Photooxidation of Unsaturated Oils: Effects of Singlet Oxygen Quenchers[1]

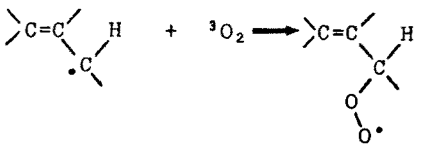
D.J. CARLSSON, T. SUPRUNCHUK, and D.M. WILES, Division of Chemistry,
National Research Council of Canada, Ottawa, Canada K1A 0R9

ABSTRACT

The photooxidative deterioration of unsaturated food oils by near UV and visible light was found to be retarded by nickel (II) chelates well known to quench singlet oxygen ($^1O_2$). The rates of hydroperoxide formation during light exposure were unaffected by efficient peroxy radical scavengers such as hindered phenols. α-Tocopherol (vitamin E) underwent a relatively rapid oxidation itself when exposed to $^1O_2$ in oil solutions, despite being able to deactivate $^1O_2$ quite efficiently. From a comparison of the rates of scavenging by stabilizers and attack on allyl groups, $^1O_2$ initiated deterioration is appreciably more difficult to retard than peroxy radical processes.

INTRODUCTION

The oxidation of unsaturated oils in the absence of light involves the formation of thermally unstable hydroperoxides and their decomposition products by the free radical processes 1 and 2 (1,2). The autoxidation of oils can be retarded by peroxy radical scavengers (usually phenols, InH) as shown in reaction 3 (1,2).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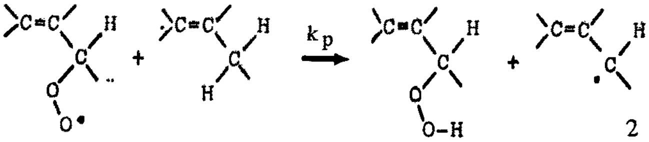
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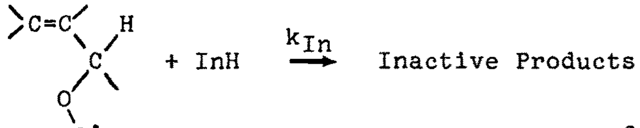
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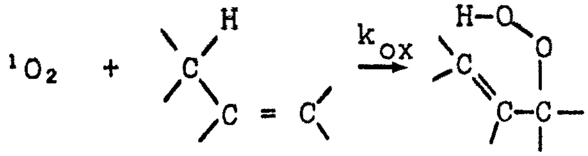
Exposure to diffuse daylight or artificial light is well known to cause a marked acceleration in the deterioration of unsaturated oils (3-5). This deterioration is probably sensitized by chromophoric impurities in the oils, especially residual natural dyes and pigments (chlorophyll, pheophytin, myoglobin, porphyrins, etc.) which absorb strongly in the visible or near ultraviolet (UV) light (3,5). In contrast to thermal oxidations, it has been suggested (3,5) that this light initiated deterioration does not involve free radicals, but instead results from the generation of singlet oxygen ($^1\Delta_g$, $^1O_2$) by the transfer of excitation energy from excited chromophoric impurities (Ch, reaction 4) to oxygen. $^1O_2$ is well known to react rapidly with C–C unsaturation to give hydroperoxides via a molecular process (reaction 5) (6,7). These hydroperoxides can then thermally cleave even at room temperature and so initiate a conventional free radical autoxidation to produce more hydroperoxide.

$$\mathrm{Ch} \xrightarrow{h\nu} [\mathrm{Ch}]^* \xrightarrow{^3O_2} \mathrm{Ch} + {}^1O_2 \tag{4}$$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Only very low concentrations of oxidation products (<0.00002% by wt) result in significant off-flavors or odors (1) so that the initial source of hydroperoxide in an oil is extremely important. It is conceivable that the early stages of light triggered deterioration involve only $^1O_2$ and molecular processes which cannot be retarded by conventional, free-radical scavenging antioxidants. In fact, Clements et al. (5) and Rawls and Van Santen (3) have shown that $^1O_2$ will react with unsaturated vegetable oils and related model compounds to give hydroperoxides, and that this oxidation can be prevented by the use of β-carotene or triethylamine. Both of these compounds are known to deactivate (quench) $^1O_2$ to the ground state (reaction 6) by different pathways (8,9).

$${}^1O_2 + \text{Quencher} \xrightarrow{k_q} {}^3O_2 + \text{Quencher} \tag{6}$$

The atmospheric concentration of $^1O_2$ is trivial in comparison with, for example, ozone (10), and $^1O_2$ reactions are probably only of commercial importance under photodegradative conditions when $^1O_2$ can be generated within the material (11). The absence of $^1O_2$ involvement in free radical autoxidations has been recently proven (2).

