane mobility in the antioxidant properties of alfa-tocopherol and alfa-tocotrienol. *Free Radical Biol. Med.* **1991**, *10*, 263−275.

(10) Suarna, C.; Hood, R. L.; Dean, R. T.; Stocker, R. Comparative antioxidant activity of tocotrienols and other lipid-soluble antioxidants in a homogeneous system, and in rat and human lipoproteins. *BBA* **1993**, *1166*, 163−170.

(11) Suzuki, Y.; Tsuchiya, M.; Wassall, S. R.; Choo, Y. M.; Govil, G.; Kagan, V. E.; Packer, L. Structural and dynamic membrane properties of α-tocopherol and α-tocotrienol: Implication to the molecular mechanism of their antioxidant potency. *Biochemistry* **1993**, *32*, 10692−10699.

(12) Yoshida, Y.; Niki, E.; Noguchi, N. Comparative study on the action of tocopherols and tocotrienols as antioxidants: Chemical and physical effects. *Chem. Phys. Lipids* **2003**, *123*, 63−75.

(13) Tan, B.; Saleh, M. H. Antioxidant activity of tocopherols and tocotrienols on plant colorant carotenoids. *Abstr. Pap. Am. Chem. Soc.* **1991**, *202* (1), 39-AGRO.

(14) Ping, B. T. Y.; May, C. Y. Valuable phytonutrients in commercial red palm olein. *POD* **2000**, *32*, 20−25.

(15) AOCS Ce 1-62. Fatty acid composition by gas chromatography. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; American Oil Chemists' Society: Champaign, Illinois, 1998.

(16) AOCS Ce 8-89. Determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; American Oil Chemists' Society: Champaign, Illinois, 1998.

(17) PORIM. *PORIM Test Methods*; Palm Oil Research Institute of Malaysia, Ministry of Primary Industries: Kuala Lumpur, Malaysia, 1995; pp 43−44.

(18) AOCS Cd 8-53. Peroxide value acetic acid-chloroform method. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; American Oil Chemists' Society: Champaign, Illinois, 1998.
(19) AOCS Cd 18-90. *p*-Anisidine value. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; American Oil Chemists' Society: Champaign, Illinois, 1998.
(20) Fuster, M. D.; Lampi, A. M.; Hopia, A.; Kamal-Eldin, A. Effects of alpha- and gamma-tocopherols on the autoxidation of purified sunflower triacylglycerols. *Lipids* **1998**, *33* (7), 715−722.
(21) Roberts, W. G.; Gordon, M. H. Determination of the total antioxidant activity of fruits and vegetables by a liposome assay. *J. Agric. Food Chem.* **2003**, *51*, 1486−1493.
(22) Mortensen, A.; Skibsted, L. H. Real time detection of reactions between radicals of lycopene and tocopherol homologues. *Free Radical Res.* **1997**, *27*, 229−234.
(23) Mortensen, A.; Skibsted, L. H. Kinetics of photobleaching of β-carotene in chloroform and formation of transient carotenoid species absorbing in the near-infrared. *Free Radical Res.* **1996**, *5* (4), 355−368.
(24) Mortensen, A.; Skibsted, L. H. Relative stability of carotenoid radical cations and homologue tocopheroxylradicals. A real time kinetic study of antioxidant hierarchy. *FEBS Lett.* **1997**, *417*, 261−266.
(25) Bisby, R. H.; Parker A. W. Reactions of the α-tocopheroxyl radical in micellar solutions studied by nanosecond flash photolysis, *FEBS Lett.* **1991**, *209* (1, 2), 205−208.
(26) Halliwell, B.; Gutteridge, J. M. C. *Free Radicals in Biology and Medicine*, 3rd ed.; Oxford University Press: Oxford, United Kingdom, 1998.
(27) Goh, S. H.; Hew, N. F.; Ong, A. S. H.; Choo, Y. M.; Brumby, S. Tocotrienols from palm oil: Electron spin resonance spectra of tocotrienoxyl radicals. *JAOCS* **1990**, *67* (4), 250−254.
(28) Yanishlieva, N. V.; Aitzetmüller, K.; Raneva, V. G. β-Carotene and lipid oxidation. *Fett Lipid* **1998**, *100* (10), 444−462.
(29) Woodall A. A.; Britton, G.; Jackson, M. J. Carotenoids and protection of phospholipids in solution or in liposomes against oxidation by peroxyl radicals: Relationship between carotenoid structure and protective ability. *BBA−Gen. Subjects* **1997**, *1336* (3), 575−586.
(30) Yanishlieva, N. V.; Raneva, V. G.; Marinova, E. M. β-Carotene in sunflower oil oxidation. *Grasas Aceites* **2001**, *52* (1), 10−16.
(31) Chen, G.; Djuric, Z. Carotenoids are degraded by free radicals but do not affect lipid peroxidation in unilamellar liposomes under different oxygen tensions. *FEBS Lett.* **2001**, *505*, 151−154.
(32) Young, A. J.; Lowe, G. M. Antioxidant and prooxidant properties of carotenoids. *Arch. Biochem. Biophys*. **2001**, *385* (1), 20−27.
(33) Edge, R.; Land, E. J.; McGarvey, D.; Mulroy, L.; Truscott, T. G. Relative one-electron reduction potentials of carotenoid radical cations and the interactions of carotenoids with the vitamin E radical cation. *J. Am. Chem. Soc*. **1998**, *120*, 4087−4090.
(34) Pedrielli, P.; Skibsted, L. H. Antioxidant synergy and regeneration effect of quercetin, (−)- epicatechin, and (+)-catechin on α-tocopherol in homogeneous solutions of peroxidating methyl linoleate. *J. Agric. Food Chem.* **2002**, *50* (24), 7138−7144.
(35) Handelman, G. J.; Kuijk, F. J. G. M.; Chatterjee, A.; Krinsky, N. I. Characterization of products formed during the autoxidation of β-carotene. *Free Radical Biol. Med.* **1991**, *10*, 427−437.
(36) Palozza, P.; Krinsky, N. I. β-Carotene and α-tocopherol are synergistic antioxidants. *Arch. Biochem. Biophys*. **1992**, *297* (1), 184−187.
(37) Wrona, M.; Korytowski, W.; Rozanowska, M.; Sarna, T.; Truscott, T. G. Cooperation of antioxidants in protection against photosensitised oxidation. *Free Radical Biol. Med*. **2003**, *35* (10), 1319−1329.

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