Quite recently, several new, highly efficient $^1O_2$ quenchers based on transition metal chelates have been discovered (12,13). In addition, the natural antioxidant α-tocopherol has been found to quench $^1O_2$ efficiently (14-16). Thus, it is of interest to compare the $^1O_2$ quenching ability of synthetic and natural compounds with their ability to retard the early stages of unsaturated oil photooxidation, using wavelengths similar to those expected during expourse on the shelf to diffuse daylight or fluorescent light (wavelengths greater than 340 nm).

EXPERIMENTAL PROCEDURES

Material

Food-grade samples of soybean (Rose and Laflamme, Toronto, Canada), peanut (Standard Brands, Montreal, Canada), olive (Bertolli, Lucca, Italy), and corn (St. Lawrence Starch, Port Credit, Canada) oils were employed. The soybean oil also contained citric acid, and the corn oil contained 2,6-di-tert-butylhydroxytoluene. Some oil samples were passed through a column of alumina before use. Hexadecane (Aldrich, Milwaukee, WI) was purified by repeated shaking with concentrated sulfuric acid (to remove olefins), distilled under reduced pressure, and passed

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TABLE I

Rate Constsnt (k_q) for the Quenching of $^1O_2$ in the Liquid Phate

	Additive	k_q x 10^8 $M^{-1}sec^{-1a}$	
I.	Octadecyl 3-(3′,5′-di-tert-butyl-4′-hydroxyphenyl)-propionate	<0.01 (i-octane)	(11)
II.	Propyl gallate	<0.01(CH_2Cl_2)	(14)
III.	α-Tocopherol	1.2 (i-octane) 6.7 (MeOH)	(15)
IV.	Ni[2,2′-thiobis(4-tert-octylphenolate)]-n-butylamine	2.0 (i-octane)	(12)
V.	Ni bis(2-hoyroxy-5-methoxyphenyl-N-n-butylaldimine)	26 (i-octane)	(12)
VI.	Ni bis(di-n-butyldithiocarbamate)	170 (i-octane)	(12)

[a] Determined in the solvents shown either in this work or in the references indicated.

through alumina immediately before use.

The additives used included α-tocopherol (Eastman, Rochester, NY) propyl gallate (Baker, Phillipsburg, NJ), octadecyl 3-(3′,5′-di-tert-butyl-4′-hydroxyphenyl) propionate (Ciba Geigy, Ardsley, NY), nickel bis(di-n-butyldithiocarbamate) (Pfaltz and Bauer, Flushing, NY), nickel [2,2′-thiobis (4-tert-octylphenolate)]-n-butylamine (Aldrich), and nickel bis(2-hydroxy-5-methoxyphenyl-N-n-butylaldimine) (prepared as reported previously [17]). All were purified by recrystallization before use.

Photooxidations

Oil samples (5 ml) were exposed to the radiation from a 500W S.P. mercury lamp (Osram) filtered through 10 cm of water (to reduce infrared) and a Corning CSO-52 filter which transmitted only above 340 nm. Quartz lenses and a front aluminized mirror were used to give a parallel beam which completely illuminated the oil sample in a 6 cm diameter Petri dish covered with a flat quartz window. The oil layer was 0.3 cm in thickness and was stirred continuously with a glass encased magnetic stirrer. The oil temperature was 27 ± 1 C during irradiation. The constancy of the incident intensity was checked with a calibrated thermopile (Eppley).

Peroxide Determinations

Peroxide buildup was measured on 0.2 ml oil samples by the spectrophotometric measurement of I_3^- released from an acidified solution of sodium iodide in isopropanol as described previously (18). This method measures all hydroperoxides and peracids but only diacyl and endo-peroxides (19). All other peroxides and peresters are not reduced under the mild conditions of this method.

$^1O_2$ Exposure

Liquid samples (2.0 ml) were exposed to $^1O_2$ generated from a microwave discharge in oxygen at 6 torr. The liquids were stirred continuously during exposure in the apparatus described previously (12). This system has been shown to be free of ozone or atomic oxygen interference when mercury is slowly distilled into the O_2 stream prior to the discharge (20).

$^1O_2$ Quenching Efficiency

The effectiveness of propyl gallate in quenching $^1O_2$ was measured by the rubrene photobleaching technique employed previously (12). Other quenching rate constants (k_q, reaction 6) were taken from the literature and are collected in Table I.

RESULTS AND DISCUSSION

Oil Photooxidations

All four oils oxidized considerably over a 60 min period when exposed to the >340 nm radiation. Hydroperoxide buildup data are shown in Figure 1. All of the commercial oils gave a positive indication of hydroperoxides even before irradiation. Passage of each oil through alumina reduced the hydroperoxide level to virtually zero but also markedly decreased their color. The alumina treated oils photooxidized at a reduced rate (Figure 1c). This decrease might result from the loss of light absorbing chromophores and/or from the loss of the hydroperoxides. Hydroperoxides do photocleave to give free radicals which may initiate oxidations (18). However, the linearity of most of the hydroperoxide concentration-time curves (despite an up to 30-fold increase in hydroperoxide concentration) and the complete absence of autoacceleration indicates that hydroperoxide photocleavage is negligible for irradiation at ⩾340 nm within the time scale of our experiments. When irradiation of each oil was interrupted after 60 min, no further change in hydroperoxide level was observed during 10 hr of dark storage, indicating the absence of a simultaneous thermal autoxidation reaction.

The solution of various additives in the oils before light exposure had widely differing effects (Fig. 1). Abbreviations for the compounds used are listed in Table I. The phenols I and II had negligible effect in all cases when used at concentrations of 0.1-0.48% by wt. Hindered phenols are known to effectively scavenge peroxy radicals and retard the autoxidation of unsaturated oils when used at $\sim$0.01% by wt (1). Thus, the light initiated oxidation of the oils is apparently not a free-radical process. The long chain hindered phenol I was chosen because of its own resistance to photodestruction compared to the simple phenols (10). The naturally occurring phenol, α-tocopherol (III), is also known to efficiently scavenge peroxy radicals (1,2) (as well as to quench $^1O_2$, discussed below), but was only marginally effective in preventing peroxide buildup during the irradiation of soybean oil containing 0.06% by wt of α-tocopherol and actually increased the measured hydroperoxide yield when used at higher concentrations (Fig. 1a).

All of the nickel chelates retarded the light initiated oxidation to a greater or lesser extent (Fig. 1). The inherent absorption of these colored compounds could simply screen out the active wavelengths during irradiation and so protect the oils solely by light absorption. To investigate this effect, additive free soybean oil was exposed to the <340 nm irradiation which had passed through a scrrening layer of oil, identical to the first layer except that this screening layer

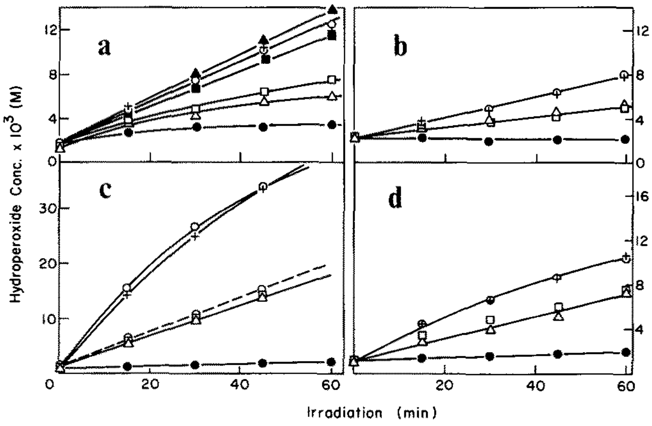


FIG. 1. Unsaturated oil photooxidation. (a) Soybean, (b) peanut, (c) olive (--after Al_2O_3 treatment), (d) corn. Additive (concentration):

0 None
+ Phenol I (0.48% by wt) or Phenol II (0.1% by wt)
■ Phenol III (0.06% by wt)
▲ Phenol III (0.75% by wt)
□ Chelate IV (0.24% by wt)
● Chelate V (0.24% by wt)
△ Chelate VI (0.012% by wt)

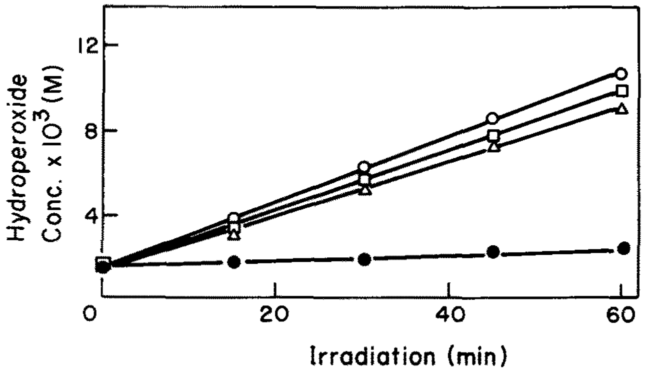


FIG. 2. Soybean oil photooxidation. Hydroperoxide formation in an additive-free oil layer irradiated through an additive-containing oil layer. Screening layer additive (concentration):

○ None or phenol III (0.75% by wt)
□ Chelate IV (0.24% by wt)
● Chelate V (0.24% by wt)
△ Chelate VI (0.012% by wt)

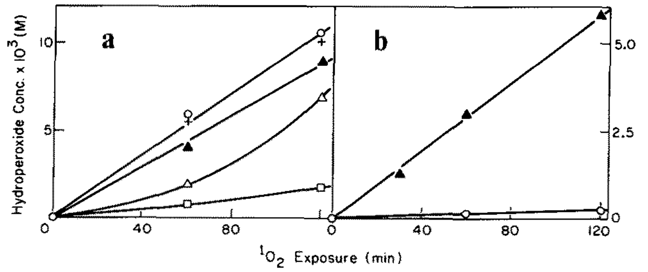


FIG. 3. $^1O_2$ exposure of solutions. $^1O_2$ from microwave discharge. (a) Soybean oil (after Al_2O_3 treatment), (b) hexadecane. Additive (concentration):

0 None
+ Phenol I (0.48% by wt)
▲ Phenol III (0.75% by wt)
□ Chelate IV (0.24% by wt)
△ Chelate VI (0.012% by wt)

contained the additives. Hydroperoxide buildup in the additive free, screened oil sample is shown in Figure 2, which clearly shows that only the nickel aldimine (V) is acting predominantly as a light absorber. Thus, the $^1O_2$ quenching effect of this additive cannot be evaluated in this light sensitized system. Similarly, chelate VI could not be studied at higher concentrations comparable to the other additives because light absorption became dominant.

The nickel chelates (including V) are all known to quench $^1O_2$ with various efficiencies, shown by the quenching rate constants k_q for reaction 6 collected in Table I. Bearing in mind the different concentrations employed, the effectiveness of nickel additives IV and VI in preventing the oil oxidation (Fig. 1) is consistent with their $^1O_2$ quenching ability. However, hydroperoxide decomposition by certain metal chelates has been previously demonstrated (21). To examine the importance of this effect, soybean oil samples were exposed to light for 60 min to give $\sim$1.2 x 10^{-2} M hydroperoxide. Chelates IV and VI (0.24 and 0.012% by wt, respectively) were then added to separate aliquots and stored in the dark. Chelate IV caused no change in hydroperoxide concentration over 5 hr. On adding VI to the peroxidized oil, no change in hydroperoxide concentration was observed for the first hour, although this was followed by an accelerating increase in hydroperoxide level, resulting in a twofold increase after 5 hr. This effect is consistent with the reported decomposition of hydroperoxides to give free-radical products by chelate VI (22), followed by a free-radical autoxidation. However, the slowness of the decomposition by VI and the failure of IV to decompose hydroperoxides implies $^1O_2$ quenching to be the key step in the retardation of oil photooxidations by chelates IV and VI for the data shown in Figure 1.

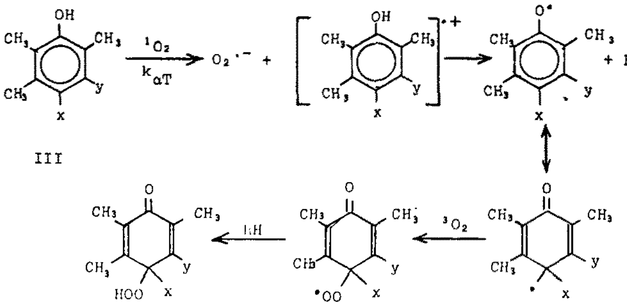
The failure of the α-tocopherol to retard the buildup of peroxide products during the photooxidations is unexpected in view of its well established ability to quench $^1O_2$ (Table I). In an attempt to resolve this anomaly and to compare the effects of the additives on the unequivocal oxidation of unsaturated oils by $^1O_2$, samples were exposed to $^1O_2$ from a microwave discharge.

Exposure to Microwave Generated $^1O_2$

The exposure of oxygen at low pressure to a microwave discharge in the presence of mercury vapor provides one of the most reliable routes for the generation of $^1O_2$ ($^1\Delta_g$) (12). The accumulation of hydroperoxides in soybean oil during $^1O_2$ exposure is shown in Figure 3a. Both chelates IV and VI were again found to be effective in preventing hydroperoxide buildup, whereas phenol I was ineffective, as expected from the $^1O_2$ quenching rate constants for these additives (Table I). α-Tocopherol was only slightly effective in preventing hydroperoxide buildup, well below that anticipated from its established $^1O_2$ quenching ability (cf. Table I comparable to k_q for chelate IV, but used at over three times its concentration).

The origin of the apparent ineffectiveness of α-tocopherol was shown when this naturally occurring phenol was exposed to $^1O_2$ in the saturated alkane hexadecane, which is inert to $^1O_2$ attack (20) (Fig. 3b). The observed rapid buildup of hydroperoxide (or an endoperoxide) can only come from $^1O_2$ attack on the phenol itself. Thus, although α-tocopherol can quench $^1O_2$ and prevent hydroperoxide formation from the unsaturated oils, at the concentration chosen the natural antioxidant itself peroxidizes to give roughly an equivalent amount of peroxidic product detected by our iodometric technique.

Phenol loss was also indicated by the associated decrease of the UV absorption of α-tocopherol. After 2 hr of $^1O_2$ exposure, the optical density change in the 290 nm α-tocopherol absorption indicated the destruction of 5 x 10^{-3} M of the phenol, close to the hydroperoxide yield observed in this time period (Fig. 3b). α-Tocopherol destruction by $^1O_2$ has been previously observed (14,15) and may follow the scheme shown in reaction 7 to give the thermally and photounstable hydroperoxycyclohexadiene-one, as proposed for simple phenols (23). Grams et al. (24) have found quinone products from a dye sensitized oxidation of α-tocopherol. Endoperoxide intermediates were suggested, and these would account for the α-tocopherol oxidation products observed after microwave exposure (Fig. 3). However, Grams et al. did not preclude the possibility of α-tocopherol oxidation by a free-radical process following a hydrogen abstraction by the sensitizing dye (25) rather than the assumed $^1O_2$ reactions.



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Published rate constants ($k_{\alpha T}$) for α-tocopherol destruction by $^1O_2$ range from $\sim 2 \times 10^6$ (in pyridine [14] and benzene [16]) to $\sim 5 \times 10^7$ $M^{-1}s^{-1}$ (in methanol [15]), whereas rate constants (k_{ox} reaction 5) of 0.7×10^5 and 1.3×10^5 $M^{-1}s^{-1}$ (in pyridine [26]) have been found for the reactions of $^1O_2$ with methyl oleate and methyl linoleate, respectively. The data shown in Figure 3 for the oxidations by identical $^1O_2$ streams of soybean oil (∿30% oleic and ∿50% linoleic acids) and α-tocopherol in hexadecane are consistent with a ratio of $k_{\alpha T}/k_{ox}$ of ∿100, intermediate between the ratios calculated from the published values (20-500). The variations in the published $k_{\alpha T}$ values have been explained in terms of marked solvent polarity effects (15).

The rapid peroxidation of α-tocopherol by $^1O_2$ in light exposed oils has three important implications. Firstly, the natural phenol cannot prevent the buildup of hydroperoxide or peroxide species during the light exposure of the oils. Secondly, its ability to act as a thermal antioxidant (free radical scavenger) will be impaired by light exposure of the oils. Finally, the vitamin E content of the foodstuff will be decreased.

$^1O_2$ Attack and Stabilization

The retardation of the photooxidation of unsaturated oils could in principle arise from one or more of hydroperoxide decomposition to molecular products, $^1O_2$ quenching, or deactivation of the light excited chromophores (presumably residual natural dyes and pigments). For chelates IV and VI, the first mechanism appears unimportant within the time scale of our experiments, although chromophore deactivation is still a possibility. In fact, most of the published evidence for $^1O_2$ involvement in oil photooxidation (3,4) is based on the use of compounds which are known to quench other light excited chromophores. For example, β-carotene can quench most polynuclear aromatics (27), aliphatic amines quench ketones (28), and nickel chelates quench anthracene (17). However, the close parallel between the ability of the chelates IV and VI to prevent the oil attack by microwave generated $^1O_2$, and their ability to protect against light initiated oxidation implies that hydroperoxide buildup is prevented by the deactivation of photogenerated $^1O_2$.

Free-radical autoxidation of olefins can be effectively retarded by low concentrations (∿0.01% by wt) of free radical scavengers (added phenols or natural α-tocopherol) (1). This effectiveness results from the low rate of peroxy radical (RO_2°) attack on an allyl methylene [rate constants k_p [reaction 2] for $RO_2\cdot$ attack on oleate and linoleate are 1 and 62 $M^{-1}s^{-1}$, respectively [29]) as compared to $RO_2\cdot$ scavenging by the more effective phenols (rate constant k_{In} [reaction 3] for linoleate $-RO_2\cdot$ scavenging by hydroquinone is 1.1×10^6 $M^{-1}s^{-1}$ [29]). Thus, the competitive rates of $RO_2\cdot$ scavenging by a phenol concentration [InH] and of $RO_2\cdot$ propagation are $k_{In}[RO_2\cdot][InH]/k_p[RO_2\cdot][{>}C{=}\overset{|}{C}{-}CH{<}]$, i.e., $\sim 2 \times 10^4\ [InH]/[{>}C{=}\overset{|}{C}{-}CH{<}]$ and $\sim 1 \times 10^6\ [InH]/[{>}C{=}\overset{|}{C}{-}CH{-}]$ for linoleate and oleate, respectively. In addition, many propagations cycles (reactions 1 and 2) can occur for every initiation event in the free radical autoxidation.

For an efficient $^1O_2$ quencher (e.g., $k_q \geqslant 1 \times 10^8$ $M^{-1}s^{-1}$), the competitive rate of $^1O_2$ quenching by an additive concentration [Q] and $^1O_2$ attack on an oil is $(k_q[^1O_2][Q]/k_{ox}[^1O_2][{>}C{=}\overset{|}{C}{-}CH{<}])$. For both oleate and linoleate [$k_{ox} \sim 1 \times 10^5$ $M^{-1}s^{-1}$ in each case [26]), the relative rate for $^1O_2$ quenching and allyl group attack becomes $\sim 1 \times 10^3\ [Q]/[{>}C{=}\overset{|}{C}{-}CH{<}]$. This should be compared with the previously calculated peroxy radical ratios for scavenging as compared to allyl attack (i.e., up to $\sim 1 \times 10^6\ [InH]/[{>}C{=}\overset{|}{C}{-}CH{<}]$). Thus, only the most efficient $^1O_2$ quenchers ($k_q \sim 10^9-10^{10} M^{-1}s^{-1}$) can prevent the one step molecular process and can provide protection at ∿0.01% by wt. Additives meeting this quenching criterion include β-carotene and chelate VI (12). However, both would impart objectionable coloration (orange and green-brown, respectively) even at low concentrations.

The oxidations of unsaturated oils at wavelengths >340 nm are not retarded by known free-radical scavengers but are retarded by compounds which quench $^1O_2$. The degree of retardation approximately parallels the $^1O_2$ quenching ability of these compounds, although the $^1O_2$ quenching effect of α-tocopherol is offset by the oxidation of the vitamin itself to give hydroperoxidic products. Extremely effective $^1O_2$ quenchers are required to give any protection against light at conventional levels (0.01% by wt) because of the high rate of $^1O_2$ attack on allyl methylene groups. $^1O_2$ initiated deterioration can be most effectively minimized by the removal of natural pigments during the refining process. However, the inclusion of a nontoxic, effective $^1O_2$ quencher in foods and beverages containing unsaturated oils could substantially improve their shelf life.

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